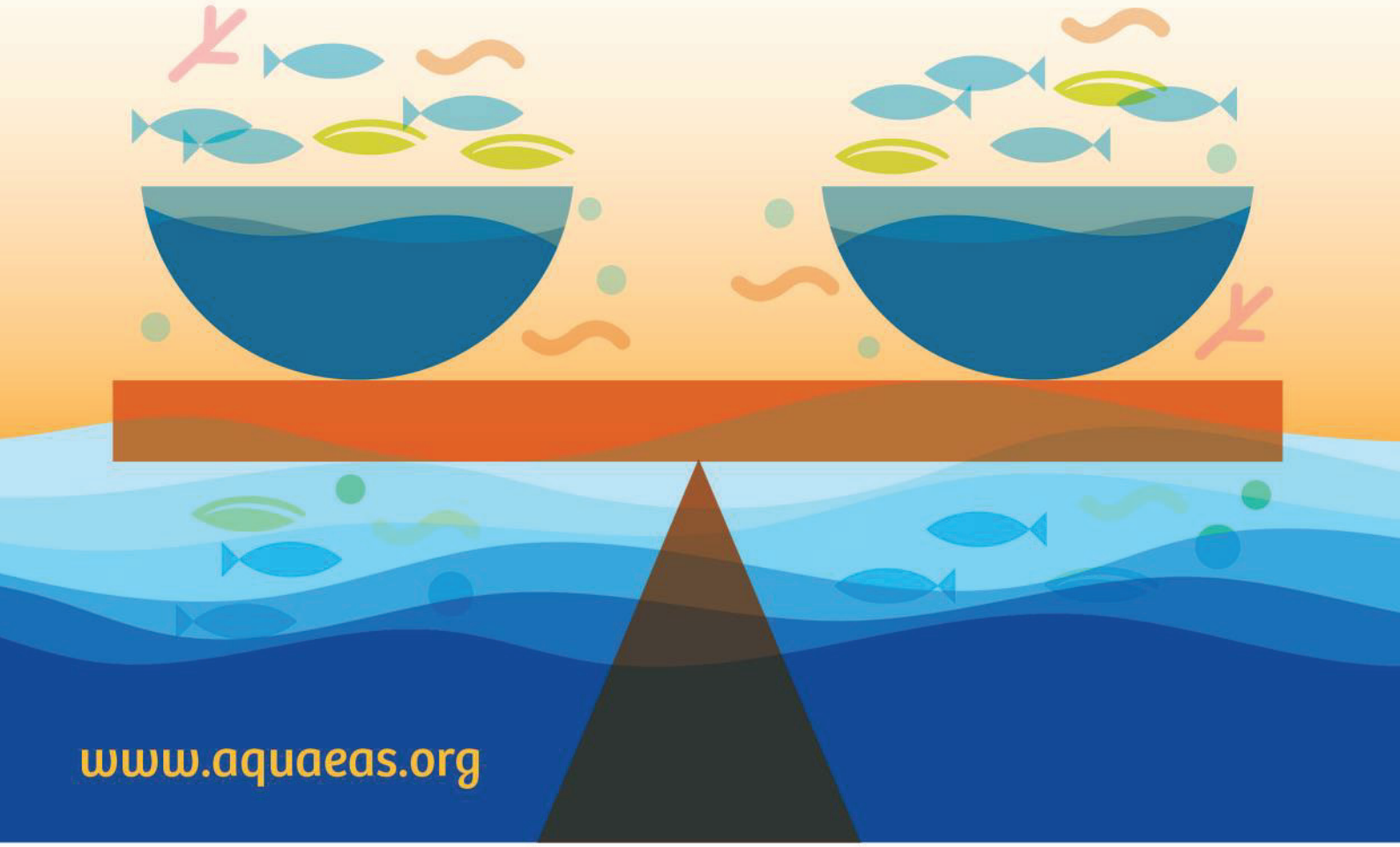
VIENNA, AUSTRIA SEPTEMBER 18-21, 2023

Balanced Diversity in Aquaculture Development

aquaculture europe 23



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Welcome to Aquaculture Europe 2023 in Austria's iconic capital Vienna

"Balanced Diversity in Aquaculture Development" is the theme of Aquaculture Europe 2023 (AE23). This theme surely highlights the potential for aquaculture, already representing the world's most diverse farming practice, to further grow sustainably utilising the opportunities that lie in diversity.

Food from aquaculture contributes to Sustainable food systems at a local and global level. Since global aquaculture production is dominated by a few dozen species, major efforts are being made to promote species diversity. To succeed, we need responsible use of resources, circular food systems, improved efficiency, and increased resilience against future challenges such as diseases and climate change. All issues that require further diversification in aquaculture also beyond species level. AE2023 will provide a great opportunity for discussing new and innovative ideas to address challenges and opportunities as well as up scaling already proven concepts and solutions of diversification in aquaculture industry.

What makes EAS annual events unique is bringing together scientists, industry leaders and entrepreneurs, governmental bodies, and regulators from all over Europe and sharing the same passion for aquaculture. This year theme of diversification will be reflected in the 32 scientific sessions over 3 days ranging from genomics to socioeconomics, covering the full scope of European aquaculture scientific disciplines and species. AE2023 will also feature an international trade exhibition with close to 170 booths, student sessions and activities, satellite workshops and updates on EU research.

In addition, two special events will take place: The AE2023 Industry Forum entitled "TRANSITION: Towards new technologies and new markets" focus on freshwater aquaculture especially targeted towards farmers from Austria, Germany, Switzerland, Hungary and Czech Republic. A unique opportunity to learn more from and about freshwater aquaculture for all AE23 delegates.

This year's program AE23 Innovation Forum is dedicated to new innovations on the theme of balancing diversity within aquaculture and the wider blue economy. The Innovation Forum includes a series of specific pitching sessions, showcasing research driven innovations as well company-driven initiatives that lead to innovation for the benefit of the entire sector. The program is exciting for all of us with a passion for knowledge that is used by and make an impact for industry and society.

This year we are expecting close to 2000 attendees with more than 540 oral presentations. Results presented as Eposters will also receive special attention at this year's conference - and the last slot in each session before the breaks is dedicated to a "Poster Focus." More than 600 scientific abstracts were received and these have been reviewed by the session chairs and integrated into an impressive programme by Bela Urbanyi (MATE, Hungary) and Nikos Papandroulakis (HCMR, Greece) as AE2023 Program co-chairs. Thank you for your hard work! I'd like also to thank our Steering and Local Organising Committees who gave their time and efforts to make AE2023 possible as for my colleagues on the Board of the EAS with several newly appointed directors. A big thanks also to our Gold Sponsors Biomar, Session Sponsors DSM and support from Meeting Destination Vienna. We are also grateful for the communication channels offered to us by our Media Partners. I hope you will enjoy the event, the people, and the science. I'm excited about the diverse aquaculture program we have for you, and I look forward to seeing you all in Vienna!

Bente E. Torstensen – EAS President 2022-2024

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ABSTRACTS

MODELLING WASTE ASSIMILATION BY BLUE MUSSELS WITHIN THE SPATIAL CONSTRAINS OF A COMMERCIAL FISH FARM: IMPLIMENTATIONS FOR MULTITROPHIC AQUACULTURE

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Blue mussels (*Mytilus edulis*) are often recommended in integrated multitrophic aquaculture (IMTA) systems to extract particulate waste from finfish production (Cranford et al. 2013), but it is debated significant mitigation can be obtained by direct assimilation of fish farm waste (Sanz-Lazaro & Sanchez-Jerez 2017). The performance of IMTA in open water systems is highly influenced by the spatial arrangement of the system and local environmental factors. Therefore, it is essential to evaluate the potential mitigation within the local environmental and spatial constraints (Kerrigan and Suckling 2018; Reid et al. 2020). The mitigation effect depends on the dispersion of waste from the fish farm to the mussel farm and the exposure time of the mussels to the waste, which depend on currents and settling velocity of the waste particles (Reid et al. 2020).

As vertical transport dominates the particulate waste distribution around fish farms, it has been suggested that higher mitigation can be achieved by locating the extractive species below fish farms (Filgueria et al. 2017), and studies have explored IMTA with extractive species at the seabed (Nederlof et al. 2020). However, the living conditions at the seabed vary considerably during the course of a production cycle, especially in sheltered areas (á Norði et al. 2011). Another approach to extract particles in the vertical waste stream is to suspend the extractive species below the fish farm, but this has received little attention.

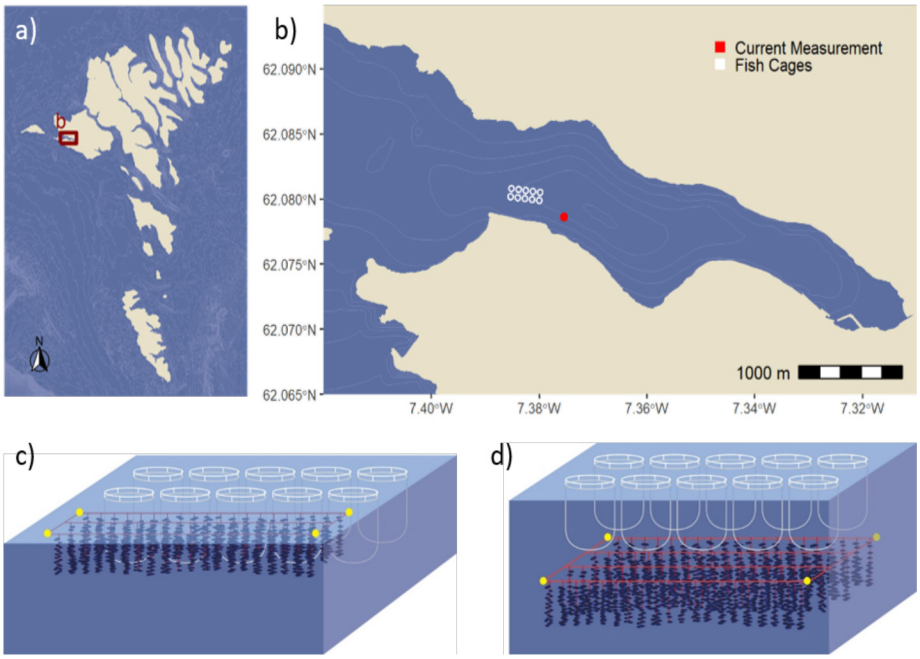

Fig. 1 Study site (a and b) and conceptual drawings of the modelled spatial locations of the Atlantic salmon farm and the blue mussel farms (c and d).

(Continued on next page)

To support the decision basis in future implementation of blue mussels to mitigate commercial fish farm impacts, a model was developed to assess the assimilation of fish farm waste by blue mussels at an operational fish farm. The waste production was modelled based on feed data, analysis of the commercial feed used, and the spatial arrangement of the net pens (Fig. 1b). Dispersion was modelled according the local hydrodynamics and assimilation of particulate waste by blue mussels was modelled according to two spatial blue mussel/salmon farm configurations; the approach with blue mussels at the surface at the long side of the fish farm (Fig. 1c) and an alternative approach with the blue mussel farm submerged directly below the net cages (Fig. 1d).

The general design of the fish farm is widely used in salmon farming and to investigate if the mitigation potential would be higher at other farms with different hydrodynamic settings, a sensitivity analysis was conducted on the current speed. A sensitivity analysis was likewise performed on the density of blue mussels as the density of mussels on passive spat collectors can be highly variable. Size and settling velocities of fish faecal particles are highly variable and depend on feed ingredients, fish size and hydrodynamics (Reid et al. 2009); and in general, the information on the size fractionations of waste particles is scarce (Nederlof et al. 2022). Thus, a sensitivity analysis was also conducted to investigate how the fraction of slowly settling particles influenced the mitigation performance of the blue mussel farms.

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TESTING ENVIRONMENTAL DNA-BASED DIAGNOSTICS FOR MULTI-PATHOGEN GILL DISEASE ASSOCIATED WITH CARP EDEMA VIRUS INFECTION IN COMMON CARP

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Introduction

Recently, environmental DNA (eDNA) analysis has been recognised as a tool for monitoring the presence of pathogens in aquaculture. eDNA-based diagnostic appears to be particularly suitable for the detection of mucosal pathogens in fish. Gill and skin diseases are often multi-pathogenic including co-infections with viruses, bacteria and parasites. For example, carp edema virus, an immunosuppressive poxvirus that infects the gills and causes koi sleepy disease (KSD), often occurs with co-infections with ectoparasites such as *Ichthyobodo necator* and the bacterium *Flavobacterium branchiophilum*, making diagnosis and treatment difficult. Furthermore, the disease occurs in common carp and koi that are less accessible and cannot always be sacrificed for sampling (e.g. high-value ornamental koi or common carp broodstock). We therefore tested the applicability and robustness of eDNA-based methods for the detection of pathogens associated with KDS.

Materials and methods

To test eDNA-based methods for rapid detection of KDS, water samples, gill swabs and gill biopsies were collected during disease outbreaks and experimental infections and stored frozen at -20°C. Several centrifugation speeds and different pore size filters were used to select the best method for concentrating pathogens from water. Detection of carp edema virus, *Ichthyobodo necator* and *Flavobacterium* sp. was performed by qPCR after DNA extraction using Qiagen DNA mini kit.

Results

Filtration (0.20 µm and 0.45 µm) appeared to be the most reliable method for concentrating the pathogens associated with KDS outbreaks. The detection of CEV, *I. necator* and *Flavobacterium* sp. was possible at very early stages of infection and the CEV concentration increased rapidly on day 4 onwards when the first clinical signs were visible. Furthermore, the DNA of all pathogens could be detected in the water for at least 8 days after removal of infected fish.

Conclusions

Concentration of all pathogens involved in multi-pathogen gill disease associated with carp edema virus infection was possible with a single water filtration procedure using a e.g. 0.20 µm syringe filter. eDNA-based diagnosis could therefore be a very efficient method for detecting outbreaks of KSD, flavobacteriosis and ichthyobodiasis, at least in relatively small water bodies such as small ponds or tanks.

LIVE ATTENUATED VIRUS VACCINE PROTECTS THE SKIN BARRIER AND GILL FUNCTION FROM DISRUPTION CAUSED BY *Cyprinid herpesvirus 3*

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Introduction

Epitheliotropic viruses can be particularly dangerous in the aquatic environment, which is osmotically and microbiologically hostile to fish. If the pathogen is able to disrupt the mucosal barrier, the breach can lead to severe secondary effects, such as disruption of the osmotic balance or induction of secondary infections. We used a live attenuated virus vaccine against cyprinid herpesvirus 3 (CyHV-3) to investigate which aspects of non-direct protection are important in the aquatic environment and to fill the remaining knowledge gaps on how these vaccines effectively protect fish against the deadly disease caused by CyHV-3.

Materials and methods

Common carp were vaccinated against CyHV-3 using a double-deletion vaccine virus KHV-TΔDUT/TK in the absence or presence of a mixture of common carp beta-defensins 1, 2 and 3. Fish were challenged with a hyper-virulent Polish isolate of CyHV-3 2.5 months after vaccination. Blood, skin, gill and kidney samples were collected at 2, 7, 14, 28 days post vaccination and challenge for monitoring of immune responses by SNT, RT-qPCR using Fluidigm and disease related pathology.

Results

Vaccination induced mild clinical signs, low viral load and slight up-regulation of cd8 and igm gene expression in vaccinated fish, while blood neutralising activity increased from 14 days post-vaccination. A challenge infection with CyHV-3 induced severe disease with 80-100% mortality in unvaccinated fish, whereas no mortality was observed in vaccinated fish and the viral load was >1000-fold lower. Histological analysis showed that vaccination protected against pathological changes in the skin and gills. In the skin of non-vaccinated fish, T and B cell responses were severely downregulated, inflammatory and stress responses were increased upon challenge, whereas vaccinated fish had enhanced neutrophil, T and B cell responses. Disruption of skin and gill barrier elements (tight and adherence junctions, desmosomes, mucins) led to a severe osmotic imbalance and an uncontrolled increase in skin and gill bacterial load, which most likely exacerbated the pathology.

Conclusions

Using a live attenuated virus vaccine, we show that increased neutrophil, T and B cell responses provide protection against CyHV-3 infection and preserve skin integrity and gill function, supporting successful protection against secondary bacterial pathogens and osmotic disruption, enabling vaccinated carp to cope with the hostile aquatic environment.

BAD NEWS - GOOD NEWS SITUATION: SALT TREATMENT OF KOI SLEEPY DISEASE DELIVERS BOTH: LONG PERSISTENCE OF CARP EDEMA VIRUS AND SUCCESSFUL PROTECTION AGAINST CEV RE-INFECTION

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Introduction
Carp edema virus (CEV) is a highly virulent fish poxvirus that frequently causes a very severe koi sleeping disease (KSD), leading to high mortality in carp populations. Its worldwide spread is due to the international trade in koi and carp. In Germany, the virus has repeatedly been found in consignments of completely asymptomatic fish. In addition, some fish farmers and ornamental fish keepers have experienced repeated outbreaks of the disease without introducing new fish. This raised several questions about the ability of the virus to persist in populations following salt treatment and the effectiveness of natural immunity following this treatment.

Materials and methods
To answer these questions, a combination of case studies documenting the outcome of salt treatments in koi and farmed carp and experimental infections were used. Virus load was measured by qPCR and the development of clinical signs was measured by observing behaviour, gill pathology and blood Na+ and ammonia concentrations. Immune responses were evaluated with qPCR and immunocytochemistry.

Results
Salt treatment was found to be highly effective in preventing most of the morbidity and mortality associated with KSD. The main mechanism of action of salt treatment was confirmed to be the prevention of osmotic disorders by stabilising blood sodium concentration and the prevention of ammonia intoxication by facilitating gill excretion of ammonia. Results from the monitoring of the KSD outbreak showed that over 25% of the fish sampled remained CEV positive for five months without clinical signs. Further studies were carried out on carp rescued from a fish farm in Saxony, Germany. Experimental reinfections of these fish three months after recovery showed that they were protected against reinfection. Compared to naive fish infected with CEV, the naturally immunised fish did not develop the clinical signs of KSD, including pathophysiology and immunosuppression. Conversely, reinfection resulted in increased expression of *gzma*, *zap70*, *cd4* and *igm*, which are normally downregulated during KDS.

Conclusions
The long persistence of CEV may explain its successful global spread. On the other hand, the protection against reinfection conferred by increased T-cell and B-cell responses gives hope for the development of effective vaccines against CEV and other poxviruses affecting fish.

DISCOVERY OF A NILE TILAPIA STRAIN WITH RESISTANCE TO TILAPIA LAKE VIRUS DISEASE - FROM IMMUNOLOGY TO CONSIDERATIONS FOR IMPLEMENTATION IN AQUACULTURE

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Introduction

The occurrence of viral diseases that cause very high mortality can disrupt aquaculture production. This recently happened in Nile tilapia aquaculture with the emergence of a disease caused by tilapia lake virus (TiLV), which has dramatically affected tilapia farms around the world. In areas where the virus is endemic, three strategies can be used to limit the losses caused by infection: 1) improved biosecurity, 2) vaccination programmes, 3) selective breeding to increase resistance. To explore the third strategy, we investigated the resistance to TiLV in three genetic strains of tilapia reared in Germany. We used two strains originating from Nilotic regions (Lake Mansala (MAN) and Lake Turkana (ELM)) and one from an unknown region (DRE).

Materials and methods

Nile tilapia juveniles were infected with TiLV by intraperitoneal injection or cohabitation. Immune responses were measured using a Fluidigm array and correlated with viral load and pathological changes.

Results

Infection by injection resulted in 100% fish mortality in all three strains. However, when using cohabitation, we found that the ELM strain did not develop clinical signs of infection and had almost 100% survival. The other two strains showed severe clinical signs and a much lower survival rate of 29.3% for the DRE strain and 6.7% for the MAN strain. Disease resistance in tilapia from the ELM strain correlated with a lower viral load in both mucosal and internal tissues. The lower viral spread was associated with a stronger *mx1*-based antiviral response in the early phase of infection in the ELM strain. In addition, lower pro-inflammatory responses in the resistant strain may further contribute to its protection against disease-associated pathology.

Conclusions

Obtained results suggest the possibility of using TiLV-resistant strains as a cost-effective ad hoc solution to the TiLV challenge. However, it is important to note that fish of the resistant strain still had a significant viral load in the liver and brain 28 days after infection and could become persistent virus carriers, potentially transmitting the virus to naive populations. Therefore, the resistant strain should be used as part of an integrated approach that includes biosecurity, diagnostic and vaccination measures as appropriate.

IS IT POSSIBLE FOR THE TILAPIA LAKE VIRUS TO HOST JUMP TO SALMONIDS DUE TO THERMAL STRESS CAUSED BY HEATWAVES IN EUROPE?

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Introduction

Many salmonids are thermally sensitive and are therefore particularly vulnerable to thermal stress caused by rising temperatures during summer heat waves. We hypothesised that the combination of thermal stress and the presence of a new viral pathogen could lead to a potential host jump of the pathogen if the fish are susceptible. Tilapia Lake Virus (TiLV) can be considered as one of the most dangerous emerging viruses affecting warm water aquaculture. This pathogen has a global distribution and a host range that remains to be defined, but preliminary *in vitro* data indicate that the virus replicates in a variety of cell lines, including those derived from salmonids. Therefore, the aim of this study was to evaluate the potential of TiLV to infect salmonids in a range of water temperatures that can be reached during summer heatwaves in continental Europe.

Materials and methods

The susceptibility of several cell lines was assessed in different temperatures. The susceptibility of juvenile rainbow trout and brown trout to infection with TiLV was investigated in infection experiments based on cohabitation of both species with infected fish or intraperitoneal (i.p.) injection of the virus at elevated water temperatures of 20°C and 25°C. The behaviour, pathology, virus load and antiviral responses were measured.

Results

TiLV can replicate *in vitro* in salmonid cells over a wide range of temperatures from 15°C to 25°C. The infection experiments showed that the susceptibility of rainbow and brown trout to the virus was low, considering the ability of the virus to enter the organism. Exposure of these fish to the virus by cohabitation did not result in high levels of virus in the liver and brain. However, the permissiveness, i.e. the ability of the virus to replicate in the body of the fish, is high because i.p. injection of TiLV resulted in high levels of virus replication in the internal organs.

Conclusions

TiLV has some pathogenic potential in salmonids, which could theoretically be enhanced by climate change and anthropogenic activities. Further studies should determine whether factors affecting the mucosal barrier allow the virus to spread to the already permissive, thermally sensitive salmonid species.

EFFECTS OF SOYBEAN MEAL (SBM) DIETARY INCLUSION ON THE INTESTINAL MORPHOLOGY OF JUVENILE RUSSIAN STURGEON (*Acipenser gueldenstaedtii*)

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Introduction

In recent years, sturgeon farming has been one of the most rapidly growing branches of aquaculture. While the production of sturgeon larvae does not present many technical difficulties, it is very costly, not only due to the relatively long administration of natural food, but also due to high prices of commercial feeds, which are largely a resultant of expensive components.

Feeds with plant protein sources have long been the subject of research in many fish species, including sturgeon fish. Studies carried out so far on *Acipenser schrenckii* and its hybrid with *A. baerii*, in which the animal protein component was replaced with soy protein concentrate, showed negative effects on immunity, gastrointestinal tract morphology and growth (Jiang et al., 2018; Wei et al., 2019). Therefore, the aim of this study was to determine the effect of using soybean meal at 10% and 20% relative to animal components on the morphology of the gastrointestinal tract of juvenile Siberian sturgeons (*Acipenser baerii*).

Material and methods:

The study was carried out as part of the project no. 0001–6521.1-OR0700001/17/20 founded by Operational Program 'Fisheries and Sea' (2014–2020) financed by the European Maritime and Fisheries Fund. Siberian sturgeon larvae were maintained in RAS (recirculating aquaculture systems). From 20 dph (days post hatching), feeding with one of three experimental feeds was initiated: a control diet with no soybean components (P0), a diet in which 10% of the animal components were replaced with soybean (P10), and a diet in which 20% were replaced (P20). The dietary experiment lasted 7 weeks. After this time, the fish were slaughtered and their digestive tracts were collected for histological examination. Analysis of digestive tract morphology was carried out on 8 individuals from each group. Paraffin-embedded tissues were stained with haematoxylin and eosin (HE) and Alcian blue with Schiff's periodic acid reagent (AB/PAS) to differentiate mucosal cells. The morphometric analysis was carried out according to Kasprzak et al. (2019). Microscopic analysis was carried out using a Nikon Eclipse 90i microscope with a camera and NIS Elements software (Tokyo, Japan). Statistical differences were calculated in Statistica (Statsoft, Tulsa, USA) using a parametric ANOVA test with Fisher post hoc test.

Table 5 Morphometrical analysis of anterior and spiral intestine.

	GROUP					
	P0		P10		P20	
	Mean	SD	Mean	SD	Mean	SD
Anterior fold height	398,87[a]	126,98	360,94[a]	92,32	243,87[b]	38,52
Anterior fold width	72,88[a]	7,74	72,50[a]	6,75	82,04[b]	7,99
Anterior enterocyte height	33,39[a]	2,73	31,06[b]	3,83	35,58[a]	3,96
Anterior muscular layer	49,88[b]	11,12	44,13[a]	8,08	43,26[a]	8,82
Anterior lamina propria width	5,82[a]	0,87	6,12[a]	1,01	8,04[b]	1,87
Posterior fold height	174,86[c]	33,45	154,61[b]	22,89	133,51[a]	28,18
Posterior fold width	70,84[ab]	7,33	74,57[b]	7,42	68,16[a]	10,27
Posterior enterocyte height	36,78[a]	10,32	36,16[b]	3,87	41,69[c]	8,99
Posterior muscular layer	42,05[b]	16,63	36,86[a]	4,26	36,32[a]	6,69
Posterior lamina propria width	5,38[a]	0,96	6,14[a]	1,35	7,62[b]	2,173

Means followed by different letters in the same row are significantly different ($P < 0.05$).

(Continued on next page)

Results

In all individuals, the intestinal folds in both examined sections were well-organised, containing not only enterocytes without signs of degenerative changes, but also mucosal cells with a normal structure. At the bases of the intestinal folds, numerous cells were undergoing mitotic division. Enterocytes showed well-developed, visible microvilli. In the spiral section, enterocytes were characterised by an enlarged, transparent supranuclear part with clearly visible absorptive vesicles. However, the morphometric analyses carried out indicate that fish from the control group had the most favourable intestinal morphological parameters.

Conclusion:

The histological analysis indicates that the use of soybean components at both 10 and 20% protein replacement adversely affects the morphology of the digestive tract of juvenile Siberian sturgeon, impairing the ability to absorb nutrients and, as a result, generating lower growth rates.

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EVALUATION OF STOMACH CONTENT AND FEEDING HABITS OF *Tilapia mariae* IN LOWER OGUN RIVER, AKOMOJE WATER RESERVOIR, NIGERIA

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Introduction

Fish is a high quality food, apart from its protein contents; it is also rich in vitamins and contains variable quantities of fat and minerals for human health (Bard *et al*., 1976). Fish is often recommended for cardio-vascular disease patients because of its unique fat, which is composed mainly of Omega- 3 polyunsaturated fatty acid.

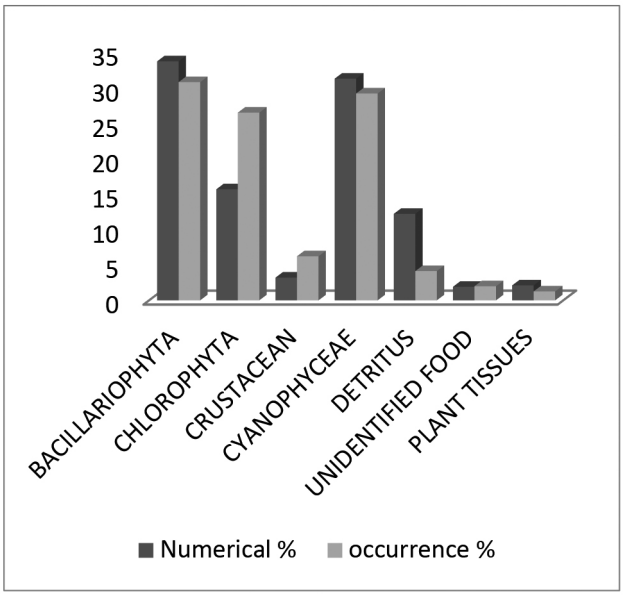
Materials and Methods

The food and feeding habits of *Tilapia mariae* in Akomoje River reservoir, Abeokuta, Ogun State, Nigeria, were examined between the months of August and December 2019. A total number of 125 fish specimens were collected on monthly basis from the commercial landings of fishermen around the water body.

Results

The results of monthly variation in food items show that Bacillariophyta, Chlorophyta, Cyanophyceae, crustacean, detritus, plant tissues, and unidentified food all occurred in varying quantities from August to December 2019. Bacillariophyta (diatoms) was the most important food item in the stomach of Tilapia mariae accounting for 14.72% and 78.10% by numerical and frequency of occurrence methods, respectively. Cyanophyceae constituted 11.43% in number and 59.63% in occurrence as the next food item in order of importance. Crustaceans occurred least in order of importance with 2.34% in numbers and 27.12% in frequency of occurrence.

Figure 2: Distribution of food items in the stomach of *Tilapia mariae* from Akomoje water Reservior.



IDENTIFICATION AND VALIDATION OF SELECTED PATHOGEN RECOGNITION AND IMMUNE RESPONSE MRNAS IN THE FRESHWATER AFRICAN CATFISH *Clarias gariepinus*

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Introduction

African catfish *Clarias gariepinus* is the dominant aquaculture species in Nigeria with over 160000 tonnes output per year (Dauda *et al*., 2018) and 2 million tonnes global output (FAO 2022). It is also intensively farmed in Ghana, Cameroon, Kenya, Mali, South Africa, Brazil, Netherlands, Hungary, Italy and Syrian Arab Republic (FAO, 2022). Disease outbreaks from pathogenic bacteria cause huge economic losses affecting all production systems with the Gram negative bacteria *Aeromonas hydrophila* the aetiological agent of motile aeromonas septicaemia (MAS) outbreaks in Clarias catfish (Jiang *et al*., 2016). Very little is known about host-pathogen interactions in Clarias species. The objective of this study was to identify mRNAs involved in pathogen recognition and response in *C. gariepinus* to provide new knowledge supporting the development of innovative disease control strategies.

Materials and methods

The study was approved by the Animal Welfare Ethical Review Board of the University of Stirling. The Clarias catfish were sourced from a stock population bred at the Institute of Aquaculture (UoS) and housed in a 300 litre stock tank, in a recirculation system on a 12:12 light:dark cycle and water temperature of 28 ± 2 °C. All fish were fed twice daily with commercial pellets at 2% bodyweight per day. Water quality parameters including temperature, dissolved oxygen, ammonia, nitrate and pH were recorded daily. A total of 18 fish were used for this experiment with 3 fish samples taken from each treatment group (TG1 = vaccinated and TG2 = non-vaccinated/control fish groups) at 24 hours, 72 hours and 41 days post vaccination. The fish were vaccinated by intraperitoneal injection using a whole cell, inactivated (heat killed) preparation of *Aeromonas hydrophila* mixed at 3:7 ration with commercial adjuvant Montanide. Blood, head kidney, spleen, liver and gill were aseptically removed from *C. gariepinus* and transferred into 1.5 ml tubes with RNA preservation buffer.

Table 1: Optimized primer sets for the different genes, the table shows the name of gene, primer sets, product size and annealing temperature.

Gene	Forward Primer	Reverse Primer	Product size (bp)	Annealing Temp (°C)	Ref
IL1B	TGCAGTGAATCCAAGAGCTACAGC	CCACCTTTCAGAGTGAATGCCAGC	128	53	Nasrullah et al., 2021
COX2	TAAGCCGTCATACGCACTCC	ATGCTTGTAGTACCCTGGCG	175	55	This study
TLR3	TCTCTGACCCGATACAACCG	AGGTCTTGGTCTTGCAGCAG	149	55	This study
MPO	CAAGGGGACAGGCTCTGGTG	AACTCTAGAAATGCCTGTGT	117	55	This study
MHC2	GTACGAGGCCAGAAACGTCA	GAGCCCACTGAGAGGAACAC	190	55	This study
NOD1	AAGCAATCGGGAACTTCTGG	TGCCAGTTGATGAGTCCTCG	-	50	This study
NOD2	AGAACAGAACTGTTGGGGGC	CTTTCTGGGCCTCGGGTAAG	241	53	This study
B-actin	AAAGGTTTCCATTCCCCCTGG	CCCCACCAAATGTCTGGCA	72	53	This study

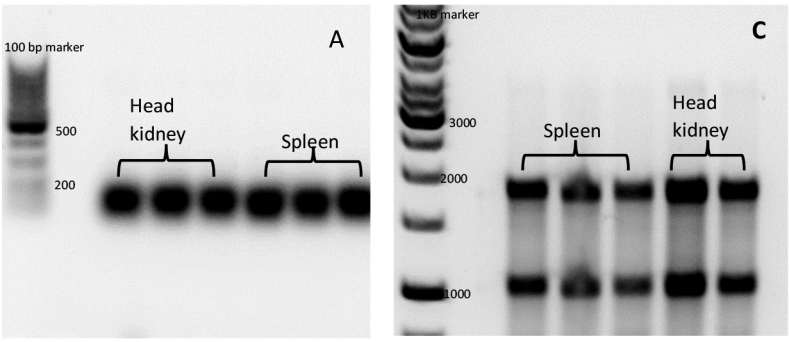

Plate 1: Shows amplicons on tissues with β-actin primer (A) and amplicons of RNA extracted from the tissues (B)

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Seven immune genes were selected based on their related functions in pathogen recognition (Nucleotide-binding oligomerization (NOD1 and NOD2), toll like receptor 3 (TLR3)), pathogen perception and integration (Major Histocompatibility Complex (MHC2)) and inflammatory response (interleukin 1β, Cyclooxygenase-2 (COX2) and Myeloperoxidase (MPO)). Predicted sequences of the genes from different species of animal (figure 1) were sourced through nucleotide query on https://www.ncbi.nlm.nih.gov and aligned using MEGA-X 10.1.8 software. The primer sets for each gene were designed from the conserved regions of the aligned sequences using https://www.ncbi.nlm.nih.gov/tools/primer-blast/ and purchased from commercial company.

Total ribonucleic acid (RNA) was extracted from 100 mg head kidney and spleen tissues using TRI Reagent® (Sigma-Aldrich T9424) following the manufacturer's protocol. Extracted RNA products were subjected to purity check and quantified using electrophotometer (Thermo Scientific Nanodrop 2000c) and the integrity tested on 1% Agarose gel electrophoresis. A pool of RNA was made with 1 μl of RNA from each tissue sample and first stranded DNA was synthesized with QuantiTect® Reverse Transcription kit according to the protocol from the manufacturer. The PCR for all the mRNAs were optimized with MyTaq™ mix using the manufacturer's description at temperature gradient of 51–65 °C and integrity checked on 1% Agarose gel electrophoresis. Optimized genes were successfully cloned using Nucleospin Gel and PCR clean up kit (Fisher Scientific 1507/001) as described by the manufacturer and the extracted plasmid DNA were sequenced by a commercial company. Sequences obtained were confirmed on https://blast.ncbi.nim.nih.gov/Blast.cgi?PROGRAM. The genes were validated using Clustal Omega multiple sequence alignment tool (https://www.ebi.ac.uk/Tools/msa/clustalo/) by aligning sequences against their respective primer sets.

Results and discussion

Seven sequence specific primer pairs were successfully designed and optimized (table 1). All the primers (except for IL1β) were designed, optimized and validated for the first time in the *C. gariepinus*. Going forward, the validated mRNA target sequences will be used to assess their distribution and expression in tissues of the fish through real time qPCR. They will also be used to determine the pathogen recognition pattern of the fish by comparing results from vaccinated and non-vaccinated groups.

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MONITORING THE PRESENCE OF *Ostreid herpesvirus,* OSHV-1 AND OSHV -1 *µ*VAR*,* IN WILD AND FARMED OYSTERS ALONG THE PORTUGUESE COAST

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Introduction

The production of mollusks in Portugal has a very high economic and social importance. The Portuguese mollusk production is represented by various species of greater socioeconomic significance such as the Portuguese oyster, *Crassostrea angulata* and the Pacific oyster, *Crassostrea gigas*.

In general, the consumption of these species has been increasing and as such their production as well. The production of these species faces some threats, such as the appearance of diseases. Several pathogenic agents can cause lesions and diseases both in wild and farmed oysters. *Ostreid herpesvirus* (OsHV -1 and OsHV-1 *µvar*) has been the cause of important mortalities in Pacific oyster, in several countries, including in Portugal. Lesions are not specific of this virus and may include, namely, discoloration of the digestive gland, presence of vacuoles in the cytoplasm of epithelia of the digestive gland diverticula and necrotic lesions in several tissues, mainly in gills epithelium and connective tissue.

Furthermore, hemocitosis and intracytoplasmatic inclusion bodies can be observed in most affected tissues.

Materials and Methods

The presence of OsHV-1 and OsHV-1 µvar in Portuguese and Pacific oysters was surveyed from 2018 to 2022. Oysters (adults) were sampled from five different sites on the Portuguese coast (water temperature: 18-20ºC; salinity: 30-34 ppm). Portuguese oysters from Sado estuary (n=159) and Mira estuary (n=66) and Pacific oysters from Formosa lagoon (n=234), Alvor lagoon (n=90), and Aveiro lagoon (n=371).

For the detection of this pathogen, we followed, since the first time the pathogen was detected in Portuguese waters (2011), the protocol set out in Commission Regulation (EU) No 175/2010 of 2 March 2010, Part B of Annex I for the DNA extraction method and PCR analysis.

For histopathology, the tissue samples were fixed in Davidson's fixative for 48h, dehydrated, embedded in paraffin and cut with a microtome (3-5 µm thick), then stained with Hematoxylin and Eosin (H&E).

Results

Slide analyses for histopathology of both oyster species revealed the presence of various ciliates such as *Trichodina sp*. in gills and in mantle epithelium and *Ancistrocoma sp*. in the digestive gland tubules and in the connective tissue, both with a moderate prevalence. The copepod *Mytilicola sp*. was observed in the intestine lumen of both oyster species. It was also observed hemocytosis in connective tissue, edema and metaplasia in the digestive gland and tissue necrosis. In the population from Mira estuary the prevalence of these lesions was slightly higher, except for individuals with metaplasia.

The lesions observed in the epithelium of the digestive gland, hemocytic infiltration and necrosis may be related to different pathogenic and/or environmental agents. In all populations analyzed in this study, the presence of the virus was not identified.

Discussion and conclusion

The first reported mortality outbreak of oysters occurred during the summer of 2012 in south coast of Portugal, in Pacific oyster populations and was related with the presence of a herpes-like virus. In 2011 was detected for the first time the presence of the microvariant (µvar) genotype of OsHV-1 in *C. angulata* produced from a broodstock collected in Sado estuary and then transferred to the Formosa Lagoon (Batista et al., 2015). This disease reached the highest impact in oyster production during 2017, when mortalities rates between 60 and 100% were registered in Alvor (south) and Aveiro lagoons. Since then, reports of massive mortalities related with the presence of OsHV-1 have been declining.

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Environmental factors, as well bad pratice management methods, namely the high animal densities in oyster tables and the intensive handling of animals in unfavorable environmental conditions, can contribute to increasing the virulence and propagation capacity of this opportunistic virus.

It is fundamental to implement biosecurity measures to prevent the spread of diseases, namely controlling the transport of animals and equipment from different countries and regions. Furthermore, regular monitoring, identification and characterization of pathological processes in oysters are important measures for sanitary control. These measures all together, as well as the collaboration of producers, have been contributing to the control and spread of Ostreid Herpsvirus in Portugal.

References

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This work was supported by MAR2020: MAR-02.05.01-FEAMP-0010

EFFECTS OF EMULSIFIER LIPOGEST® ON GROWTH PERFORMANCE OF SIBERIAN STURGEONS (*Acipenser Baerii*)

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Introduction

Siberian sturgeons are considered as one of the most prolific fish species. This is mainly because of its relatively rapid growth in maturity which, by producing caviar in shorter period of time, ensures sooner come back of investment. Among diverse factors affecting the feed efficiency, emulsifiers play an important role. In this study, Lipogest®, a synthetic emulsifier, was tried on Siberian sturgeons. Lipogest® is a bile salt-based emulsifiers and promotes formation of small size lipid droplets and aid fat digestion.

Materials and Methods

The current study was done on 750 Siberian sturgeons (mean weight 250±20 grams) on a period of 84 days. Fish were divided into five treatments (each in three replicates). Treatments consisted of 0 (control group), 0.5, 0.8, 1 and 1.5 g of Lipogest® per kg of basic diet (BEYZA 21®). To exclude the role of Lipogest®, the control group were fed on mere basic diet. On the last day of study fish were weighed under anesthesia and the results were analyzed by SPSS.

Results

According to this study, all treatments showed significant changes compared to control group. Control group showed the least feed efficiency. While group 1 and 1.5 g/kg Lipogest® showed the most which was 7% improvement in compare with control group). SGR and FCR was also affected by different dosages of the addetive. The results showed that both reached its best in 1 g/kg Lipogest® which was 6% and 1.5% respectively better than control group. Based on the results the difference between 0.6 and 0.7 of Lipogest® was not significant. So, the recommended dosage for adult Siberian sturgeon based on our results is 0.6 gram/kg of diet.

Conclusion

Lipogest® significantly increased the feed efficiency and SGR AND decreased FCR of Siberian sturgeons in the current study within suggested ranges (best at 1 g/kg of food). Since dose 1.5% did not significantly affect the feed efficiency, for economic purposes dose 1% may be more reasonable. This additive also has shown good results in carnivores fish such as rainbow trout and seabass and herbivores tilapia, with significant changes in growth performance in compare with control group.

DIETARY OREGANO (*Origanum vulgare*) ESSENTIAL OIL IMPROVED RESISTANCE TO *Aeromonas hydrophila* INFECTION IN STERLET STURGEON (*Acipenser ruthenus)*

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Introduction

With regard to side effects of antibiotic therapy, there is an increasing attention to herbal plants as a substitute for synthetic medications. The oregano plant (*Origanum vulgare*), because of its antioxidant, antimicrobial and anti-inflammatory features, has been surveyed in different fish species and proved to have significant effects in improving immune system and accelerating growth rate. In this study, the effect of oregano essential oil is examined in sterlet sturgeon for the first time.

Materials and Methods

Diets were fortified with five levels of Oregano essential oil (0, 2, 5, 8, 10 g/kg). Two hundred and twenty five sterlet sturgeons (25±1 g) were divided into five treatments (in 3 replicates) and fed with the diets for twelve weeks. At the end of feeding trial, the activity of liver enzymes (SOD, CAT, MDA) was assessed. After a fifteen-day-challenge, the cumulative mortality rate with *Aeromonas hydrophila* was recorded.

Results

The results of the trial revealed that oregano essential oil increased the immunity levels of sterlet sturgeon. The levels of CAT and SOD in fish fed with 10 g/kg oregano significantly increased compared with other treatments ($p<0.05$). Also, the 2 and 5 g/kg groups did not show a significant difference compared with the control group ($p>0.05$).

After challenging with *Aeromonas hydrophila*, cumulative mortality decreased significantly as the dose increased ($p<0.05$). The results of the experiment showed positive effects of oregano essential oil in improving the antioxidant defense capability and increasing survival rate of sterlet sturgeon challenged with bacterial infection.

CAN WE TRUST DIGITAL PHENOTYPING FOR ESTIMATING WHOLE BODY FAT IN ATLANTIC SALMON? LARGE-SCALE GENETIC VALIDATION PROVIDES PROMISING RESULTS

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Introduction

Maintaining an optimal level of body fat is essential for the survival in Atlantic salmon, but higher levels can result in costly harvest waste and economic losses. Breeding programs could in principle, incorporate whole body fat as a trait and thus contribute to more sustainable and cost-efficient fish farming. However, high throughput and cost-effective methods for phenotyping whole-body fat in thousands of fish are currently lacking. The reference Folch lipid extraction method (Folch *et al*., 1957) is destructive, highly laborious, and expensive. Two rapid non-invasive methods, namely the Distell fat meter which is a highly portable dielectric spectrometer (DS) and a new semi-automated Near-infrared (NIR) system offer the possibility to change this but require further validation. To address this issue, we conducted a large-scale genetic validation study on thousands of Atlantic salmon with the goal of 1) comparing the accuracy and reliability of digital NIR and DS methods for estimating total body fat; and 2) examining the pattern of total body fat across three different life phases in Atlantic salmon.

Materials and Methods

The large-scale genetic validation study is done on ~3000 Atlantic salmon belonging to 35 fish families of 2017-year class at MOWI Genetics, Norway. From fertilization the families were reared in separate trays, while from the eyed egg stage families were pooled and reared in a common garden tank. At an average body weight of 40 grams fish were PIT tagged with and a fin clip taken for genotyping with MOWI's customized single nucleotide polymorphisms SNP array which contains 55735 SNP markers.

In August 2020 at an average body weight of 48.9 (SD= 7.9) grams, the fish were transported to the freshwater facility of Nofima Research Station for Sustainable Aquaculture, located at Sunndalsøra (62°40N 8°31E), Norway. After a three-week acclimation period, the fish were sorted into 3 phase (Parr, Pre-Smolt and Post-Smolt) groups as shown in figure 1.

The Whole-body fat traits were recorded on different numbers of participating fish in different phases through reference Folch lipid extraction, DS and NIR spectroscopy. Genetic, Biometric, spectroscopic and chemical measurements were taken at all life stages. The DMU software and Rdmu package were used for genetic estimates and multivariate analysis (Madsen *et al*., 2014).

Results

Both the NIR and DS methods showed strong agreement with the reference method (r_p= 0.80 -0.88). Moderate to high genetic estimates were obtained for whole body fat estimation through NIR and DS as presented in the table 1 below.

We found substantial variation in total body fat across the different life phases, with an average of 11.32 ±1.23 % in parr, 33.80 ±2.6 % in pre-smolt, and 14.98 ±1.27 % in post-smolt fish. The genetic and phenotypic agreement between whole-body fat in different phases are presented in table 2. A significant decrease in phenotypic and genetic correlation was observed between fresh water and sea water stages.

Conclusion

For the first time, genetic parameters are presented for whole-body fat in Atlantic salmon in multiple life phases. The substantial genetic estimates demonstrate significant potential in selective breeding for whole-body fat, but cognizance must be taken of the life phase. The genetic validation proves the capability of non-invasive DS and NIR methods for measuring whole body fat at different life stages without killing or filleting, and their usefulness depends on the user's specific needs and preferences.

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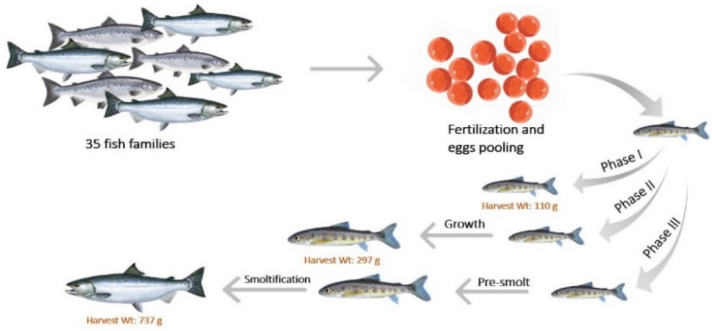

Figure 1: Overview of the experimental design

Table 1: Descriptive statistics and genetic parameters of whole body fat estimation at different time point in Atlantic Salmon

Life stage$_{Method}$	No. of fish	Mean (whole body fat) ± SD	CV %	Min-Max	Heritability ± SE
Parr$_{NIR}$	1742	11.32± 1.23	10.86	8.40-16.40	**0.57±0.04**
Pre-Smolt$_{NIR}$	680	33.77±2.60	7.70	27.4-43.61	**0.63±0.06**
Pre-Smolt$_{DS}$	370	34.03±1.20	3.53	30.6-38.05	**0.48±0.08**
Post-Smolt$_{DS}$	358	14.86±1.27	8.54	8.19-18.76	**0.56±0.10**

* SD= Standard deviation, CV= Co-efficient of variation, SE= Standard error

Table 2: Genetic and phenotypic agreement between whole-body fat at different time points

Correlations (Phenotypic & Genetic)	**Parr$_{NIR}$**	**Pre-Smolt$_{NIR}$**	**Pre-Smolt$_{DS}$**	**Post-Smolt$_{DS}$**
Parr$_{NIR}$	1	0.71±0.07	0.65±0.09	0.23±0.13
Pre-Smolt$_{NIR}$	0.49	1	0.93±0.03	0.30±0.12
Pre-Smolt$_{DS}$	0.42	0.70	1	0.32±0.13
Post-Smolt$_{DS}$	0.14	0.22	0.22	1

Reference(s):

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META-ANALYSIS OF GENOME-WIDE ASSOCIATION STUDIES OF AMOEBIC GILL DISEASE RESISTANCE IN ATLANTIC SALMON

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Introduction

Amoebic gill disease (AGD) is becoming a growing concern to the salmon industry in Northern Europe due to its increasing incidence and resulting consequence on fish health and welfare. Genetic variation of resistance to this disease is documented (Ajasa et al., 2023), and resistance can thus be improved by selective breeding. Genome-wide association studies (GWAS) of AGD can help unravel the genetic basis of resistance to the disease, and identified QTLs can be used for marker or gene-assisted selection. Most of the GWAS on AGD resistance (Aslam et al., 2020; Boison et al., 2019; Robledo et al., 2018) have until now not identified QTLs reaching genome-wide significance for resistance to this disease. Many of these studies are lacking a sufficient sample size (i.e. < 1500) which may limit the power to detect QTLs. A useful approach that can increase the power of detecting QTLs is combining several GWAS through meta-analysis. In this study, we conducted a meta-analysis GWAS of AGD resistance in six Atlantic salmon populations in Norway.

Materials and methods

The dataset consists of categorical gill score records (0-5) of six Atlantic salmon populations collected during natural field outbreaks of AGD. The six populations were all genotyped with custom 55k SNP array. Quality control procedures included removing markers and samples with call rate < 95%, minor allele frequency < 1%, and Hardy Weinberg p value (Fisher's exact test) < 10^{-25}. Finally, only samples with > 0.25 and < 0.45 heterozygosity were retained so as to limit the impact of poor-quality samples (Weale, 2010). After quality filtering, 50 456 SNPs remained, which was used for further analysis. We performed genome-wide association analysis (GWAA) on each of the six populations independently using the model described below, applying the software GCTA (--mlma option) (Yang et al., 2011):

$$\mathbf{y} = \mathbf{xb} + \mathbf{Zu} + \mathbf{e}$$

where **y** is a vector of gill scores, **x** is a vector of SNP genotypes (coded 0|AA, 1|AG, 2|GG), **b** is the allele substitution effect of the SNP, **Z** is an incidence matrix relating the phenotype to the residual polygenic effect **u**, and **e** is a vector of the residual environmental effect. $\mathbf{u} \sim (0, \mathbf{G}\sigma_u^2)$, $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$, where **I** is an identity matrix, σ_e^2 is the residual variance, **G** is the genomic relationship matrix, and σ_u^2 is the additive genomic variance. The resulting summary statistics from the GWAA were then combined using the sample weighted Z-score method of the METAL software (Willer et al., 2010). Bonferroni correction of 0.05/number of SNPs (50456) was used to correct for multiple testing. Chromosome wide significance level was derived from 0.05/average number of SNPs per chromosome (1740).

Result and discussion

Two QTL regions on chromosomes 2 and 12 were identified in this study (Figure 1). The most significant region was located on chromosome 12, with the most significant SNP in this region located at 61,580,655 bp. We identified 6 protein-coding genes within 100kb region of this SNP.

Conclusion

We identified novel QTLs with strong signals that have not been identified in previous studies. Further studies will be conducted to fine-map causal variants in the regions identified.

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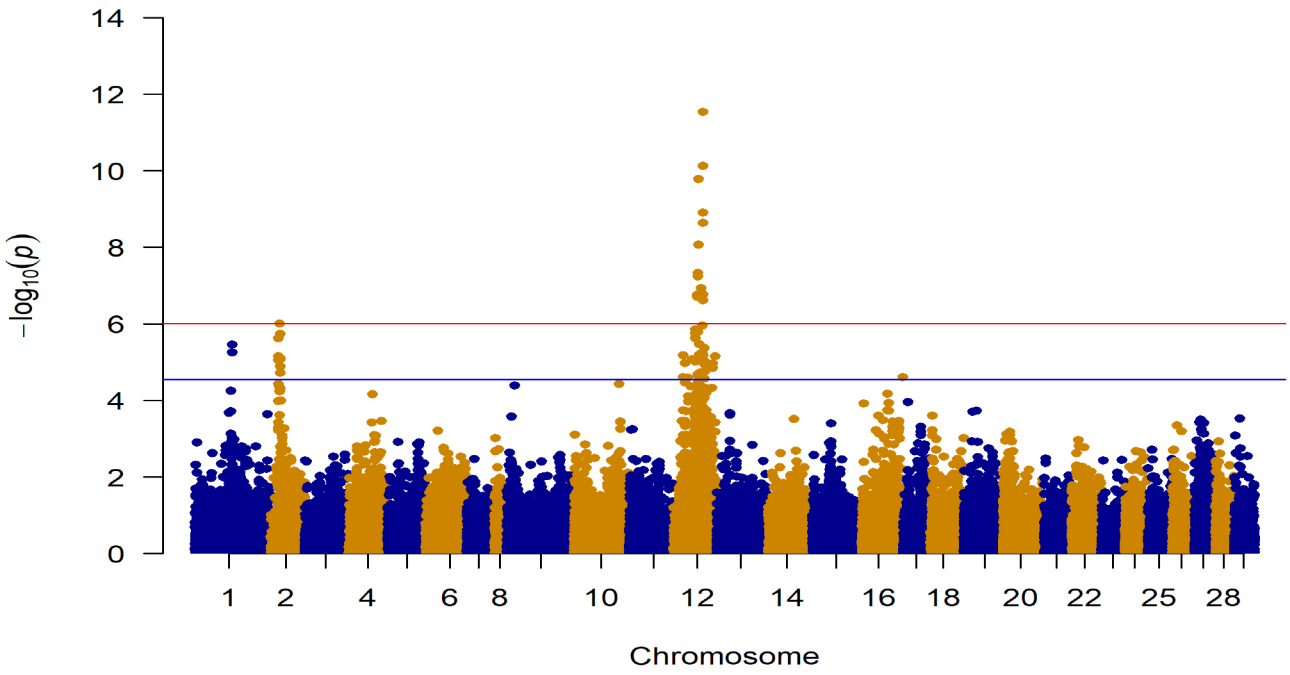

Figure 1: Manhattan plot from the genome-wide marker meta-analysis of resistance to AGD. The red and blue represent Bonferroni and chromosome-wide significance.

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DESIGN, VALIDATION and VERIFICATION of a MOVING BED BIOMEDIA REACTOR (MBBR) UNIT a CASE STUDY: 220 m^3/h

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Total Ammonia Nitrogen (TAN), which occurs due to various reasons especially fish defecation and death, is crucial for life and bring about death of living creatures. Therefore, it is very important to reduce the amount of TAN in water and it is called as Nitrification process. In a system inhabited by living organisms, even a very low amount of TAN can have fatal consequences. For this reason, it is very important that the system responds to the requirement with pinpoint accuracy.

Design validation gains meaning by evaluating the designed system in terms of Computational Fluid Dynamics (CFD). Within the scope of this evaluation, after deciding on the final three designs with validation, prototypes were manufactured, and the error margin of the validation part was evaluated as verification.

Within the scope of the study, the dimensioning of the system depending on the biological load was created and structured with the approaches in the literature. Life Cycle Inventory Assessment (LCIA) and Life Cycle Cost Assessment (LCCA) studies on the MBBR unit to ensure the principle of eco-design were structured during the design phase and optimization studies on the system were carried out in this way within the scope of sizing.

The aim of the study is to produce a user-friendly technology that complies with the eco-design directive, is environmentally friendly and has low energy consumption compared to its counterparts. With the result obtained in this context, it has been confirmed with experimental results that water consumption is reduced by 90%, the loss of nitrification bacteria is minimized, and the nitrification process is carried out in a quality manner. The margin of error of CFD and experimental studies was obtained as 3.4%.

BEHAVIOURAL CHANGES AND HISTOPATHOLOGICAL EFFECT OF GLYPHOSATE AQUATIC HERBICIDE (FORCE UP) ON TISSUES OF SUB ADULT AFRICAN CATFISH (*Clarias gariepinus*)

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This experiment determined the toxicity and behavioural changes of glyphosate aquatic herbicide (Force up) on African catfish *Clarias gariepinus* sub adult. A total of 120 live and apparently healthy *Clarias gariepinus* sub adult measuring 18.3-30cm standard length and average weight of 123g was randomly distributed into twelve (53.5cm X 33cm X 34cm) glass tanks of 60litres capacity. Each were filled with 20litres aerated bore-holes water and six treatment in triplicate was set up for the experiment. Ten (10) *Clarias gariepinus* sub adult was distributed randomly in triplicate per treatment for experiment involving the sub adult *Clarias gariepinus*. The toxicant was introduced at different concentrations (0mg/l, 40mg/l, 70mg/l, 100mg/l, 130mg/l, 160mg/l) in triplicate per treatment. Fish mortality and behavioral changes was monitored and recorded for the first 24hours, 48 hours, 72 hours for the next 96hours. The inability of the fish to respond to external stimuli was used as an index of death. Dead fish were removed immediately with a scoop net to avoid contamination due to rotting. Behavioral changes exhibited by the fish include erratic swimming, air gulping, loss of reflex, molting, discoloration, barbel deformation and excessive mucus secretion in fish exposed to higher concentration of glyphosate aquatic herbicides. Histopathology of the organs after 96 hours in the liver shows nuclear vacuolization (NV) with irregular shaped nuclei, moderately damaged tissue and hepatocyte (H) regeneration which indicates that recuperation is still possible. The effect of the toxicant on the skin reveals there was cellular abnormalities, shrinkages, hypertrophy of tissue, absence of dermal layer and necrosis. The 96h LC_{50} of glyphosate aquatic herbicide (Force up) to Sub Adult African Catfish *Clarias gariepinus* is 123.784mg/l with the maximum safe concentration ranged between 1.24mg/l to 12.38mg/l, the safe level of a compound is derived by multiplying the 96h LC50 with an application factor of 0.01-0.1. Such application factor are applied to acute toxicity test data to estimate the concentration that is safe for chronic exposure. The results of the study revealed that Glyphosate aquatic herbicide (Force up) is toxic to fish organs and causes histopathological changes in different organs such as skin and liver; therefore, indiscriminate use by farmers should be discouraged particularly in aquatic bodies.

DOES THE FISH TANK HISTORY HAVE AN INFLUENCE ON THE MICROBIAL DIVERSITY IN RECIRCULATING AQUACULTURE SYSTEMS (RAS) AND HOW TO LIMIT THIS INFLUENCE?

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Introduction

Aquaculture is undergoing rapid development worldwide (FAO, 2020), but the sustainability of aquaculture systems is more and more questioned in view of the many changes taking place (climate, animal welfare, antibiotic resistance, etc.) (Sudarshan et al., 2021). To reduce their dependence on the surrounding unstable environment, the Recirculating Aquaculture Systems (RAS) have been developed. Those systems allow to reuse 90-99% of water but also to create an optimal growth environment for fish (temperature, nutrients, etc.). The functioning of RAS is highly dependent on the equilibrium between fish and microbial communities (e.g.: biofilter, associated biodiversity) (Kamali et al., 2022). This equilibrium could become more complex to achieve with the development of the polyculture in RAS (Thomas et al., 2020).

The fish microbiota (tract, skin, and gills) is highly diverse, including bacteria, fungi, viruses (Merrifield and Rodiles, 2015; Dulski et al., 2020; Dai et al., 2021) and is affected by intrinsic factors including trophic level, fish species (mainly linked with gastrointestinal tract morphology and diet) and environmental factor as season and captive-state (Egerton et al., 2018). Fish have an intimate interaction with their surrounding environment resulting in a shaping effect on water microbial communities (Fourrier et al., 2022). This study suggest also that the RAS history (linked with biofilm memory) seems to influence water microbiome. A reliable protocol is required to stabilize and equilibrate initial microbial communities before studying the effects of fish communities on microbial communities.

An experiment was performed to select a standardized procedure to obtain tanks with a microbial diversity as close as possible regardless of the history of the tanks. The objective here is not to have a perfect disinfection of the tanks, but rather to have a starting situation (t0) in the tanks that is as similar as possible either by homogenizing the filtration substrates or either by replacing them, limiting variability due to fish rearing.

Materials and methods

We compared 3 disinfection/homogenization modalities (M) using 4 replicates for each. That's why 12 tanks were selected based on their rearing history of different fish species over the past 3 years.

1. M1: Emptying and filling of the tanks after cleaning (raw cleaning),
2. M2: Raw cleaning, homogenization of active biofiltration substrates between fish tanks, standard protocol disinfection which consists of chemical treatments (hydrogen peroxide and chloramine T) and physical treatments (high temperature and drying) (protocol currently used at the Aquaculture Experimental Platform PEA of the University of Lorraine).
3. M3: Same as M2 but using new and inactive biofiltration substrates.

The experiment lasted 30 days and water was sampled (days 0, 16, 23 and 30) to access physico-chemical parameters of water and microbial communities dynamic. The physicochemical parameters of water: temperature, dissolved oxygen, pH, NH4+, NO2- and NO3- will be measured during the experiment. The microbial diversity of tank water (sampled on days 0, 16, 23 and 30) was assessed by metabarcoding approaches. Sequencing (Miseq 2x300 bp) was performed at the GIGA Institute (University of Liège) and bioinformatic analysis was performed using FROGS and R software.

Results

We observed a significant effect of the treatment on the dynamic of the microbial communities. The most homogenous starting point in all replicates was obtained using the second modality M2. The results allowed us to select the most reliable tested protocol that can be used to study microbial communities regardless of the tank rearing history.

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REDUCING RISK IN DESIGN AND OPERATION OF OCEAN AQUACULTURE

HOW TO MAINTAIN SAFETY OF ASSET, PERSONNEL AND PREVENT FISH ESCAPE FOR EXPOSED HIGH VOLUME, ADVANCED FISH FARMS

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INTRODUCTION

We see a growing trend that mariculture is growing out of sheltered, near-shore areas and moved to locations further offshore. There are several advantages applying this solution. Better fish welfare, less conflicts with interests in the ocean space as well as possibility to scale up output volume without compromising environmental sustainability are some of them. As the world's population grows, the options for producing enough food are becoming limited. The oceans offer a vast opportunity to meet this demand with sustainable, safe and efficient offshore fish farming. Adding safety to offshore fish farming projects is mainly ensured by providing uniformity, transparency, and predictability and thereby reducing project risk. We need to be ensured that facilities for aquaculture can handle harsh environment and still contain the fish safely.

MAIN APPROACH

The main areas of concern when it comes to ensuring safe and reliable offshore fish farming units may be categories into: Asset integrity, personnel safety, fish welfare and prevention of fish escape.

Asset integrity includes structural strength, stability, mooring, technical arrangement, and solutions on board together with reliability of essential equipment installed.

Personnel safety is mainly addressing arrangement for emergency escape and fire safety. This included lifesaving appliances, launching equipment and similar as well as fire detection and -extinguishing. It is common to apply well know maritime codes as acceptance criteria for personnel safety. SOLAS is a good example followed by local flag- or shelf states interpretation of requirements embedded in this maritime code.

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

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

The four different items are considered equally important for safe and sustainable fish farming offshore. These elements are also closely interconnected where integrity of one may support several others.

CONCLUSION

There is a significant potential to utilize competence from traditional offshore and maritime industry to help operators of exposed fish farming units to identify operational risks by applying technical rules and requirements from classification. As opposed to the offshore oil and gas industry, classification may not be obligatory in aquaculture, but it turns out that many developers and operators nonetheless choose to follow class requirements and recommendations.

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

INDUSTRIALIZATION OF OCEAN FISH FARMING CONTRIBUTING TO BOOST SUPPLY OF MARINE PROTEINS – SOLUTIONS AND CHALLENGES

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INTRODUCTION

All aquaculture market projections uncover a dramatic supply-demand imbalance in the next two to three decades. Focusing on fin fish supply specifically uncovers that output level is close to a saturation point for traditional areas with sheltered aquaculture. Currently the response to this situation is the fact that fish farming is growing out of sheltered, near-shore areas and moved to locations further offshore together with several new land-based RAS/flow-through plants being established around the world. A number of innovative solutions is being tried out in offshore fish farming in the process of industrialization. The objective is a higher output volume of fin fish combined with improved fish welfare. As the world's population grows, the options for producing enough healthy, sustainable food are becoming limited. Several interesting solutions for efficient, large-scaled fish farming in offshore/exposed environment are in the pipeline.

MAIN APPROACH

There are several advantages applying the solution with advanced, high volume fish farming installations to increase supply of marine proteins. Better fish welfare, less conflicts with interests in the ocean space as well as possibility to scale up output volume without compromising environmental sustainability - to mention a few.

The numerous consepts planned and implemented may be split into three main categories: Open trusswork with traditional net or grating, semi-closed units and at last closed aquaculture installation. The first may be split into two where most are operating at the ocean surface while an increasing number of designs have the option of submerged position to avoid splash-zone issues at rough sea states. These have all a number of advantages and weaknesses that have to be considered and evaluated. The various solutions ability to perform depends on a long list of aspects. The essential parameters to be taken into consideration assessing performance are among other things:

- Oxygen level in sea water. Essential for fish welfare is the ability to keep continuous O2-level above lower limit in all parts of the fish farming installation. In-depth analysis has to be performed to ensure no "dead pockets" where the fish density at periods of low water circulation may be too high.
- Crowding and live transfer of fish. These are processes that cause significant stess and physical strain. Again, resulting in reduced resistance towards diseases, etc and may lead to great losses of fish combined with low fish welfare. The industrialized solutions applied for offshore fish farming often utilized advanced mechanical systems to facilitate these processes, failure will lead to operational interruption and fish escape.
- Dead fish handling and ensilage processing. Typically, automated processes that involves much less manual handling compared to traditional sheltered methodology where these task are not carried out at the pen, but rather on the feed barge, etc.
- Logistics of supplies and goods going both on and off the fish farming installation result in several and more advanced marine operations compared with traditional fish farming. Higher sea states with more dynamics in both supply vessel and offshore fish farming installation is a major risk contributor to be mitigated when emergency preparedness is planned.
- Personnel safety. Personnel safety is mainly addressing arrangement for emergency escape and fire safety on board. This included lifesaving appliances, launching equipment and similar as well as fire detection and -extinguishing. In addition, other common safety items such as falling objects, working at hights and pinching/crushing have to be taken into account.

Other similar issues such as hygiene and cleaning as well as feed handling & -control could also be included discussing the total operational performance of a high-volume fish farming installation.

CONCLUSION

To address and mitigate issues mentioned above there is a significant potential to utilize competence from traditional offshore- and maritime industry to support operators of exposed fish farming units in design and tuning operational modes for efficient production. The industry is currently very much exploring possibilities and collecting experience from the various concepts being planned and put in operations. This learning journey is an important phase in the development of offshore fish farming to become a significant contributor in feeding future generations with sustainable and healthy marine proteins.

A MULTIPLEXED, TILED PCR METHOD FOR RAPID WHOLE-GENOME SEQUENCING OF INFECTIOUS SPLEEN AND KIDNEY NICROSIS VIRUS (ISKNV) IN TILAPIA

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INTRODUCTION

Tilapia farming is one of the most important sectors in finfish species cultured worldwide and of major importance to global food security, with total global production estimated at more than 6 million tonnes in 2018 (FAO; Martínez-Cordero et al 2021). Infectious spleen and kidney necrosis virus (ISKNV) has been identified as an agent of high morbidity and mortality, threatening tilapia aquaculture. ISKNV was detected in Lake Volta, Ghana, in September 2018 and spread rapidly, with mortality rates between 60 and 90% and losses of more than 10 tonnes of fish per day resulting in the closure of more than 50 farms and the disruption of the livelihoods of the communities along the lake (Okai 2021). Understanding the spread and evolution of viral pathogens is important for control strategies. With the information obtained from genomic surveillance and epidemiological data, it is possible to reconstruct chains of transmission (Quick et al. 2020), (Gardy et al. 2015). Here, we assess the epidemiology of an ongoing epidemic of ISKNV in Nile tilapia in Ghana by developing a tiled-PCR sequencing approach for whole-genome sequencing of ISKNV to enable field-based, real-time genomic surveillance. Additionally, this method was used as a monitoring scheme and an alternative to destructive sampling, by concentrating viruses from the water of floating cages. Despite the low mutation rate of dsDNA viruses, 20 single nucleotide polymorphisms (SNPs) accumulated during the sampling period, with two non-synonymous SNPs were seen in the major capsid protein. Droplet digital PCR identified a minimum requirement of template in a sample to recover 50% of an ISKNV genome. Overall, tiled-PCR sequencing of ISKNV provides an informative tool to assist in disease control in aquaculture (Alathari et al. 2023).

METHODS AND MATERIALS

This study was performed on samples collected from seven farms in Lake Volta/Ghana, during an outbreak of ISKNV between October/2018- May/2022. Furthermore, water samples were collected to test the feasibility of this method in the field. Primers were designed with the PrimalScheme (v 1.3.2) to produce 2kb amplicons spanning the genome, for WGS of ISKNV from infected fish using a tiled PCR approach. Library preparation was conducted on the generated amplicons. Samples were multiplexed and sequenced using the MinION sequencer. A consensus genome was generated according to the ARTIC bioinformatics pipeline (artic.network/ncov-2019/ncov2019-bioinformatics-sop.html). Phylogenetic analysis of ISKNV within the Ghana outbreak of 2018–2022 was performed using Augur and visualised in Auspice. Finally, the number of ISKNV viral templates from 6 ng to 6×10^{-6} ng was measured using the ddPCR, to determine the minimal input for genome recovery of ISKNV using the tiled PCR protocol.

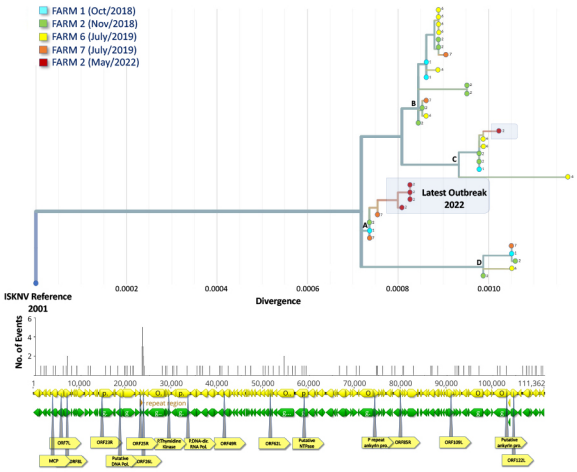

Figure 1. Phylogenetic placement of ISKNV genomes from Ghana and their associated farms, using Augur bioinformatics toolkit. The horizontal axis indicates divergence relevant to the root of the tree. Clades are labelled A–D. The colour of the tips represents the date of sample collection; the number and location of mutational events are shown in the diversity panel below.

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RESULTS AND DISCUSSION

In this study, we were able to recover up to ~96% of the ISKNV genome, directly from fish samples collected from Ghana during the outbreak, using the tiled PCR protocol. Approximately 137 SNPs were detected, when compared with the original reference genome, and it has shown that changes were sufficient to detect a phylogeographic signal during this outbreak. Some of these non-synonymous mutations were associated with the MCP, ATPase gene and OFR022. Phylogenetic analysis of ISKNV within the Ghana outbreak of 2018–2022, performed using Augur and visualised in Auspice, showed the initial outbreaks in Lake Volta clustered into four distinct clades, and each clade had a mix of samples from different farms. To evaluate the optimal concentration of ISKNV needed for genome recovery using the tiled PCR method, we measured the number of ISKNV viral templates. The minimum requirement to recover 50% of an ISKNV genome was ~2410 viral templates. This work shows that PCR tiling approaches used successfully to track the evolutionary rate, signatures of host adaptation, and transmission patterns of RNA viruses (Quick et al. 2016) can also be applied to monitor infections of large dsDNA fish viruses. Thus, this method can be utilised as a surveillance tool for other viral infections threatening the growth of the aquaculture industry.

FUTURE RESEARCH

Further studies will include determining the sensitivity of our developed protocol for different viruses affecting the growth of aquaculture. We have tested this method on Tilapia Lake Virus (TiLV) samples- an RNA virus affecting tilapia in several continents. This work provides a platform from which it is feasible to replicate the Artic-Network "lab-in-a-suitcase" approach to disease tracking and management in aquaculture in remote and resource-limited locations. With appropriate training and guidance, this workflow represents a suitable framework for local authorities in lower- and middle-income countries to contain and track different viral diseases in their localities.

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DIETARY B-GLUCAN AMELIORATES METABOLIC STRESS CAUSED BY A HIGH DIETARY CARBOHYDRATE LEVEL IN NILE TILAPIA

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Introduction

Carbohydrates are an excellent cost-friendly energy source for fish, and they have been used to improve growth performance and reduce dietary protein levels. However, the aquaculture industry has frequently overused high dietary carbohydrate levels, which has caused metabolic disturbances in fish. This deleterious effect is often diagnosed at the end of the production cycle, affects fish health and causes significant losses to farmers. In this context, □-glucan, which is broadly known as an immunomodulator, may ameliorate the deleterious effects caused by high levels of dietary carbohydrates. Therefore, we investigate the effect of dietary □-glucan on the reduction of metabolic disturbances in Nile tilapia fed with a high carbohydrate diet.

Material and methods

A total of 352 juvenile Nile tilapias (25.39 ± 0.83 g) were fed with isoproteic (280 g kg^{-1} PD) and isoenergetic diets (3130 kcal kg^{-1}) containing low amounts of carbohydrates (CHO-L 270 g kg^{-1}) or high amounts of carbohydrates (CHO-H 620 g kg^{-1}) with four levels of β-glucan (βG; 0, 1, 3 and 6 g kg^{-1}) for 10 weeks. We evaluated the productive performance, plasma glucose, serum triglycerides, hepatosomatic index, total liver and muscle lipid, hepatic glycogen, innate immune responses, hepatic activity of hexokinase, glucokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, malic enzyme and liver histopathology (not all data discussed here). The data were verified regarding normality (Kolmogorov-Smirnov test) and homoscedasticity (Brown-Forsythe test) of variances. Thereafter, the results were analyzed using two-way ANOVA with CHOs and βG levels as factors followed by Tukey's test to identify differences among the treatments. When the interaction was found between the factors, a One-Way ANOVA was performed to compare the βG groups and a t-test to compare the CHO levels. The effects of the treatments were considered significant at a 5% probability. All the analyses were done using the ExpDes.pt package (Ferreira et al., 2018) in software R®, version 3.5.1.

Results

Fish fed with a high carbohydrate diet had significantly increased productive performance (Weight gain – figure 1A and Feed conversion ratio - 1B) and lipogenesis, presenting higher muscular lipid (1C), hepatosomatic index (1D), hepatic activity of glucose-6-dehydrogenase phosphate, and malic enzyme. Additionally, histological analyses indicated expressive cytoplasm vacuolization. The innate immune responses such as leukocyte respiratory activity (1E), complement and lysozyme were also significantly decreased for the high carbohydrate diet, indicating the occurrence of immunosuppression in fish. These results also indicate impaired metabolic functioning, however, β-glucan ameliorated metabolic stress.

Figure 1. Weigh gain (A), Feed conversion ratio (B), Muscle lipid (C), Hepatosomatic index (D) and Leukocyte respiratory activity (RAL) of Nile tilapia juveniles fed for 10 weeks with diets containing different levels of carbohydrates (CHO-L vs. CHO-H; 270 and 620 g kg^{-1}, respectively) and β-glucan (βG: 0, 1, 3 e 6 g kg^{-1}). Capital letters indicate statistically different means between carbohydrate levels ($P < 0.05$). Lowercase letters indicate statistically different means between glucan ($P < 0.05$). Values are means ± 1 standard error (S.E.).

Conclusion

β-glucan mitigated the metabolic disturbances caused by the high levels of carbohydrates in the diet of Nile tilapia. This finding indicates that besides having an immunomodulating effect, β-glucan also has metabolic functions/benefits such as reducing glycolysis and lipogenesis.

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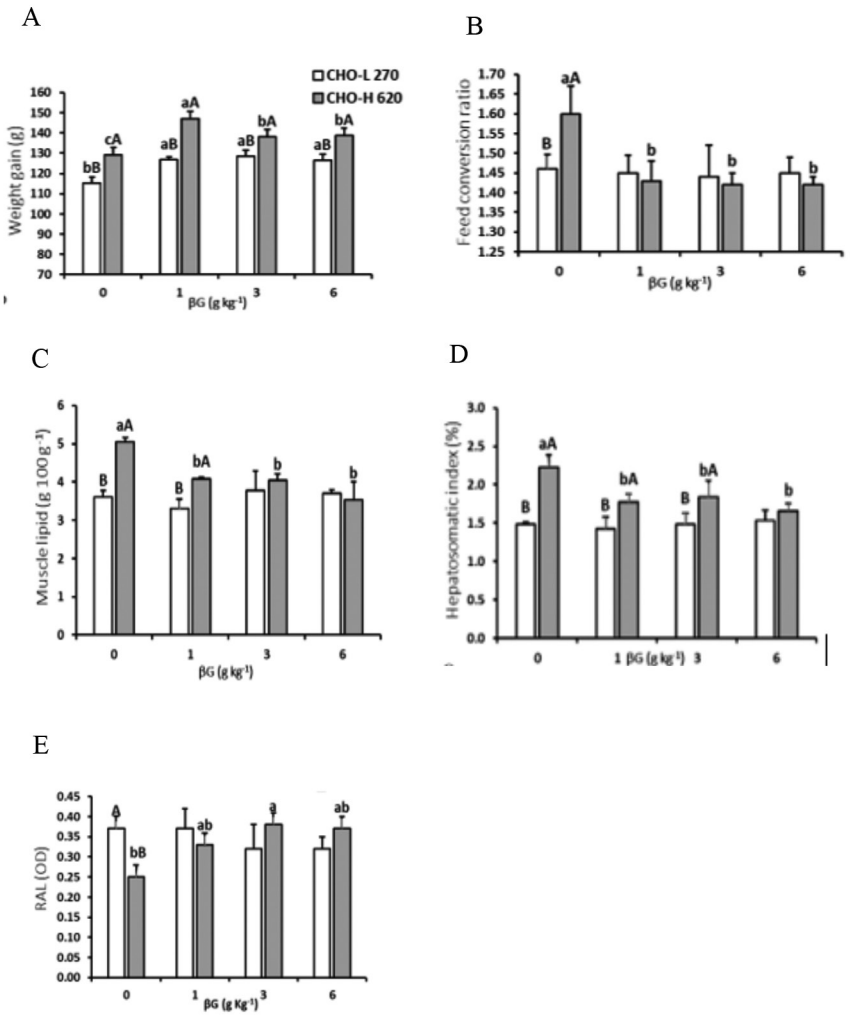

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SYSTEMATIC REVIEW OF ENABLING AND CONSTRAINING FACTORS FOR THE DEVELOPMENT OF ORGANIC AQUACULTURE IN THE EU

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Introduction

Although global aquaculture is setting production records, the organic aquaculture still represents a niche market. Indeed, the total organic aquaculture production of EU-27 is only accounting for 6.4% of the total production, and this appears to be not sufficient to fit the objectives of at least 25% of the agricultural land by 2030 according to the EU's Farm to Fork Strategy. Historically, the organic aquaculture sector has faced many difficulties hampering the development of the sector as e.g., technical difficulties in production, lower profitability, limited market demand, strict regulations or competition with other certification schemes (EUMOFA, 2022). In this study, we have conducted a meta-analysis of the available literature to investigate the enabling but also constraining factors on the organic aquaculture development in the EU. We focused particularly on the main farmed species, including freshwater and marine species (i.e. shellfish, salmon, trout, carp, sea bass and sea bream).

Materials and methods

Impact factors (IFs) were retrieved from the 82 documents reviewed, which include scientific papers, national, European reports and classed them as enabling or constraining as presented in the documents. List of IFs able to support/constrain the development of the EU organic aquaculture used in this study was defined according to Michelsen, (2001). Briefly, the IFs (n=59) were defined to encompass the interrelationship between the farmer and the institutional environment by conceptualizing society to be composed of three parts: the state (based on political authority), the market (based on economic competition) and civil society (e.g. based on civil solidarity within families, social groups) at micro-, meso- and macro level (Michelsen, 2001).

Each mentioning of IF was coded according to the document content and to the way the factor was defined in the document: "Supporting" or "Constraining" with three sub-categories which were "Supporting/Constraining but insufficient", "Supporting/Constraining" or "Very Supporting/Constraining". In more details, an IF was coded as "Supporting/constraining but insufficient", when the authors did not explicitly refer to the IF as supporting or constraining but when this was implicit or when there was some specific caution about the IF made by the authors. IF was coded as "Supporting/constraining" when the authors explicitly referred to the IF as supporting/constraining. An IF was coded as "Very supporting/constraining" when the authors explicitly referred to the IF as main supporting/constraining factor to the development of organic aquaculture.

Results and discussion

We have identified 470 impacting factors (IF) as supporting or constraining to the development of organic aquaculture in the EU. Overall, the five most mentioned supporting factors to the development of the organic aquaculture in the EU were the "consumer demand and/or willingness to buy" (n=35), the "marketing strategies for organic products" (n=24), "accessibility to communication and marketing" (n=20), "consumer attitude & belief" (n=19) and "innovation in farming" (n=17). Other significant supporting factors (n>10 mentions) highlighted are related to the "price relation between organic and conventional products" (n=14), "offer of organic products/sortiment" (n=12), the "need of research effort, funds for organic research and development" (n=12), "private label and criteria" (n=11), "need for clearer and simpler organic farming regulation/rules" (n=10), "public awareness" (n=11), and "environmental benefit and ecosystem services" (n=10) and the "availability of incentives (e.g., eco-premium)" (n=14) (Figure 1).

At the contrary, the most mentioned constraining factors to the development of the organic aquaculture in the EU were the "(quality) requirements for organic products and/or price" (n=32), the "price relation between organic and conventional products" (n=29), the "high bureaucracy level in organic farming regulation/rules, including high cost for certification" (n=18), the "unavailability of organically produced inputs (e.g. animals, seeds, feeds)" (n=16), the "competition and/or confusion with other labels (e.g. MSC, ASC, label rouge)" (n=15), "consumer demand, willingness to buy" (n=15) and the "lack of public awareness" (n=13) (Figure 2).

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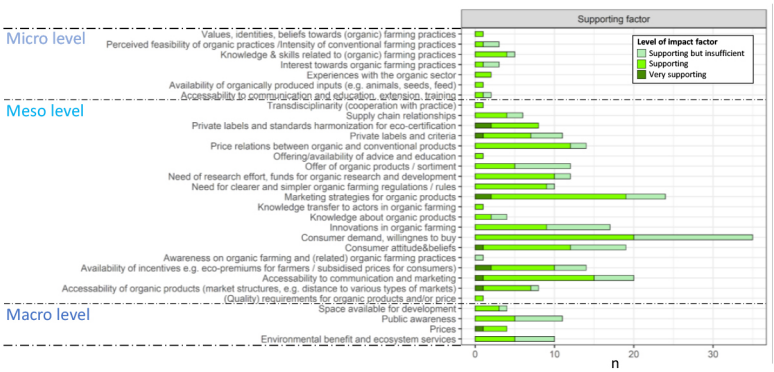

Fig. 1. Number (n) of mentions of the impact factors supporting the development of the organic aquaculture in the EU at micro-, meso- and macro-level. The level of impact factor is indicated ("supporting but insufficient" colored in light-green, "supporting" colored in green, "very supporting" colored in dark-green).

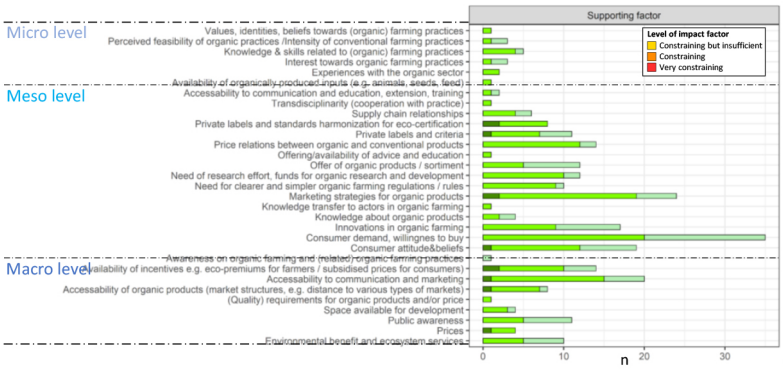

Fig. 2. Number (n) of mentions of the impact factors supporting the development of the organic aquaculture in the EU at micro-, meso- and macro-level. The level of impact factor is indicated ("constraining but insufficient" colored in yellow, "constraining" colored in orange, "very constraining" colored in red).

In conclusion, results showed a certain demand for aquaculture organic product does exist and is expected to growth in the EU. There still are, however, important constraining factors to the development of the organic aquaculture, i.e., quality requirements for organic products, harmonization of the standard for certification, competition with other sustainable labels (e.g., ASC, MSC) and the price relation between organic and conventional products. Strong effort from the EU policy will be needed in term of incentives, simplification of regulation, harmonization of standard, effective marketing strategies and availability of research funds to support innovation. In addition, institutions to support organic aquaculture need to be built at different policy levels in collaboration with different stakeholders to promote sound development of the organic aquaculture sector

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SNEAK-PICK INTO COMMON OCTOPUS *Octopus vulgaris* PROTEOME AFTER A BACTERIAL CHALLENGE: UNRAVELING PUTATIVE BIOACTIVE PEPTIDES WITH POTENTIAL FOR AQUACULTURE

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Introduction

Limiting the spread of classical and emergent microbial infections in aquaculture is a challenge the scientific community is increasingly aware of, looking for new efficient preventive and therapeutic approaches. Several synthetic antimicrobial agents have been used to control bacterial infections in shellfish and fish cultured worldwide. However, around 90% of aquatic bacteria are resistant to at least one antibiotic, threatening human health shortly (Elbashir *et al*., 2018). Marine species compose around half of the total global biodiversity, and considering their unique living environment (composition, close contact with microbes, and properties, *e.g.,* antibacterial, antiviral, antitumoral), these organisms have gained substantial importance as a "gold mine" for the search of new natural bioactive compounds (Cheung *et al*., 2015). Therefore, in the BIOPTAL *"Bioactive Octopus peptides with potential for aquaculture"* Marie SkłodowskaCurie project (https://cordis.europa.eu/project/id/101026577), we are focused on high-throughput protein search from an under-explored marine reservoir of peptide diversity – the common octopus (*Octopus vulgaris*). *O. vulgaris* is one of the most demanded cephalopod species for human consumption and represents a challenge for aquaculture farming. Octopuses routinely edit their RNA sequences to adapt to their environment (Alon *et al*., 2015), meaning that some pertinent bioactive compounds with antimicrobial or other relevant properties to several industries, such as aquaculture, must only occur under particular environmental conditions. Unraveling putative bioactive compounds from *O. vulgaris* can help develop novel therapeutic solutions for several marine species to fight the appearance and persistence of multidrug-resistant bacterial strains (*e.g.,* the pathogenic gram-negative bacterium, *Vibrio parahaemolyticus*, found in marine and estuarine environments which causes a varying degree of illness including gastroenteritis) in the aquaculture sector. Thus, aiming to detect natural compounds highly expressed under challenging conditions and stimulate bioactive peptide secretion to produce different kinds of compounds involved in host defense mechanisms, we accomplished an *in vivo* bacterial challenge with *V. parahaemolyticus* and generated the *O. vulgaris* proteome to investigate the differential expression of several antimicrobial and toxins-related genes in the skin, posterior salivary glands (PSGs), and hemocytes of the common octopus.

Materials and methods

Ten adult specimens of common octopus were obtained from the Mediterranean Sea (Valencia, Spain), maintained in the Marine Fish Facility at the University of Murcia (Spain), and randomly distributed into two groups: *i)* control group (unchallenged, immersion with sterile sea water) and *ii)* bath-challenged with *V. parahaemolyticus* (challenged, immersion with a sub-lethal bacteria concentration). After 6 h bath-challenged with the bacteria, octopuses were humanely sacrificed (Fiorito *et al*., 2015) and dissected. Hemolymph, skin, and PSGs fragments were collected to obtain the proteome of these tissues and hemocyte cells. Digestion of the samples was performed with trypsin/LysC overnight following SP3 – solid-phase-preparation (Hughes *et al*., 2019), and the concentration of the resulting peptides was measured by fluorescence. The shotgun proteomics analysis of the two tissues and cells was performed by injection of peptides in a nano-LC (Ultimate 3000, Thermo Fisher Scientific, Bremen, Germany) connected to a Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The LC-MS/MS raw data were analyzed by Proteome Discoverer SP1

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3.0 software (Thermo Fisher Scientific) for protein identification against the following databases UniProtKB/Swiss-Prot, UniProtKB/Swiss-Prot Tox-Prot, Starpep (Aguilera-Mendoza *et al*., 2019), and a comprehensive non-redundant antimicrobial peptides (AMPs) database (Almeida *et al*., 2020) together with a database of common contaminants from MaxQuant.

Results and discussion

AMPs present great diversity considering their structure, activity, mode of action, and genetic origin (Almeida *et al*., 2020). These peptides are widely distributed among prokaryotes, animals, and plants, establishing the first line of defense against microorganisms (bacteria, viruses, parasites, and fungi) as part of their primary immune system. A previous proteomics study of unchallenged *O. vulgaris* from eastern Atlantic waters (Portuguese waters) suggests the putative production of AMPs by *O. vugaris* PSGs as part of their primary immune system (Almeida *et al*., 2020). That study revealed that the *O. vulgaris* PSGs antimicrobial repertoire is composed of AMPs found in relatively high abundance, such as ubiquitin-derived peptides (cgUbiquitin), histone-derived peptides (buforin-II, H2A, and H2B), peptides similar to bovine pancreatic trypsin inhibitor (BPTI/Magainin), among others (Almeida *et al*., 2020). In this project, we are implementing a high-throughput approach for discovering new antimicrobial and immunomodulatory peptides potentially expressed under challenge (*V. parahaemolyticus*) from tissues and cells of *O. vulgaris*. We are looking specifically for differential expression of the previously mentioned AMPs and other AMPs not yet characterized in this species. Our results (*work in progress*) will help shed light on the underexplored antimicrobial repertoire of the common octopus, uncovering potential new peptides and simultaneously elucidating the role of glands, skin, and hemocytes in the species' molecular environmental adaptation, which remains poorly known (Fingerhut *et al*., 2018), opening the opportunity to develop alternatives to fight the synthetic antimicrobial resistance problem currently impacting several industrial sectors such as aquaculture.

Conclusion

This study offers a comprehensive proteomic view to enlarge the understanding of the common octopus's adaptive mechanisms, broadening the knowledge on the first line of defense against multi-antibiotic resistant bacteria affecting the aquaculture sector. The information provided by this study represents a pilot prospect that could help optimize culture conditions and disease prevention procedures affecting aquaculture-relevant species.

Acknowledgments

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ASSESSING THE IMMUNE RESPONSE OF THE COMMON OCTOPUS (*Octopus vulgaris*) UPON BACTERIAL CHALLENGE

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Introduction

Aquaculture represents one of the most promising sectors in the global food industry, driven by the rising demand for increased production. Among the species garnering significant interest in the Mediterranean area is the cephalopod common octopus (*Octopus vulgaris*), known for its short life cycle and high reproductivity. However, a major challenge faced in its aquaculture is the emergence of infections caused by bacteria that have developed resistance to commonly used antibiotics, leading to substantial economic losses and jeopardizing the survival of the animals [1]. In invertebrates, such as cephalopods, stress triggers an innate immune response, encompassing cellular and humoral reactions, as they lack an adaptive immune system [2]. To enhance animal welfare in aquaculture and gain insights into the immune defense mechanisms of cephalopods, it becomes essential to study defense mechanisms in the skin, which features a mucus layer in the epidermis acting as a barrier against pathogens [3], and the plasma, which carries substances that support the immune response [2]. Thus, to better understand cephalopod immunity, we conducted tests to identify potential differential responses in the expression of immune-related genes in the posterior salivary glands (PSGs) and assessed immune-related parameters and the bactericidal activity in the skin mucus and plasma of the *O. vulgaris* upon a bacterial challenge with the pathogen *Vibrio parahaemolyticus*. By investigating these aspects, we aim to shed light on the immune defense mechanisms of the common octopus and potentially discover new strategies to strengthen disease resistance in aquaculture production.

Materials and methods

Ten adult *O. vulgaris* specimens, previously acclimatized, were randomly divided into two experimental groups: *i)* unchallenged and *ii)* challenged group subjected to immersion with a sublethal concentration of *V. parahaemolyticus*. After a 6-hour bath-challenge, the animals were anesthetized and euthanized to collect PSGs, skin mucus, and hemolymph samples. The immune response of *O. vulgaris* was assessed by studying the gene expression in the PSGs and evaluating several immune-related parameters in skin mucus and plasma. In particular, PSGs samples were used to evaluate the expression of the following genes by RT-qPCR: *i)* immune-related genes – heat-shock protein 90 (*hsp90*), toll-like receptor (*tlr*), peptidoglycan recognition protein 3 (*pgrg3*), C1q binding protein (*C1q*), β-actin (*β-act*); *ii)* stress-related genes – superoxide dismutase (*sod*), peroxiredoxin-5 (*prdx5*), lysosomal-trafficking regulator (*lyz*) and clotting factor C1 (*C1-like*), and *iii)* antimicrobial peptide (AMP) genes –ubiquitin-40S ribosomal protein S27a (*rps27a*) and histone H4 (*h4*). To study the humoral response, innate immune parameters such as protease, antiprotease, lysozyme, peroxidase, esterase, and alkaline phosphatase activities were measured in skin mucus and plasma samples to assess potential differences between the unchallenged and bacterial-stimulated animals. Finally, the bactericidal activity in skin mucus and plasma samples was evaluated against *V. parahaemolyticus*, *V. anguillarum*, *V. alginolyticus*, *V. harveyi*, *Escherichia coli*, and *Photobacterium damselae* subsp. *piscicida*.

Results and discussion

The expression of immune-related genes in PSGs showed variations between both experimental groups for *rps27a*, *prdx5*, *lyz*, and *C1q* genes, which were down-regulated in the challenged group compared to the unchallenged one. The *rps27a* downregulation could suggest that the presence of bacteria may exert an inhibitory effect on the antibacterial function associated with the ribosomal protein, as reported by Hurtado-Rios *et al*. (2022) [4]. *Prdx5* showed a significantly lower mRNA expression in challenged octopi, as observed in the research conducted by Castellanos-Martínez *et al*. (2014) [5]. In

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their study, this gene was found to be repressed in infected hemocytes with *Aggregata octopiana*. In contrast to the findings of Vizcaíno *et al*. (2023) [2] and Castellanos-Martínez *et al*. (2014) [6], where the expression levels of *lyz* and *C1q* increased in octopi hemocytes under stressful conditions, in the present study, we observed a decrease in the expression of both genes within the challenged group. The immune-related parameters measured in the plasma and skin mucus did not indicate variations among the unchallenged and challenged groups. This fact could be due to the exposure time, which might be insufficient to trigger a local and systemic response, as reported by other authors considering several challenges[2]. Finally, no variations were observed between the experimental groups in the bactericidal activity measured in the skin mucus and plasma against the six marine pathogenic bacteria tested. This finding aligns with our results and previous studies, which have indicated that significant changes in bactericidal activity are typically observed after five days of challenge [2]. Thus, the relatively short 6-hour challenge period employed in our study may not have provided enough time for the substantial accumulation of natural compounds exhibiting significant bactericidal activity.

Conclusion

In this study, the expression of immune-related genes in both unchallenged and challenged groups exhibits variations in genes encoding ubiquitin 40S ribosomal protein S27a, peroxiredoxin-5, lysosomal-trafficking regulator and C1q binding protein, which showed a down-regulation in the challenged group. This finding leads us to hypothesize that these genes repression in challenged octopi may occur because of the weakening of the immune system of the specimens, caused by the recent infection with *V. parahaemolyticus* prior to the onset of the immune response, which has been seen at around five days in other studies[2]. Furthermore, at 6-hours post-challenge, we did not observe variations in the immune-related parameters. Consequently, future studies should consider analyzing these markers at different time points post-exposure to better understand *O. vulgaris*'s defense mechanism against this multi-drug-resistant pathogen.

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PROTEOMICS INSIGHTS OF THE INVASIVE JELLYFISH *Phyllorhiza punctata*: UNVEILING THEIR POTENTIAL FOR BIOACTIVE PEPTIDES DISCOVERY

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Introduction

Global public health is being threatened by multidrug-resistant pathogens fueled by the overuse of traditional antibiotics. To combat this challenge, antimicrobial peptides (AMPs) derived from natural sources are currently being studied as viable alternatives to traditional antibiotics (Browne *et al*., 2020). AMPs are key components of the innate immune response, with the remarkable ability to target multiple sites in pathogens, reducing the risk of resistance development.

Marine invertebrates, widely distributed and highly diverse, face constant microbial challenges due to altered environments driven by human activities. Their innate immune system, involving cellular and humoral responses, defends them against infections. Cellular immunity employs hemocytes for processes like encapsulation and phagocytosis, while humoral immunity involves hemolymph coagulation mechanisms and AMPs that disrupt microbial membranes. Invertebrates are the primary source of aquatic AMPs contributing with around 40 characterized AMP families (Bertrand and Munoz-Garay, 2019). Within the phylum Cnidaria, a group of primarily marine invertebrates, is the underexplored white-spotted jellyfish *Phyllorhiza punctata* (Scyphozoa, Rhizostomeae), an invasive jellyfish originally from the western Pacific Ocean that it's rapidly spreading through the Mediterranean Sea (Kaminas *et al*., 2022) even reaching the Northeast Atlantic Ocean (Enrique-Navarro and Prieto, 2020).

The primary goal of this work is to unveil the proteomic profile of the gonads of *Phyllorhiza punctata,* aiming to explore the AMPs produced by this organism and assess their potential as effective alternatives to currently used antibiotics in aquaculture.

Materials and methods

Two adult specimens of *Phyllorhiza punctata* were obtained from Oceanário de Lisboa (Lisbon, Portugal), their gonads were dissected and stored at -80 ºC. The proteins from these samples were extracted using two protocols FASP – *Filter Aided Sample Preparation* (Wiśniewski *et al*., 2009) and ISD – *In-Solution Digestion* with some adjustments, and further processed using a nano-LC coupled to a Q Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Waltham, MA, USA). The resulting raw files were searched against three databases consisting of *i)* AMPs from the starPep database (Aguilera-Mendoza *et al*., 2019), *ii*) several proteins from the UniProtKB database, and *iii*) reviewed venom proteins and toxins from Tox-Prot, together with a database of common contaminants (CRAP: https://www.thegpm.org/crap/), using Proteome Discoverer 3.0 SP1 software. The search parameters for protein identification included precursor and fragment mass tolerances of 10 ppm and 0.02 Da, respectively, allowing up to two missed trypsin cleavage sites, cysteine carbamidomethylation was selected as static modification, while methionine oxidation and asparagine and glutamine deamidation were chosen as variable modifications. Peptide-spectrum matches (PSMs) and peptides were accepted at a 1% false discovery rate (FDR), and peptide confidence was set to "High".

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Results and discussion

Several AMPs share common structural characteristics, like their overall size (~10-100 amino acids), molecular weight (<25-30 kDa), and net positive charge. However, they display a wide variety of structural motifs that, even similar sequences, may have completely different conformatures. Aurelin, a peptide with 40 amino acids, six cysteine residues, and active against gram-positive and negative bacteria, remains the only AMP originally extracted from a jellyfish (Ovchinnikova *et al*., 2006). In fact, there are very few jellyfish proteomic studies, and the exceptions are mostly focused on studying their venom proteomes (Leung *et al*., 2020).

In our study, we identified a total of 5,805 putative proteins belonging to 700 protein groups. Out of those, 2,633 proteins distributed among 409 protein groups were found with both FASP and ISD methodologies: 2,603 proteins are led by putative proteins identified against the UniProt database, while the remaining 30 were found against the starPep database. No toxins from the Tox-Prot database were retrieved. Antimicrobial peptides, such as Acipensin 6, Aurein-1.2, and Brevinin-2-RA13, were identified.

Conclusion

Jellyfish studies have critical implications for conservation and environmental awareness. Gaining new insights into the unique molecular properties of the invasive *Phyllorhiza punctata* contributes to the study of its biotechnological potential and the preservation and conservation of marine ecosystems and their biodiversity.

Acknowledgments

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MODELLING DISSOLVED OXYGEN CONCENTRATIONS IN AN ATLANTIC SALMON SEA CAGE FARM IN NORWAY

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Introduction

Dissolved oxygen (DO) is a key variable in Atlantic salmon sea cages, as sufficient oxygen is needed to ensure good fish welfare and high growth. The effects of insufficient oxygen leading to hypoxia range from reduced appetite, stress responses, reduced feed conversion and growth to acute mortality, depending on how low DO levels are encountered (Oppedal et al., 2011, Remen et al., 2016). Alver et al. (2022) presented a mathematical model of 3D distributed DO levels in salmon cages, seeking to combine our knowledge of the advective and diffusive transport of oxygen, the oxygen consumption by individual fish, and the spatial distribution of the fish, to estimate DO levels as a function of environmental conditions. In this study, the model is used along with an extensive measurement program to investigate oxygen conditions in one of six commercial scale cages containing biomass at a location in Mid-Norway.

Materials and methods

The mathematical model is based on an advection-diffusion equation operating on a regular grid with horizontal and vertical resolution of 2 m, with a simplified representation of the fish to compute feed ingestion and oxygen consumption rates. Feeding dynamics are computed based on Alver et al. (2016), and oxygen dynamics based on Alver et al. (2022). Model inputs for temperature, ambient oxygen level, current speed and direction are based on measurements over a 9 day period in June 2022, and model output is compared to measured oxygen levels at 12 points for one of the cages (centre and three points along the edge of the cage, each at 5, 10 and 15 m).

The model is tested when simulating the observed cage only, and when simulating the full farm (Fig. 1). The effect of neighbouring cages partly depleting the water of oxygen needs to be taken into account when simulating a single cage only, and this was addressed with an *ad hoc* model modification that reduces boundary levels of oxygen dependent on current direction and speed. An additional modification was tested to account for increased biofouling over the study period leading to gradually more damping of the current speed within the cage. The different model varieties were evaluated based on their agreement with measured oxygen.

Results and discussion

Comparisons between simulated and observed DO levels at the sensor positions are summarized in Fig. 2. The model variants are denoted **basic** (single cage model, no modifications), **VAB** (single cage model with adjustment to boundary conditions and current damping rate) and **VFB** (full farm model with adjustment to current damping rate). The **basic** model has a clear overall positive bias, with a positive trend over the study period. The **VAB** model has near zero bias and no similar trend, showing that the *ad hoc* modifications lead to better model fit. The **VBF** model also significantly reduces the bias and the positive trend, showing that taking the full farm into account clearly improves the overall model fit. All model variants have highest RMSE at 5 m and lowest at 15 m, indicating that dynamics in the upper part of the water column in the cage is the most difficult to capture in the model.

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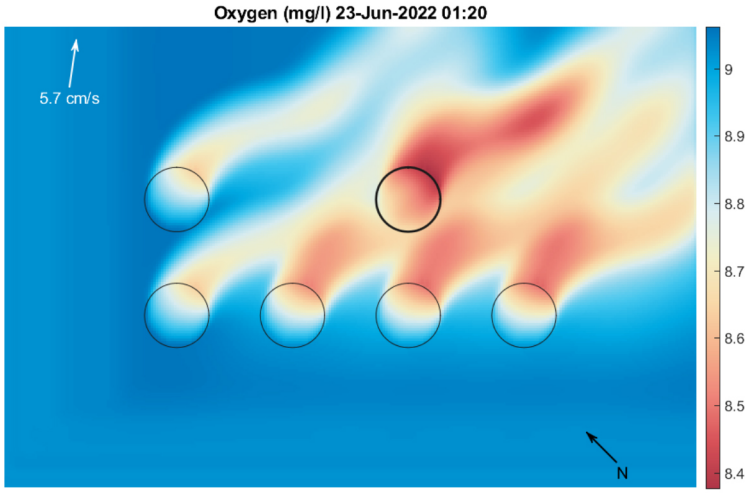

Fig. 1: Snapshot of simulated oxygen levels in surface layer when simulating the full farm. Cages are indicated by circles. The observed cage is the rightmost cage in the upper row. The current speed and direction are indicated by the white arrow.

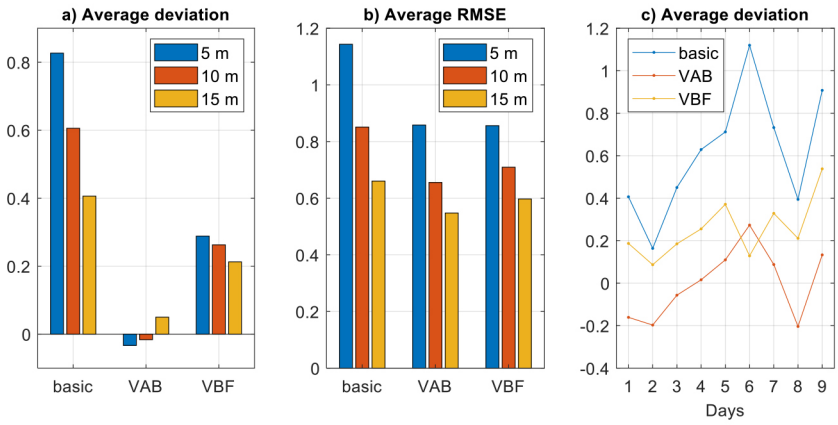

Fig. 2: a) Average dev. across sensors at each depth b) Average RMSE across sensors at each depth. c) Average dev. across all sensors per day for each model variant.

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QUALITY AND FISH WELFARE – LINKING CAUSES FOR QUALITY DOWNGRADING AND PRODUCTION RELATED DRIVERS IN NORWEGIAN ATLANTIC SALMON FARMING

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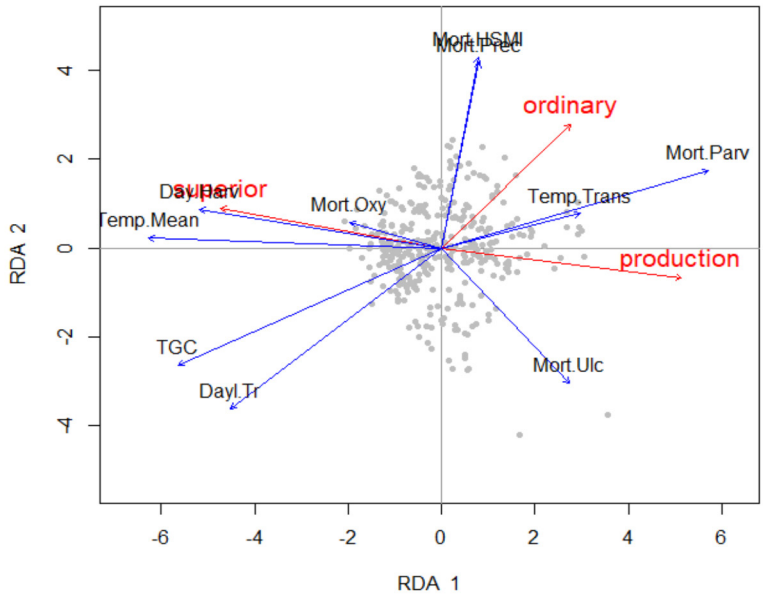
Quality downgrading of fish with quality defects is a considerable cause for economic losses to farmers due to reduced marketability and sales prices, as well as increased processing costs. Several causes for quality downgrading can be linked to adverse welfare states during fish rearing and should therefore be considered as welfare indicators. In this study, we linked the production records of 24 sea cage sites to slaughter records from a processing plant in northern Norway. We quantified the prevalence of proximate causes for downgrading and used ordination and regression-based approaches to model the variation in quality traits due to selected production related drivers. The most important causes for downgrading were ulcers (39 % of downgraded fish), dark spots (17 %), deformities (12 %), and early maturation (10 %). The presence of ulcers was by far the most severe cause for downgrading. Important drivers for overall quality reduction were growth during the seawater stage, seawater temperature (mean and standard deviation), certain cause specific mortality counts, and day of harvest. Ulcers were linked to low seawater temperatures during production.

Our study demonstrates that proximate causes for quality downgrading at slaughter are relevant retrospective welfare performance indicators and provides an overview of how production related drivers can affect slaughter quality throughout the year. It highlights the link between improved welfare and improved production outcomes, as well as the utility of systematic data collection and analysis in aquaculture production.



BIOCHEMICAL COMPOSITION OF MILT AND REPRODUCTIVE PERFORMANCE OF MALE *Clarias gariepinus* BROODSTOCK FED WITH A DIETARY SUPPLEMENTATION OF *Cocos nucifera* POWDER

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Introduction

Good nutrition in aquaculture correlates with better sperm quality and reduced abnormalities in parameters such as sperm count, sperm concentration, and motility (Oke *et al*. 2019). Some plants have been reported to enhance gonadal development, induce maturation and increase growth in fish species (Oke *et al*. 2019). These plants contain inherent active ingredients (sterols, flavonoids, saponins) and nutrients for their actions. *Clarias gariepinus* is a very important freshwater fish in Nigeria that enjoys wide acceptability in most parts of the country because of its ease of cultivation and unique taste, flavour, and texture. World Health Organization encourages the use of medicinal plants to minimize the use of chemicals as fertility enhancers, hence the use of *Cocos nucifera* in this study. The study aims to determine the dietary effect of *C. nucifera* on the biochemical composition of milt and the reproductive performance of male *C. gariepinus* broodstock.

Materials and methods

Sixty male *C. gariepinus* (446.56 g) were randomly distributed into 15 concrete tanks (1×2×1.5m3) at four fish per tank at the Teaching and Research Farm, Federal University of Technology, Akure. Five isonitrogenous diets at 40% crude protein level with different inclusion levels of *C. nucifera* powder were formulated (Table 1). The experimental fish were randomly assigned to five treatment groups (n = 3). The fish were fed at 3% biomass daily spread over two rations (0900 and 1400) for 56 days. The fish were batch-weighed bi-weekly and the water quality was monitored weekly (Temp 26.65±0.02, DO 5.98±0.04, pH 7.85±0.05). At the end of the trial, reproductive indices, hormone level, biochemical indices of milt of the fish, and phytochemical screening of the plant were investigated using standard methods as described by Hussain *et al*. (2018). The total protein, glucose, cholesterol, calcium, and magnesium of the milt were measured using the spectrophotometric method. The concentration of sodium and magnesium was determined with a flame photometer. Data were analyzed with SPSS v22 using one-way ANOVA.

Results

There were significant differences ($P<0.05$) in the values obtained for milt volume, milt count, motility duration and weight of testes of *C. gariepinus* fed *C. nucifera* endosperm residue (Table 2). Testosterone level, % fertility, hatchability, and survival were highest in fish fed CN1.5. All treatments displayed normal biochemical composition. There was no significant difference ($P<0.05$) in most analysed biochemical parameters except for magnesium and sodium (Table 3).

Conclusion

The study showed that the inclusion of *C. nucifera* endosperm powder in the experimental diets improved the milt quality, however it was observed that when *C. nucifera* inclusion level was above 1.5g/kg, the weight of the testes and milt volume decreased. The inclusion level of 1.5g/kg gave the best performance for the milt count, % motility, fertility, hatchability, survival, and testosterone level of *C. gariepinus*.

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Table 1: Experimental diets formulation (g/100g)

Ingredients (g/100g)	Control	CN0.5	CN1.0	CN1.5	CN2.0
Fish meal (65)	26.00	26.00	26.00	26.00	26.00
Soybean (45)	35.00	35.00	35.00	35.00	35.00
Groundnut cake (48)	12.50	12.50	12.50	12.50	12.50
Yellow maize (10)	15.00	15.00	15.00	15.00	15.00
Fish oil	4.00	4.00	4.00	4.00	4.00
Vegetable oil	3.00	3.00	3.00	3.00	3.00
Vitamin premix	2.50	2.50	2.50	2.50	2.50
Starch	2.00	2.00	2.00	2.00	2.00
C. nucifera powder	0	0.50	1.00	1.50	2.00

*Vitamin premix- A Pfizer livestock product containing the following per kg of feed: A = 4500 I, U, D = 11252 I.U, E = 71I.U, K3=2mg, B12=0.015mg, pantothenic acid = 5mg, nicotinic acid = 14 mg, folic acid = 0.4mg, biotin = 0.04 mg. choline = 150mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20mg, iodine = 0.6 mg, selenium = 2.2 mg, zinc = 20 mg, antioxidant = 2 mg

Table 2: Reproductive performance (Means ± SD) of *Clarias gariepinus* male broodstock fed dietary inclusion of *C. nucifera* powder

Parameters	Control	CN0.5	CN1.0	CN1.5	CN2.0
Final weight (g)	525.20±5.55^{c}	537.78±0.96^{c}	556.28±1.94^{b}	560.05±6.23^{b}	580.37±2.78^{a}
Weight of testes (g)	1.50±0.38^{b}	2.29±0.19^{b}	3.35±0.51^{a}	4.06±1.28^{a}	2.97±1.15^{b}
Milt volume (ml)	0.11±0.01^{b}	0.30±0.09^{a}	0.50±0.09^{a}	0.51±0.19^{a}	0.39±0.21^{a}
Milt count ($\times 10^4$spz/ml)	12.45±0.32^{b}	12.27±0.87^{b}	16.81±1.53^{a}	18.62±0.66^{a}	17.36±0.79^{a}
Motility duration (mins)	2.88±0.89^{c}	2.68±0.78^{c}	3.75±0.28^{b}	4.21±0.45^{a}	4.21±0.61^{a}
% Motility	42.99±11.90^{c}	54.68±2.86^{bc}	57.13±4.74^{bc}	69.92±5.13^{a}	64.41±5.84^{b}
% Fertility	45.85±15.51^{c}	50.88±4.28^{bc}	66.92±4.89^{b}	88.04±4.19^{a}	83.17±4.76^{a}
% Hatchability	32.90±5.09^{c}	40.18±2.53^{c}	53.66±12.53^{bc}	85.66±5.12^{a}	61.95±8.96^{b}
% Survival	44.93±3.95^{b}	48.14±1.50^{b}	55.93±11.95^{b}	70.92±4.44^{a}	50.76±8.01^{b}
TT level(ng/ml)	1.60±0.06^{b}	1.70±0.03^{b}	1.80±0.87^{b}	3.10±1.10^{a}	2.66±0.43^{a}

Values in the same row with different superscripts are significantly different ($P < 0.05$)
TT= Testosterone

Table 3: Biochemical composition (Means ± SD) *Clarias gariepinus* milt fed dietary inclusion of *C. nucifera*

Parameters	Control	CN0.5	CN1.0	CN1.5	CN2.0
Cholesterol	134.19±36.52	135.07±4.83	131.16±44.58	129.58±17.38	125.12±33.57
TP	17.91±0.29	18.06±0.68	17.92±0.17	17.79±0.19	19.18±1.46
Glu	35.40±2.25	36.45±5.28	35.25±1.30	34.35±1.47	37.15±11.34
Ca	11.06±2.08	12.03±4.87	10.93±1.20	10.10±1.36	14.05±10.46
K	2.80±0.24	2.91±0.56	2.79±0.14	2.69±0.16	3.84±1.20
Mg	74.44±4.17^{b}	76.39±9.78^{b}	74.17±2.41^{b}	72.50±2.73^{b}	80.50±21.01a
Na	137.14±5.08^{b}	143.64±2.46^{a}	144.20±0.61^{a}	144.62±0.69^{a}	139.59±5.29^{b}

Values in the same row with different superscripts are significantly different ($P < 0.05$)
TP= Total Protein, Ca= Calcium, K=Potassium, Mg=Magnesium, Na=Sodium, Glu=Glucose

CHANGE - AN UNDERWATER ROBOTICS CONCEPT FOR DYNAMICALLY CHANGING ENVIRONMENTS

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Introduction

As salmon farm sites are moved further offshore and to more exposed locations, working conditions become increasingly challenging. Farmers therefore aim to automate certain operations to facilitate safer working conditions. Automation and autonomous unmanned underwater vehicles (UUVs) are key elements in meeting this goal, and will contribute to increasing precision in finfish farming operations that in turn will enable the aquaculture industry to advance operational efficiency, safety and thus sustainability [1]. In this paper, an advanced control scheme for UUVs operating in complex environments has been investigated. The proposed scheme is suited for enabling verifiable collision-free navigation in dynamically changing environments. During demonstrations the UUVs were successful in autonomous navigation while successfully avoiding both static and moving obstacles.

This work was financed by the Research Council of Norway through the project: CHANGE − An underwater robotics concept for dynamically changing environments [2].

Materials and methods

The elastic band method has been a suggested method for planning collision-free paths [3] and was included in an adapted guidance, navigation, and control (GNC) architecture shown in Figure 1. The guidance system featured the elastic band path planner and a guidance law. Waypoints and positions of obstacles and the vehicle were used to calculate the control system reference signals. The low-level control system then used these signals and feedback of the vehicle states to calculate the control input for each thruster. Vehicle states were estimated using an Extended Kalman Filter (EKF) based on sensor readings.

Results

Extensive simulation results were obtained using FhSim [4] as shown in Figure 2. In addition, lab and field trials were conducted in the NTNU Marine Cybernetics Lab (MCLab) and the SINTEF ACE full-scale aquaculture laboratory to investigate the performance of the proposed control scheme for obstacle avoidance of UUVs in fish farms. Figure 3 and Figure 4 show some demonstrated case studies with a BlueROV2 vehicle from MCLab and field trials using an Argus Mini in an industrial scale fish farm at SINTEF ACE, respectively. All simulations, lab and field trials showed that the robot was able to avoid both static and moving obstacles during autonomous navigation of UUVs. The results demonstrate that the proposed method worked well at obstacle avoidance, and suggest that the elastic band method is a viable method for underwater collision avoidance in dynamically changing environments.

Conclusion and future work

Management of sea-based fish farms typically entails manual, and often challenging, inspection operations to monitor equipment, structures and biomass, which may result in sub-optimal and costly operations, insufficient maintenance, a general lack of control in daily routines and potential high risks for welfare of personnel and fish. This implies a need for new methods and technology for operations in modern fish farms, especially when moving operations to more exposed locations with more challenging environmental conditions, and new farm designs. The proposed methods and demonstrations show the great potential towards increasing the level of autonomous during daily operations in fish farms.

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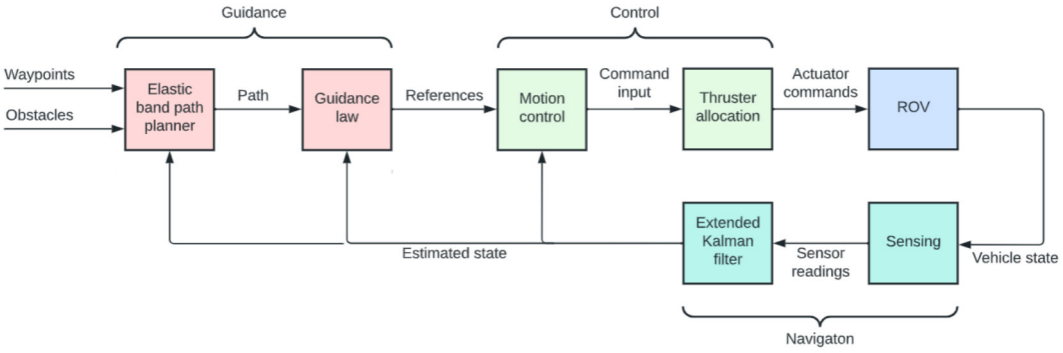

Figure 1 Adapted control scheme for obstacle avoidance of UUVs operating in fish farms

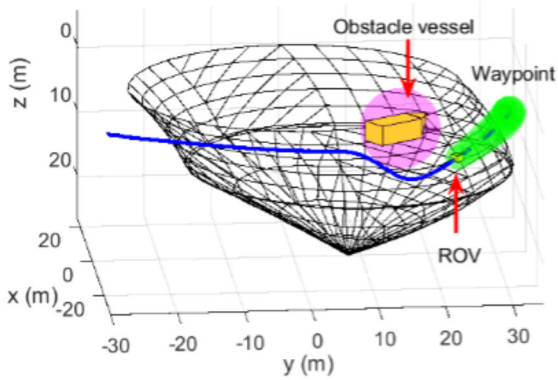

Figure 2 Simulated case study for obstacle avoidance of Remotely Operated Vehicle (ROV) in the FhSim simulation environment

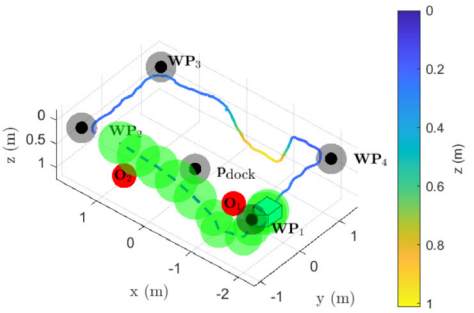

Figure 3 Demonstration of obstacle avoidance during autonomous navigation of BlueROV2 at the MCLab

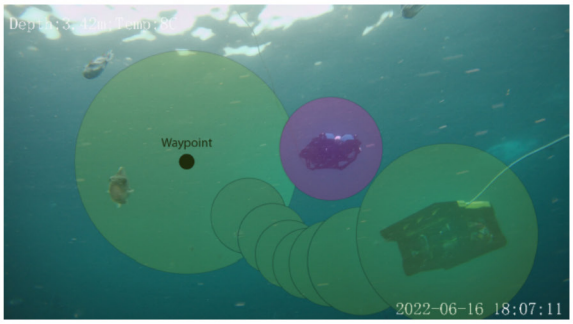

Figure 4 Demonstration of obstacle avoidance during autonomous navigation of Argus Mini at SINTEF ACE

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EXPLORING THE ANTIMICROBIAL POTENTIAL OF MICROALGAE AGAINST FISH PATHOGENIC BACTERIA

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Introduction

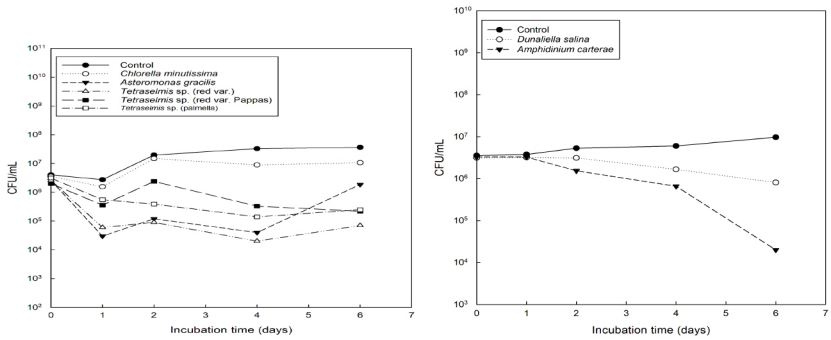
Intensive fish farming involves high population densities and stressful conditions, can lead to disease outbreaks, and may result in the use of antibiotics. However, the development of antibiotic-resistant strains in fish farms has rendered the use of antibiotics ineffective. This has also contributed to a negative perception of the aquaculture industry among consumers (Martinez et al., 2012). In this study we conducted two series of experiments to monitor the antimicrobial activity of microalgae species, isolated from lagoons in Western Greece against four fish pathogenic bacteria (*Vibrio anguillarum*, *Aeromonas veronii*, *Vibrio alginolyticus*, and *Vibrio harveyi*), using axenic cultures and extracellular assays in two experimental series.

Materials and methods

In this first experiment series, we tested the antimicrobial activity of the axenic microalgae strains: *Amphidinium carterae*, *Asteromonas gracilis*, *Tetraselmis* sp. (red var.), *Tetraselmis* sp. (palmella), *Tetraselmis* sp. (red var., Pappas), and *Dunaliella salina*, at different salinities. The microalgae were cultured under aeration, and their cell numbers were counted using a microscope at the start and end of the six-day experiment (Stanier et al., 1978). Experiments were conducted with and without light, using *Chlorella minutissima* as a reference microalgae (Makridis et al., 2006). In the second series of experiments, supernatants from *Amphidinium carterae*, *Asteromonas gracilis*, *Tetraselmis* sp. (red var. Pappas), *Nephroselmis* sp., *Phormidium* sp., *Anabaena* sp., and *Cyanothece* sp. were tested against the same fish pathogens (Katircioglu et al., 2006, Jlidi et al., 2022). The optical density was measured at 600 nm at 0, 2, 4, 6, 21, 23, 25, 48, 72, and 168 h after inoculation and calculated the inhibition efficiency using a formula according to Jlidi et al. (2022).

Results and Discussion

In the first series of experiments, all microalgae tested reduced the growth of bacteria compared with the control treatments. The largest differences were observed on the 4th day of the experiment for all microalgae tested, with the best results observed against the pathogen *V. anguillarum*.



(a) (b)

Figure 1. Colony-forming units (CFU) per mL of *V. anguillarum* in cultures of *Chlorella minutissima*, *A. gracilis*, and *Tetraselmis* species (red var., palmella, red var. Pappas) (a), *A. carterae,* and *D. salina* (b), compared with sterile seawater 25 ppt added Walne's medium (control), through time in light conditions.

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In the case of *V. anguillarum* (Fig.1a,b), the control treatment had a microbial concentration of 33×10^6 CFU/mL, while in exposure to light, the microalgae *A. gracilis*, and *Tetraselmis* species (red var., palmella, red var. Pappas) resulted in a concentration range of 2×10^4-89×10^5 CFU/mL. Similarly, in exposure to light, the microalgae *A. carterae* and *D. salina* resulted in a concentration range of 66×10^4-$16{,}6\times10^5$ CFU/mL, compared to the control treatment of 6×10^6 CFU/mL. In the absence of light, the microbial concentration range for *A. gracilis*, and *Tetraselmis* species (red var., palmella, red var. Pappas) was 3×10^4–77×10^5 CFU/mL, and the concentration range for *A. carterae* and *D. salina* was 78×10^4-$18{,}8\times10^5$ CFU/mL.

Seven species of microalgae were then tested for extracellular antimicrobial activity, where our experiments with *V. alginolyticus* yielded promising results compared with the control treatment, particularly for the cyanobacteria strains. The inhibitory effect on *Phormidium* sp. and *Anabaena* sp. was observed during the period from 21 to 48 hours, and for *Cyanothece* sp. it was observed during the period from 25 to 48 hours ($p<0.05$). The samples containing *Tetraselmis* sp. (red var., Pappas) with *V. alginolyticus* showed statistically significant inhibition between 4 and 25 hours. *A. gracilis* showed inhibition at 72 hours, while *Nephroselmis* sp. had a peak inhibition at 48 hours.

Our results with *V. anguillarum* indicate that *A. carterae* demonstrated inhibitory activity between 48 and 72 hours while in the experiment with *Tetraselmis* sp. (red var., Pappas), the growth of the pathogen was inhibited during the period from 6 to 21 hours. The results of our experiments with *V. harveyi* revealed statistically significant inhibition of growth for the *Anabaena* sp. strain between 23 and 25 hours while in our experiments with the *Tetraselmis* sp. (red var., Pappas) strain, inhibition was observed between 21 and 23 hours. *Nephroselmis* sp. exhibited inhibition against *V. harveyi* between 4 and 6 hours.

Acknowledgements
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ELECTRO-STUNNING PARAMETERS AND WATER TEMPERATURE SIGNIFICANTLY AFFECT FLESH QUALITY IN MEDITERRANEAN MARINE FISH SPECIES

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Introduction

Fish fillet proteolysis is a complex process that involves various biochemical and structural changes post-mortem, along with microbial activity. These changes can result in a decline in fish fillet quality and shelf life, often manifested as muscle softening and the creation of gaps in the myocommata [1]. Proteases responsible for muscle deterioration, namely, calpains, cathepsins and metalloproteases, can originate from both muscle tissue and the digestive system, and their activity is influenced by factors such as stress, temperature, and handling during harvesting and transportation [2], [3]. In Mediterranean aquaculture, species such as Sparus aurata, Dicentrarchus labrax, and Pagrus major are harvested using ice slurry that can cause stress on fish. This stress can exacerbate proteolytic cleavage and muscle softening, leading to further degradation of fillet quality [4]. The need for a more humane method of fish harvesting has led to the application of electro-stunning, suggested by both EFSA and OIE. However, before proceeding with the wide application of electro-stunning in Mediterranean marine fish farming, it is necessary to ensure that fish fillet quality is not compromised [5]. In this direction, the effects of electro-stunning on fish fillet proteolysis and flesh quality are investigated in the above Mediterranean farmed fish species, the gilthead sea bream, European seabass and the red seabream.

Materials and methods

The fish were harvested at the same fish farm in Astakos, Aitoloakarnania, Greece, during three different temperature periods: Warm (August, 25°C), Moderate (June, 21°C), and Cold (February/March, 15.5°C). Three harvest methods were applied: the conventional method of ice slurry and two electro-stunning settings of higher and lower voltage, with the same flow rate. White muscle samples were excised from each fish on slaughter day (Day 0) and on days 1, 2, 5, 7, and 13 post-harvest and were snap-frozen in liquid nitrogen. The activity of Calpain, Collagenase, Cathepsin B, and L was assayed using the Barrett and Kirschke method with minor refinements, and protein content was quantified using the Bradford method. Activity was expressed as fluorescence units change per minute per mg protein. Additionally, white muscle samples at harvest day (Day 0) and on days 7 and 13 post-harvest underwent histological analysis to assess flesh quality [5].

Results

The post-mortem activity of proteolytic enzymes in European seabass, gilthead seabream, and red seabream at different water temperatures and harvest methods was investigated. The results showed that calpain and collagenase activities were activated early post-mortem, and species-specific variations were observed in enzyme activity levels. Cytoplasmic calpains had the highest average activity among proteolytic enzymes, while collagenase activity shared a similar temporal pattern with calpain. Cathepsin B and L also showed a positively correlated activation regardless of the harvest method. Changes in muscle histology caused by the action of proteolytic enzymes can lead to flesh softening and loss of texture. The myofibrils, which make up most of the muscle fiber volume, are particularly vulnerable to degradation by endogenous proteases [1]. In our study, we observed an increase in the average single fiber volume density between days 0 and 7, irrespective of the harvest method used, though this increase was significant in electro-stunned groups. The temperature had a significant effect on enzyme activity and the histological phenotype, with the highest activities observed in the moderate temperature period for all species. Harvest method had a milder effect than the water temperature on enzymatic activities and the histological phenotype.

Discussion and conclusion

In the present study the effect of electro-stunning as a harvest method was investigated on the proteolytic activity and flesh quality of three Mediterranean farmed fish species. It is known that electro-stunning can have an impact on pre-slaughter stress and pH levels, which in turn, may affect the activation of proteolytic enzymes [5]–[8]quality and shelf life of whole fish (gilthead sea bream, European sea bass and red sea bream. The observed variations in enzyme activity among different species of fish, water temperatures, and harvest methods can be attributed to differences in muscle composition, physiological status, and post-mortem metabolism. Previous studies have suggested that this increase may be due to osmotic phenomena resulting from changes in intracellular membrane permeability and disruptions in ion balance in the cytoplasm [5].

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These findings suggest that water temperature, species and harvest method have an impact in the histological phenotype due to protease activity and they highlight the importance of developing species-specific humane post-harvest strategies to effectively preserve fish fillet quality ensuring animal welfare.

Acknowledgements
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BIOREMEDIATION OF CARBON, NITROGEN, AND PHOSPHORUS FROM AQUACULTURE SLUDGE USING THE POLYCHAETE *Hediste diversicolor*

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Introduction

Focusing on circular bioeconomy and solutions for managing finite and scarce resources such as phosphorus are issues that need to be addressed across sectors to obtain food security (Cordell et al., 2009). Land-based aquaculture of Atlantic salmon (*Salmo salar*) smolt and post-smolt in flow-through and recirculated system results in a large output of aquaculture sludge (Aas and Åsgård, 2017). These nutrient-rich side streams from aquaculture production constitute a valuable resource for cultivation of polychaetes *Hediste diversicolor* (Wang et al., 2019). The aim of this study was to recycle carbon (C), nitrogen (N), and phosphorus (P) contained in aquaculture sludge by production of polychaetes. Hereby, the bioremediation potential of *H. diversicolor was* assessed and a nutrient budget for recycling of C, N, and P was established.

Material and methods

Two different experiments, where feed levels were calculated based on estimated nitrogen content of diets and polychaetes, were carried out to investigate our research question. In a first experiment, we studied how the composition of two different diets, smolt sludge (S) and post-smolt sludge (PS) at four different feed levels (5-47% N) affected growth and C, N, and P recovery in *H. diversicolor* over a feeding period of 30 days. In a second setup, nutrient budgets of individual polychaetes supplied with two different quantities of smolt sludge (5% N, 40% N) were investigated to gain further knowledge on feed ingestion rates, feces production, respiration, excretion and assimilation of C, N, and P in polychaete biomass. Growth was calculated following Jørgensen (1990). Carbon and nitrogen in polychaetes, sludge, and feces were analyzed using an elemental analyzer. Phosphorus was oxidized with potassium peroxydisulfate and analyzed photometrically as phosphate in the same way as sea water samples containing nitrate, ammonium, and phosphate, using an autoanalyzer.

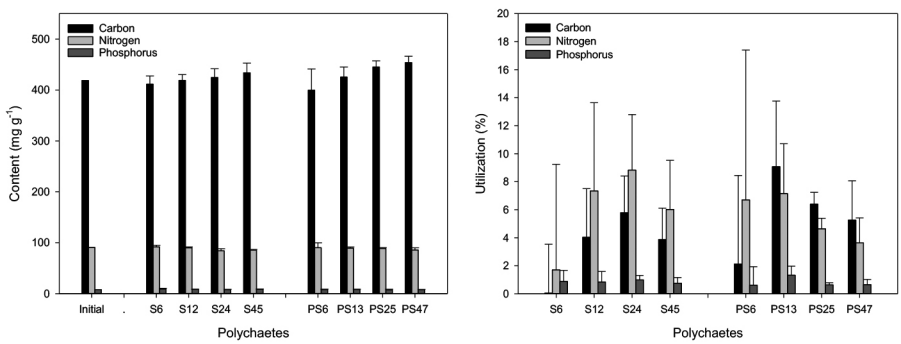

Figure 1 Carbon, nitrogen, and phosphorus composition of initial polychaetes (n=1), smolt sludge (n=4), post-smolt sludge (n=4) and polychaetes *H. diversicolor* fed with different levels of smolt sludge (S6-S24 n=4; S45, n=3) and post-smolt sludge (PS6-PS47, n=4) (left) and utilization of carbon, nitrogen, and phosphorus by polychaetes *H. diversicolor* fed different levels of smolt sludge (S6-S24 n=4; S45, n=3) and post-smolt sludge (PS6-PS47, n=4) (right).

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Results and discussion

In the 30-day trial, polychaetes fed with both types of aquaculture sludge were shown to incorporate C, N, and P from their diets into biomass. Specific growth rates increased with increased feed supply for polychaetes fed with both diets, and incorporation of C, N, and P in polychaete biomass was highest at the highest feed levels. C, N, and P contents in polychaetes were not affected by type of diet or feed level (Figure 1, left). Conversion factors of diet C, N, and P into polychaete C, N, and P differed between nutrients but were not significantly different between feed levels. On average, carbon and nitrogen conversion rates amounted to 2-10% and 2-15%, respectively, while values for phosphorus conversion rates were lower at 0.5-1.5% (Figure 1, right). When determining individual nutrient budgets, polychaetes supplied with more feed showed higher ingestion of smolt sludge, however, a smaller relative ingestion rate, suggesting overfeeding. Assimilation rates of C, N, and P were significantly higher in the high feeding treatment compared to the low. Accordingly, relative feces production was higher in the low treatment. Respiration was not affected by feed supply.

Conclusion

The presented findings suggest that *H. diversicolor* can successfully grow on aquaculture sludge. Nutrients C, N and P were incorporated successfully, however utilization rates varied between the three, with P being most poorly utilized. The species can be considered beneficial for bioremediation of aquaculture sludge, however, should not be regarded as the sole solution for efficient recycling of side streams from Atlantic salmon production.

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EFFECTS OF MICROALGAE SUPPLEMENTED COMPLETE FISH FEED ON CARP FINGERLINGS MEAT QUALITY

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Introduction

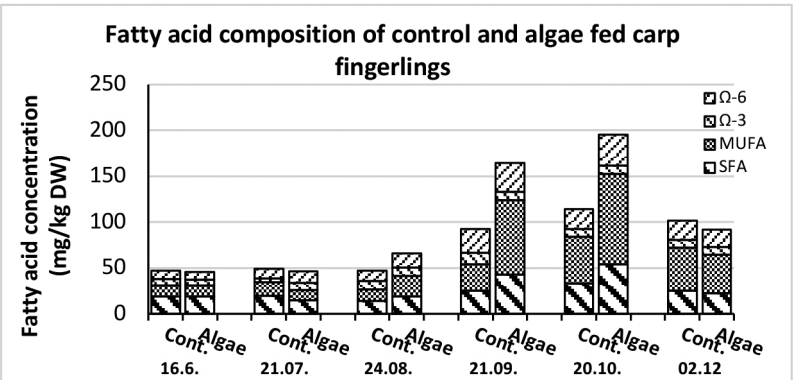
The natural sources of marine and freshwater fish have been over-exploited in the last decades, therefore aquacultural fish production's role in food security is becoming more important which led to aquaculture becoming the fastest growing segment in food industry. At global level freshwater fish production increased from 55,6% to 61,2% in total aquaculture production between 1995 and 2019 (FAO 2021), which shows that freshwater aquaculture has become more significant compared to mariculture in recent years. Aquacultural fish production has to face the challenges of increasing consumption and sustainable production at the same time, which can only be achieved by innovation and high efficiency production. To increase production complete feeds and feed supplements are being used in many cases despite their unsustainable production. Recently, microalgae are being investigated as a potential bulk-feed ingredient for fingerlings and adult fishes (Hodar et al., 2020). The aim of our study was to investigate the effects of microalga supplemented feed on common carp fingerlings (*Cyprinus carpio*) meat quality. The studies involved the production of freshwater microalgae in open pond systems (Vegetable Trading Centre Ltd.), sample analyses (Bay Zoltan Nonprofit Ltd.), fish feeding tests conducted in fishponds (Szegedfish Ltd.) and the professional supervision of the Institute of Aquaculture and Environmental Safety (MATE).

Materials and methods

The microalgae powder was a mixture of autotrophically open pond-grown unicellular microalgae (*Chlorella spp; Scenedesmus spp; Coelastrella spp; Acutodesmus spp*). The biomass was concentrated with a flow through centrifuge, dried at 70 °C to constant weight. In the final step the dried microalgae biomass was grinded to a powder (particle size <0,8 mm). The algae supplemented complete fish feed contained 3% w/w alga powder.

The carp fingerlings used during the experiment were kept in 10-hectare natural fishponds.

Fish samples were prepared from whole carp fingerlings, the fish were cut to smaller pieces, then homogenised in a bead beater. Representative portions of the homogenised fish samples were dried to weight constant in a lyophiliser and stored at -20 °C until analysed.



1. Figure: Total fatty acid contents of carp fingerlings during the examined period. Control carp (Cont.): fish fed with a complete diet; algae fed carp (Algae): fish fed with 3% microalga supplemented diet. Ω-6, Ω-3: omega 6 and 3 fatty acids, MUFA: monounsaturated fatty acids, SFA: saturated fatty acids

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FAME measurements were conducted of given amounts of dried samples, which were extracted in methanol-chloroform-hidrogen chloride at 90 °C, in the presence of an antioxidant. The carboxyl group containing compounds present in the extracted samples were trans esterified to methyl esters. The fatty acid methyl esters present in the chloroform phase were separated with a gas chromatograph and were detected with a mass spectrometer.

The total protein content of the samples was determined by the Lowry method, the color intensity of the solutions was measured with a Helios Alpha UV-Vis Spectrometer.

Results

Our results showed that the total fatty acid content of both control and fish fed with algae supplemented diet was almost identical during the first two sampling (1. Figure). After the 24th of August the total fatty acid contents of fish started to increase rapidly, however, the increase was much greater in the fish samples the had alga supplemented diet, although this difference was only observable until the 20th of October as the samples obtained on the 2nd of December had no significant difference in their total fatty acid contents. We also found that in the beginning, the fatty acid composition regarding saturated, unsaturated, poly unsaturated, omega-3 and omega-6 fatty acids was very similar in control and algae fed carp fingerlings, however the proportions of the fatty acids changed during the examined period. The proportion of saturated fatty acids decreased by about 15%, a similar decreasing trend was observable in the case of omega-3 fatty acids. In contrast to the previously mentioned fatty acids the average proportion of monounsaturated fatty acids increased by 16,1% ±SD 1,0. In general the carp fingerlings which had algae supplemented diet had an average of 34,7% higher total, 38,46% higher unsaturated and 18,65% higher omega-6 fatty acid content compared to control fish during the examined period. The protein content, which is an indicator of meet quality, was also higher due to algae supplemented diet compared to control fish samples.

Conclusions

The fatty acid content of first year common carp fingerlings is increasing during the warm seasons in natural fishponds, probably due to plankton consumption.

The fatty acid composition of first year common carp fingerlings grown in ponds changes, the proportion of saturated fatty acids decreases with the omega-3 fatty acids while the proportion of monounsaturated fatty acids increases.

The 3% w/w microalga powder supplemented diet increased meat protein levels and the total fatty acid content of the carp fingerlings, especially in the case of monounsaturated and omega-6 fatty acids.

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COMPARATIVE TRANSCRIPTOME ANALYSIS REVEALS A SEROTYPE-SPECIFIC IMMUNE RESPONSE IN NILE TILAPIA (*Oreochromis niloticus*) INFECTED WITH *Streptococcus agalactiae*

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Introduction

Streptococcus agalactiae, a Gram-positive pathogenic bacterium also known as group B streptococcus, is recognized as a causative agent of zoonosis (Gauthier, 2015). With a broad host range, it is one of the major causes of infection outbreaks in Nile tilapia (*Oreochromis niloticus*) with significant morbidity and mortality, resulting in great economic loss and threatening the development of tilapia aquaculture worldwide (Zhang, 2021). This bacterium species can be subdivided into ten serotypes (Ia, Ib, and II–IX) based on its capsular polysaccharide composition (Poyart et al., 2007), and among these serotypes, Ia, Ib, and III are the most frequent in aquatic animals (Li et al., 2003). The infection leads to septicaemia and encephalitis in fish, with noticeable clinical signs including lethargy, erratic swimming, exophthalmia, and ascites (Pretto-Giordano et al., 2010). Our research group recently isolated two distinct serotypes of *S. agalactiae* in Brazil. The first, E8ang2 (serotype Ib), was isolated in the northern state of Paraná, while the second, Maranhão (serotype III), was isolated in the Maranhão state. Upon further investigation, we noticed different patterns of responses to infection in tilapia with these strains. The Maranhão strain induces significant brain damage, resulting in severe signals of erratic swimming and a higher mortality rate in the initial days of infection. In contrast, E8ang2 kills the same number of animals but more slowly and with a lower incidence of noticeable brain-related symptoms. This study aims to characterize the brain transcriptomes of tilapia after infection with *S. agalactiae* and understand the reason for the different outcomes by comparing gene expression profiles between animals infected with strains of different serotypes.

Materials and Methods

This study followed the recommendations established by the Animal Ethics Committee, State University of Londrina (process nº CEUA 45/2017), Brazil. Nile tilapia were randomly divided into three groups: i) control, ii) challenged with the E8ang2 strain (serotype Ib), and iii) challenged with the Maranhão strain (serotype III). The fish were anesthetized and intraperitoneally injected with 0.1 mL/fish of bacterial suspension, whereas the control group fish were injected with an equal volume of broth medium. After bacterial exposure, the fish were monitored daily, and animals that showed signs of erratic swimming were removed from the aquaria and killed by medullary section. The brain was collected and immediately frozen in liquid nitrogen. The RNA was extracted, and 14 Illumina libraries were prepared (4 for Control, 5 for E8ang2, and 5 for Maranhão groups) and sequenced as paired-end reads (150-bp). After quality control and trimming of adapters using the fastp software, the reads were mapped to the Nile tilapia genome using HISAT2 and annotated using featureCounts. The differential expression of genes across treatment groups was determined with DESeq2. Enrichment of KEGG pathways and gene ontology were performed in g:Profiler.

Results

In response to exposure to strains of *S. agalactiae*, several immune response-associated genes were upregulated in the tilapia brain. These genes are mainly related to phagocytosis, cytokine production, and immune cell recruitment, suggesting that the immune response of tilapia to *S. agalactiae* infection is predominantly pro-inflammatory. However, genes involved in tissue repair and oxygen transport were downregulated. Additionally, the energetic demands of the immune response may affect the expression of genes related to the feeding behavior of tilapia during *S. agalactiae* infection.

Comparing the results of exposure to both strains, the animals exhibited an increase in the expression of pathogen recognition-related genes and inflammation-associated genes when exposed to the E8ang2 strain. These results suggest that the E8ang2 strain can be readily identified by the immune system triggering a more robust immune response. Furthermore, the upregulation of genes involved in neuroplasticity indicates a possible faster recovery of the injured tissue in the exposure to E8ang2. Meanwhile, the downregulation of some hormone-related genes may indicate a disturbance in regulating hormonal pathways involved in stress responses.

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Overall, our results suggest that the two strains of *S. agalactiae* may elicit different responses from the host immune system and neuroendocrine pathways. The study of this disease holds significant importance for aquaculture and human medicine. Additionally, elucidation of the mechanisms involved in the host–pathogen response is crucial for the success of new therapies. Our research is not only relevant for a better understanding and description of the tilapia immune system, but it also has the potential to provide new insights into the development of prophylactic measures for this species.

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USE OF OPTIMIZED CONVENTIONAL AND SENSITIVE SPERM QUALITY DIAGNOSTIC TOOLS TO ESTABLISH BASELINE REPRODUCTIVE DATA IN WILD-CAUGHT REDCLAW CRAYFISH (*Cherax quadricarinatus*)

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The Australian redclaw crayfish has great potential for aquaculture intensification and global market expansion. However, traditional production methods, subfertility, and high embryo mortality could curtail industry growth. This study evaluated sperm quality using optimized conventional and sensitive tools in redclaw crayfish. Healthy and robust male redclaw (n = 33) were collected from the Ross River Dam, Northern Queensland, and immediately subjected to electroejaculation to yield spermatophores for sperm quality assessment. Sperm concentration, count, morphology, viability, DNA fragmentation, and total potential fertile sperm cells (TPFSC) were determined. Under phase contrast microscopy, spermatozoa were visible with an elliptical shape of varying diameters and a tail-like structure. The mean ± SEM of sperm concentration, TPFSC, DNA fragmentation, and sperm viability was $42.5 \times 10^4 \pm 5.1 \times 10^4$ cells/ml, $23.6 \times 10^4 \pm 3.4 \times 10^4$ cells/ml, 17.2 ± 2.5 %, and 65.2 ± 3.9 %, respectively. Spermatophore weight was positively associated ($p < 0.05$) with sperm concentration and TPFSC and inversely associated with sperm DNA fragmentation ($p < 0.05$). Sperm viability was negatively associated with body mass ($p < 0.05$) but not spermatophore weight ($p > 0.05$). In conclusion, the weight of spermatophores provides a guide to sperm quality prior to spermatozoa extraction and may serve as a preliminary indicator of sperm quality. In addition, this study validated species-specific diagnostic tools for sperm quality assessment that may help improve productivity through selective breeding programs in redclaw aquaculture.

THE FIRST LINE OF DEFENSE AGAINST THE TOXIC GAS HYDROGEN SULPHIDE IN ATLANTIC SALMON

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Introduction

The need for sustainable and efficient aquaculture production to meet global food demand has led to the development of new technologies, such as recirculating aquaculture systems (RAS). In recent years, advancement in Norwegian land-based aquaculture has been highlighted by the implementation of RAS to produce Atlantic salmon (*Salmo salar*) in a more controlled environment, thereby addressing recurrent issues such as diseases and escapes. Despite its promise of a superior rearing environment compared to that of the traditional flow-through systems, several challenges have presented themselves, one of which is hydrogen sulphide (H_2S)-related mass mortalities. Currently, we have a significant knowledge gap on the physiological mechanisms of how Atlantic salmon respond to prolonged exposure to H_2S.

Mucosal organs such as gills, skin and olfactory organs represent the primary barrier against noxious compounds present in the aquatic environment. They can mount strong immune responses against immunotoxicants, including H_2S. Previously we have shown that mucosal organs of Atlantic salmon exhibit differential sensitivity to transient exposure to H_2S. Also, distinct molecular and structural alterations have been observed in these organs after transient exposure to H_2S. However, whether these organs exhibit the same sensitivity to prolonged sub-lethal H_2S exposure remains to be elucidated. Therefore, this study investigated how prolonged exposure to H_2S could impact the barriers at mucosal sites of Atlantic salmon.

Materials and Methods

A group of Atlantic salmon post-smolts (35 ppt) were reared under three H_2S level conditions – 0 μM/L (Control), 0.05±0.02 μM/L (Low) and 0.12 ±0.02 μM/L (High) – for 12 days. Each treatment group had 3 replicate tanks (Figure 1). After the exposure period, samples from the gills, skin and olfactory organ were collected for gene expression analysis, histology and RNAscope/*in situ* hybridisation. Moreover, mucus samples from the gills and skin were also collected for proteomics analysis.

Results and Discussion

The prolonged exposure to H_2S led to changes in gene expression, mainly affecting genes crucial for stress response, xenobiotic detoxification and immunity (Figure 2). Multi-tissue analysis revealed that the gills and olfactory organs were highly sensitive to H_2S where more than half of the genes analysed were altered by both H_2S doses. The expression of these marker genes showed minimal variations in the skin. These transcriptional profiles were not in agreement with previous transient exposure studies suggesting that the differential sensitivity of mucosal organs to H_2S was influenced by the duration of exposure. Mucosal structural integrity remained favourable following H_2S exposure since histological and morphological analyses did not show any relevant changes in H_2S-exposed fish compared with the control group. RNAScope identified and localised the transcripts important for sulphide detoxification in mucosal organs, including *suox*, *sqor1*, and *sqor2*. High throughput proteomics of gill and skin mucus revealed significant changes in mucosal proteome following H_2S exposure. 129 and 160 proteins were differentially altered in gill and skin mucus, respectively. Reactome pathway analysis revealed significant alterations in ribosomal processes and reactive oxygen species detoxification in the skin mucus, while drug metabolism was affected in gill mucus. Gene Ontology analysis further elucidated that some of the substantially affected proteins were crucial for toll-like 9 signalling and hydrogen peroxide catabolism, while affected proteins in gill mucus were important for lipoprotein metabolic process and regulation of endocytosis.

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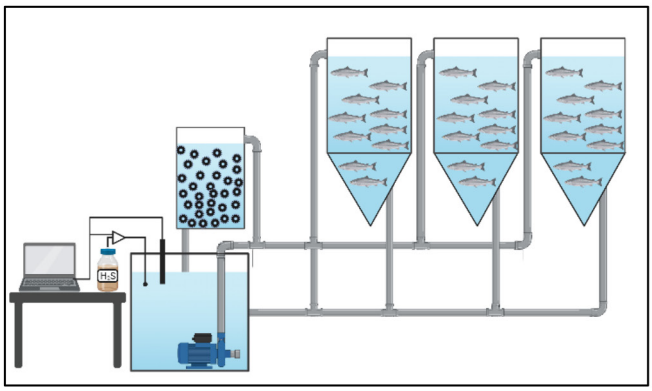

Figure 1. Set-up of the trial. H2S dose is controlled and monitored by computer. Created with BioRender

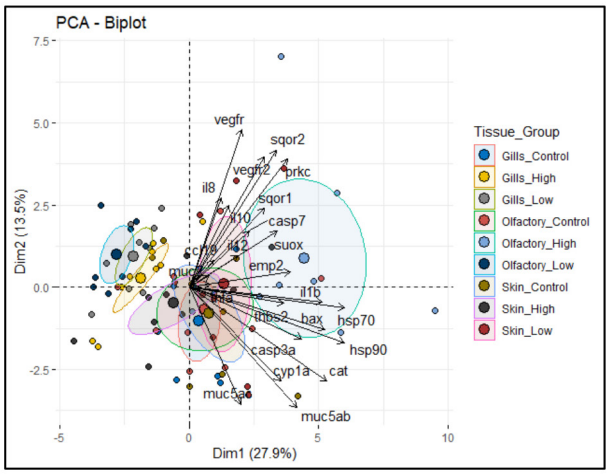

Figure 2. Principal component analysis (PCA) of the relative gene expression.

Conclusion

Overall, the results demonstrate that prolonged exposure to sub-lethal doses of H_2S altered the mucosal defences of Atlantic salmon. The mucosal changes were reflected in the gene and protein expression profiles of molecules important for stress, immunity and xenobiotic metabolism. Nonetheless, H_2S exposure revealed no substantial structural changes in mucosal organs. These results indicate that Atlantic salmon are capable of coping with low levels of H_2S, but it remained to be investigated whether this inherent mechanism at mucosal sites can persist for longer period. The study significantly advances our understanding of how Atlantic salmon interacts with H_2S at mucosal surfaces.

Acknowledgements

This work was supported by the Norwegian Research Council No. 300825 and Erasmus Mundus Joint Master Degree Scholarship.

METABOLISM OF TRIOLEIN IN SENEGALESE SOLE JUVENILES: EFFECTS OF FISH SIZE, DIETARY PROTEIN SOURCE AND TAURINE SUPPLEMENTATION

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Introduction

Contemporary feed formulations, which predominantly use plant protein sources and minimize marine ingredients, often result in reduced taurine content. Previous works on Senegalese sole (*Solea senegalensis*) juveniles have shown that dietary inclusion of high levels of plant protein sources to replace marine ingredients resulted in adverse effects on lipid metabolism (Richard et al., 2017; Aragão et al., 2023). Taurine supplementation to these high-plant diets mitigated part of the adverse effects, leading to better lipid utilization (Aragão et al., 2023). Therefore, the objective of this work was to evaluate the metabolism of triolein in Senegalese sole juveniles of two different sizes, considering dietary formulations with different protein sources and lipid levels, and the impacts of taurine supplementation.

Material and Methods

The different experiments performed used two basal diets: a marine ingredient-rich diet (FM), containing 69% of marine ingredients (fishmeal, fish soluble protein concentrate, squid meal and fish gelatine), and a plant protein-based diet (PP), in which plant protein sources replaced 85% of proteins from marine origin. Taurine content in PP and FM diets was 0.08 and 0.43%, respectively. The PP diet was further supplemented with taurine (PPT), resulting in a dietary content of 1.40%. All diets were isonitrogenous (55% crude protein, CP) and isolipidic (8.6% crude fat, CF). Additionally, based on the FM and PPT formulations, fish oil was increased at the expense of gelatinized pea starch, resulting in diets with similar CP content (55%), but with a CF content of 16.8% (FMF and PPTF, respectively).

Senegalese sole were adapted to these diets for 8 to 12 weeks and when presenting 11.9 ± 1.0 and 19.3 ± 5.5 g, metabolic trials were performed. For the metabolic trials, six fish per treatment were tube-fed with the respective diet labelled with ^{14}C-triolein and after tube-feeding the fish were placed in metabolic chambers with seawater and oxygen. After 24 h, samples were collected to analyse the effect of the different variables (fish size, protein source or taurine supplementation) on lipid evacuation, catabolism and retention. Details on the method may be found in Aragão et al. (2023).

Table 1: Changes in ^{14}C-triolein metabolism when comparing Senegalese sole with 12 versus 19 g, fed diets with low-fat levels.

Treatment	Evacuation (%)	Catabolism (%)	Retention (%)
Overall	↑	↓	=
FM	=	=	=
PP	↑	↓	=
PPT	=	↓	=

FM = marine-based diet, PP = plant-based diet, T = taurine-supplemented diet.

Table 2: Changes in ^{14}C-triolein metabolism when comparing Senegalese sole with 12 versus 19 g, fed diets with low or high-fat levels.

Treatment	Evacuation (%)	Catabolism (%)	Retention (%)
Overall	=	↓	=
FM	=	=	=
FMF	=	=	↑
PPT	=	↓	=
PPTF	↑	=	=

FM = marine-based diet, PP = plant-based diet, T = taurine supplemented diet, F = high-fat diet.

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Results and Discussion

The results showed that, when considering all the fish solely based on their size, the percentage of triolein evacuated increased concomitantly with fish size, when fed the low-fat diets (Table 1). However, when analysing each treatment separately, only fish fed the PP diet exhibited an increase in the proportion of triolein evacuated with increasing fish size (Table 1). Looking at the influence of diet, no significant differences were observed in the evacuation of triolein among fish of the same size. In terms of catabolism, the overall results indicated that the proportion of triolein that was catabolised decreases as fish size increases (Table 1). Upon closer analysis, it was noticed that larger fish presented lower triolein catabolism when fed PP diets, irrespectively of taurine supplementation (Table 1). Nonetheless, no significant differences were found in the percentage of triolein catabolised in fish of the same size among the various dietary treatments. Interestingly, the retention of triolein retention was not affected by the fish size (Table 1) or dietary treatment.

Comparing the use of low versus high-fat diets, in overall terms, the proportion of triolein evacuated was unaffected by the fish size (Table 2). However, when examining each dietary treatment separately, the percentage of triolein evacuation increased concomitantly with the increase in fish size when these were fed high-fat PP-based diets supplemented with taurine (PPTF; Table 2). Irrespective of the fish size, the proportion of triolein that was evacuated was higher in fish fed high-fat diets compared to those fed low-fat diets, regardless of the protein source. Additionally, the overall catabolism of triolein decreased as fish size increased and this was particularly evident in fish fed the taurine-supplemented PP-diet (PPT; Table 2). In terms of retention, no significant differences were found among treatments for overall fish. However, in fish fed the high-fat FM (FMF) diet, the proportion of triolein retained increased concomitantly with fish size (Table 2). Interestingly, dietary protein source and lipid content did not affect the catabolism and retention of triolein among treatments at each fish size.

In conclusion, the evacuation of triolein in fish fed the PP diet shows an increase as fish size increases, but the dietary inclusion of taurine mitigates this effect. Additionally, irrespective of the protein source, high-fat diets lead to higher triolein evacuation in fish of both sizes. In general, it is evident that triolein catabolism decreases with an increase in fish size. Careful must be taken, as triolein retention increases with fish size when fed high-fat FM diets, which may affect the quality of the fish.

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Acknowledgements

This study was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal to CCMAR (UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020) and CA (DL57/2016/CP1361/CT0033).

IMMUNOMODULATORY PROPERTIES OF A NEMATODE HOST DEFENSE PEPTIDE ANISAXIN IN FISH PERIPHERAL BLOOD CELLS

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Introduction

Antimicrobial resistance is a major health issue of this era, affecting the lives of millions of people worldwide. Alternative solutions have been proposed, such as the use of host defense peptides (HDPs), which are vital components of innate immunity, with antibacterial, antifungal, antiviral, and antiparasitic, as well as immunomodulatory properties (Mladineo et al., 2023; Shi et al., 2022). In particular, anisaxins, a cecropin-like helminthic HDPs from the zoonotic marine nematodes, *Anisakis simplex* and *Anisakis pegreffii*, have shown high efficacy against clinical and referent human bacterial Gram-negative isolates (Rončević et al., 2022).

Materials and methods

In order to evaluate the immunomodulatory properties of HDP anisaxin-2S (A-2S), the blood of the common carp *Cyprinus carpio* have been drawn from four groups of fish: i) specific pathogen free fish (SPF); ii) fish infected by the myxozoan *Spaherospora molnari* blood stages (BS); iii) fish infected by *S. molnari* blood stages and immunosuppressed (IS+BS); and iv) non-infected immunosuppressed fish (IS). Blood collected at four time points (T0, week 2, 3, and 4) was separated by Ficoll, and white (WBC) and red blood cell (RBC) suspensions were *in vitro* stimulated by A-2S. Flow cytometry was used to measure cellular changes and reactive oxygen species (ROS) production. Gene expression of innate immunity targets (*il6, il10, il1b, tnfα, infγ*) was quantified by qPCR and log-transformed relative gene expression data was used to test the statistical difference. Data were analysed in Prism 9 and presented as mean values ± SD.

Results and discussion

S. molnari infection decreased the number of RBCs in the blood by approximately 10% (100×10^6 RBC/mL), with a significant difference observed 2 weeks post-infection (wpi). In infected immunosuppressed fish, a steep decline in erythrocytes (loss of 84%) was observed 3rd wpi onward. On the contrary, non-infected but immunosuppressed fish exhibited mild but significantly higher numbers of erythrocytes. The decrease of erythrocytes during late-stage infection was accompanied by a release of the erythroblasts. This indicates a strong interaction of *S. molnari* and WBC/RBC as reported earlier (Korytář et al., 2020). High activity of both pre- and mature RBC in the IS group suggests that in the absence of the lymphocytes, erythrocytes contribute to an immune response in a much larger range (Fig. 1). All groups treated with A-S2 showed higher ROS production, in particular the IS+BS group, inferring the immunomodulatory role of this HDP (Fig. 2).

Conclusions

Immunomodulatory properties of the A-2S to the immune response in infected fish are promising, especially if we consider the fact that anisaxin predominantly stimulates red blood cells which are the most abundant population in the blood.

Funding

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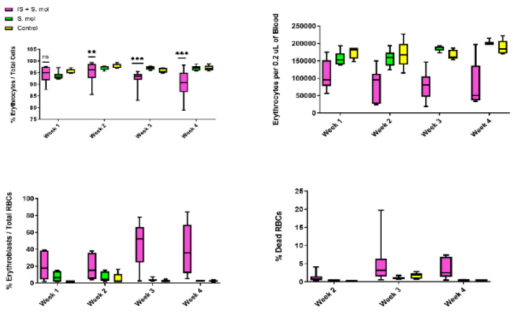

Fig. 1 Cellular changes of the RBC compartment throughout the infection in vivo.

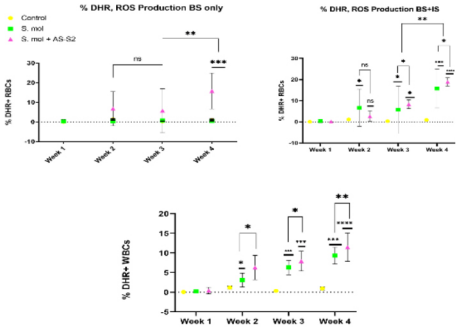

Fig. 2 ROS production of WBC and RBC stimulated by A-2S for both BS and BS+IS groups

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COMPARATIVE GENE EXPRESSION AND REGULATION ANALYSIS OF THE RESPONSE TO COMMON VIRUS (POLY I:C) AND BACTERIA (*Vibrio spp*) TRIGGERS AFTER *IN VITRO* AND *IN VIVO* CHALLENGES OF HEAD KIDNEY IMMUNE-RELATED CELLS IN TURBOT (*Scophthalmus maximus*)

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Introduction

Understanding immune response is of utmost relevance for all farmed animals. In turbot aquaculture, infectious diseases are caused by a broad spectrum of well-studied pathogens, from viruses and bacteria to different parasites (Aramburu et al., 2022). Knowledge of the transcriptomic basis for immune responses, both at general and pathogen-specific levels, is essential for a comprehensive understanding of host defence in turbot and can be helpful for other flatfish species. Moreover, dynamic changes in chromatin accessibility influence gene expression by granting or preventing binding by transcription factors (TF) and the transcription preinitiation complex (Herrera-Uribe et al., 2020; Jiang & Mortazavi, 2018). This work, framed in the AQUA-FAANG project for the functional and regulatory annotation of the six main species of European pisciculture, aims at establishing a regulatory map of the innate immune response of turbot through the immunostimulation of live (*in vivo*) and head kidney-extracted primary leukocytes (*in vitro*) using mimics of viral (Poly I:C) and bacterial (inactive *Vibrio spp*) infection.

Material and Methods

The immune challenges were carried out by i.p. injection (*in vivo*) and cell stimulation (*in vitro*). Head kidney and cell cultures were collected and frozen after 24 hpi, to be later used for RNAseq, ATACseq and ChIPseq procedures. Libraries were sequenced, with the resulting data being processed using nf-core pipelines (https://nf-co.re/).

A reference head kidney transcriptome was constructed from RNAseq data retaining genes when i) TPM > 5 and ii) present in at least two replicates in any of the conditions tested. The transcriptome was used as the reference to define differential expressed genes (DEGs) using DESeq2 ($P < 0.05$), and the resulting DEGs lists were subjected to gene ontology (GO) enrichment analysis ($P < 0.05$, GO terms with > 3 genes) using ShinyGO (http://bioinformatics.sdstate.edu/go/).

ATACseq and ChIPseq peaks were filtered following the Irreproducibility Discovery Rate method (https://github.com/nboley/idr) to obtain Highly Reproducible (HR) peaks for each condition and mark. Chromatin state discovery and characterization for the *in vivo* and *in vitro* experiments was modelled using ChromHMM (http://compbio.mit.edu/ChromHMM/) and the HR-peaks. Differential histone modification regions and differentially accessible regions were identified with DiffBind (https://bioconductor.org/packages/release/bioc/html/DiffBind.html; $P < 0.05$) using the unfiltered peaksets. Both HR-peaks and differentially accessible regions were annotated using HOMER's *annotatePeaks* function (http://homer.ucsd.edu/homer/index.html).

DEGs showing differential accessibility in their promoter regions (-1000 to -100 bp) were identified from the intersection of differentially accessible regions and the DEGs list, for each comparison. Transcription factor motif analysis was carried out for each condition using HOMER'ss *findMotifsGenome.pl* function.A turbot-specific blacklist of high-signal and low-mappability regions for ChIPseq analysis was generated using the ChIPseq controls to avoid artifacts and noisy regions.

Results

We identified 8,797 DEGs across all *in vivo* and *in vitro* conditions from a total filtered transcriptome of 12,152 genes. A significant enrichment of transcriptional activation immune response pathways was observed, such as the IFN-gamma pathway (particularly in PolyI:C stimulation) in upregulated DEGs, and metabolic pathways and cell cycle in downregulated DEGs.

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We constructed a regulomic atlas of immune stimulation in turbot using highly reproducible peaks of open chromatin regions (52,585 HR peaks) and three histone marks: H3K4me3 (14,741 peaks), H3K27ac (10,584 peaks) and H3K27me3 (33,843 peaks). Two different chromatin state models of 10 and 8 states (*in vivo* and *in vitro* respectively) were identified characterized by their transcription start site regions, potential enhancers, repressed polycomb and low signal regions. Roughly, 6.98% of the turbot genome was included in the blacklist of high ChIP signal/ low mappability regions, mostly comprised of telomeric and centromeric regions.

The differential binding analysis revealed significant differences in chromatin accessibility and H3K4me3-binded regions when comparing immune stimulated samples against the controls (particularly for *Vibrio* stimulation), with less differential binding for H3K27ac and H3K27me3. To identify potential genes with high DE and differential aperture, we identified the open chromatin, H3K4me3 and H3K27me3 differentially binded peaks annotated as promoter regions and compared them with the corresponding DEGs list (Hypergeometric distribution test, $P < 0.05$), showing high significance for the upregulated, differentially accessible genes for both marks and ATAC.

An analysis of the known transcription factor (TF) motifs identified in each of the peaks revealed a high representation of TF families associated with IFN regulation (IRF), defense and stress response (bZIP), cell cycle and differentiation (ETS), hematopoiesis (bHLH) and angiogenesis (Homeobox).

Conclusion

Our results provide the first chromatin state description of immune stimulated turbot as well as an overview of the genomic resources generated in the AQUA-FAANG project, providing brand new information on the regulomics of the species across different conditions.

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Acknowledgements

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TECHNICAL, ECONOMIC and ENVIRONMENTAL ASSESSMENT OF BIOBASED AND BIODEGRADABLE ROPES (BIOGEARS) IN LONGLINE MUSSEL (*Mytilus galloprovincialis*) PRODUCTIONS: A SHIFT TOWARDS AQUACULTURE DECARBONIZATION

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Introduction

Low trophic aquaculture, such as bivalve species, can be candidate species for the minimization of environmental impact of worldwide growing aquaculture, while providing consumers with high quality and nutritive seafood. Nowadays, mussels are cultured suspended mainly in raft and longline systems, using ropes made of non-biodegradable fossil-based plastics, which use could result in augmenting marine litter and microplastics entering the oceans. The EU BIOGEARS project addresses the challenge of minimizing the use of fossil-based plastics by developing alternative biobased and compostable ropes, biogears. The aim of this study is to assess and compare the sustainability of two biogears prototypes vs. commercial fossil-based rope counterparts in mussel offshore longline productions, under technical, economic and environmental perspectives.

Material and methods

Two biogears ropes prototypes (B1 and B2) were developed based on compounds of commercially available biopolymers and manufactured with industrial processes to be fit-for-purpose, with technical and mechanical properties similar to commercial counterparts, for mussel productions. For the technical assessment, the upscaling of their potential implementation by minimizing technical risks at rope production and the aquaculture performance phases were studied. For aquaculture validation, a one-year mussel longline production was performed using biobased B1 and B2 ropes and commercial fossil-based rope counterparts (GROPE). In the economic assessment, the costs and benefits of the use of biogears in mussel aquaculture were analysed along the value chain, comparing them to fossil-based ropes (market trends of raw materials, rope processing costs, aquaculture production, and End of Life (EoL) options). Additionally, the eco-efficiency indicator of the ropes was calculated (ISO 14045:2012). In the environmental assessment, rope biodegradation trials and the environmental profile via Life Cycle Analysis (LCA) methodology (ISO 14040/14044) were studied.

Results and discussion

Technical assessment: Biobased ropes promoted similar mussel growth but higher mussel productions per rope linear meter (85% in B2 and 23% in B1) with respect to the fossil-based rope counterpart (4.29 kg/m). Overall, mussel abundance per rope linear meter decreased over the experimental period in all types of rope, and especially in fossil-based ropes. Mussel quality was not compromised by using biobased ropes, as similar Condition Index, meat yield, proximal composition and fatty acid profiles were observed among experimental groups. Mechanical properties (Load at Break and elongation) of biogears decreased in the first two months of the sea tests, although not compromising rope functionality in one-year mussel production. The correlation between total mussel weight held per rope and the Load at Break results demonstrated that a 40% higher mussel weight held by B2 ropes had no significant effect on the variation of their mechanical properties.

Economic assessment: Currently, the raw materials used in biogears are economically more costly than the raw materials of fossil-based counterparts. Future trends envisage increasing biopolymer productions to meet market demand, which could in turn reduce their price and make biogears even more competitive than fossil-based ropes. Mussel productivity gains in biobased B2 ropes offset the over-cost of the biogears production, making mussel production more profitable with biogears than with the commercial fossil-based ropes. B2 prototype rope production and use should be encouraged, rather than biobased B1 and fossil-based ropes, due to their best eco-efficiency indicator (environmental impact and economic value of the ropes by kilogram of harvested mussel) (Figure 1).

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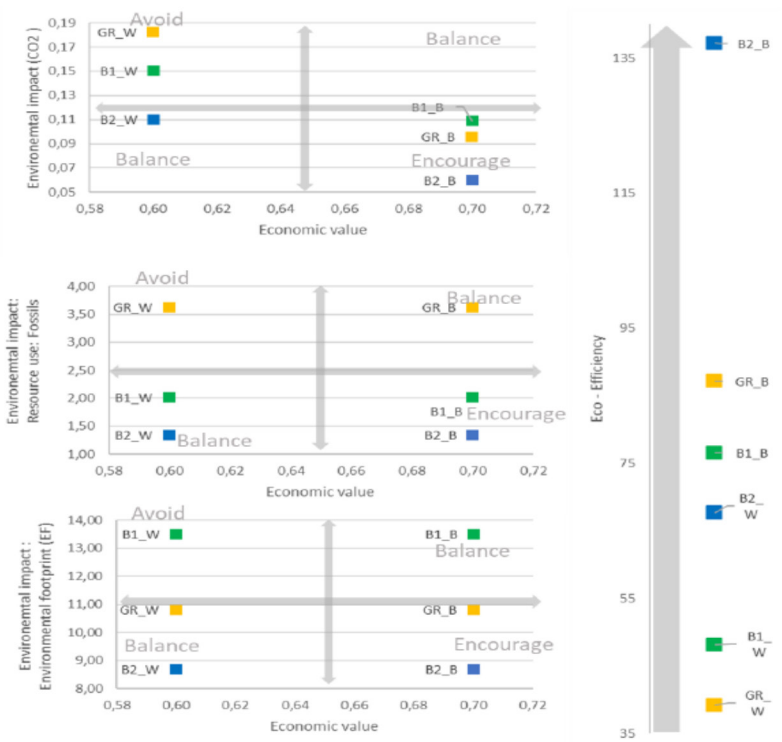

Fig. 1. Eco-efficiency portfolio for biogears (B1 and B2) and commercial conventional fossil-based rope (GR) per rope linear meter in worst (W) and best (B) rope production costs, rope price and mussel productivity scenarios.

Environmental assessment: Biodegradability tests in marine conditions indicate that biogears will not biodegrade in sea water at 20-30 °C or below. Compostability tests simulated in laboratory conditions (58±2 ºC) concluded that industrial composting of biogears is technically feasible. However, it should be validated in industrial composting facilities. Composting, as EoL option for biobased ropes, would reduce by 10% the carbon footprint of their life cycle and the impact on the use of fossil resources, due to the benefits of avoided impacts and the use of the produced compost. So, actions for the collection, sorting and transport of biogears to industrial composting facilities are encouraged for a correct management at their EoL. Finally, considering rope aquaculture production (impact/kg mussels produced per rope linear meter), B2 rope shows the best environmental performance, reducing by 34% the carbon footprint of mussel productions compared to conventional fossil-based ropes.

It can be concluded that biogears, and specifically biobased B2 ropes, can be technical, economic and environmentally sustainable alternatives to currently used fossil-based ropes in mussel offshore productions promoting aquaculture decarbonization.

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EVALUATION OF THE STRESS RESPONSE AT SLAUGHTER IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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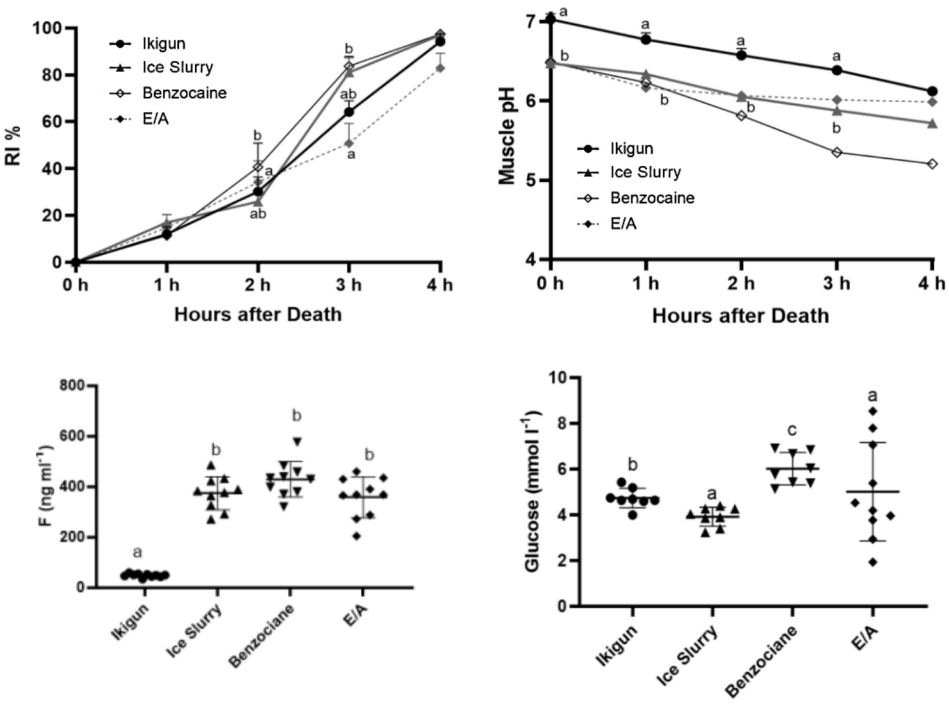
Introduction

Fish welfare is a major component of sustainable fish farming and research to gain an in-depth understanding of the factors affecting the welfare of farmed fish is of high priority. Guidelines from the World Organisation for Animal Health and European Food Safety Authority of economically important aquaculture species are available, including harvest and slaughter, however, there is still a need to develop efficient humane slaughter methods (Hastein, 2007). Existing methods for European sea bass consider to be inhumane and not conclusive (de la Rosa *et al.,* 2021). Therefore, there is an urgent need to develop and evaluate stunning/slaughter methods that will ensure immediate loss of brain function, insensibility to pain and maintenance of flesh quality. The aim of the study was to evaluate the impact of different stunning methods prior to slaughter on the onset of rigor mortis, muscle pH and stress indicators in European sea bass.

Materials and Methods

European seabass (*Dicentrarchus labrax*) ranging in weight from 250 to 300 grams were used to evaluate the effect of three different stunning methods: chemical (Group Benzocaine), electronarcosis (Group E/A: electrical stunning, 1.5 V/cm), and thermonarcosis (Group Ice Slurry: immersion in ice-slurry without prior anaesthetization). Following crowding, all fish were captured by net and slaughtered by immersion in ice-slurry. Capture of fish by hook and line and immediate slaughter by pithing (ikijime), was used as control (Group Ikigun). Muscle pH and rigor mortis were performed at regular intervals between 0- and 4-hours post-mortem. Blood samples were taken to determine haematological (haematocrit), biochemical (glucose, lactate, osmolality) and hormonal indicators (cortisol). All data are expressed as means with the standard error of the means (SEM, n = 10 per group). Data analyses were based on ANOVA or Duncan's multiple range test and significance ascribed to differences at the 0.05 level.

Results



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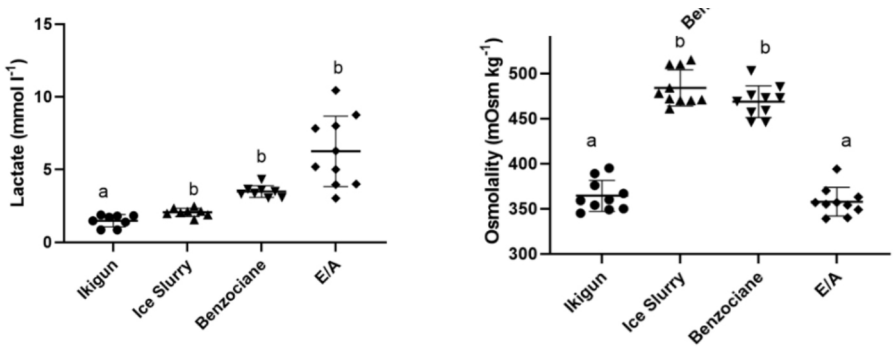

Figure 1. Scatter plot (x ± SD, n=10) of post-mortem changes in stress and quality indicators in European sea bass stunned and slaughter by different methods.

Discussion

Development of humane stunning/slaughter methods for farmed Mediterranean fish species is of high priority. E/A has shown a great potential as an alternative stunning method in European sea bass (Zampacavallo et al., 2015). Our results show that handling stress prior to stunning significantly enhances the stress response and has a negative impact on quality indicators (RI% and muscle pH). Electrical stunning delays the time needed to reach full rigor mortis and maintain muscle pH in comparable levels with those obtained in sunning/slaughter fish in ice-slurry. Chemical anaesthesia is also stressful for the fish and affects negatively flesh quality. Finally, the study confirmed that pithing, used by experienced staff, does not evoke an increase in stress indicators and ensures rapid insensibility.

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Acknowledge

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SETTING THE GROUND FOR PLANNING THE FUTURE OF AQUACULTURE IN GREEK SEAS – KEY DRIVERS OF CHANGE

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Sustainable blue economy sets the ground for maritime investments in a number of sectors, among which falls also aquaculture, as a complementary dimension to the fishery sector. In fact, interest in aquaculture is nowadays steadily growing as a result of, among others, the: globally rising healthy dietary patterns and related demand; policy directions at the global and the European/national level, seeking to achieve a sustainable future of the sector that additionally contributes to the integrated coastal zone management and the protection of marine resources; radical technological advances that can abrogate adverse environmental repercussions of aquaculture; etc. The aforementioned developments frame, in a way, the future developments of the sector. Taking into consideration the dynamics of the broader decision-making environment, as well as the current spatial and developmental policy contexts in Greece, the present paper attempts to explore the key drivers of change that are decisive for shaping the future of aquaculture in the Greek marine environment; while keeping track with its multiple dimensions, i.e. spatial, developmental, social, economic, environmental, technological, etc. That said, a thorough exploration of the external decision environment is carried out that is grounded on: i) sector-related documentation, emanating by international organizations (e.g. FAO) and literature review, policy recommendations and institutional developments; and ii) developments that are taking place in related fields (blue economy, marine spatial planning, integrated coastal zone management, etc.) at the global, European and national level and have a direct or indirect impact on aquaculture development. Results identify key drivers that need to be taken into account for feeding the revision of the currently out-dated Special Framework for Spatial Planning and Sustainable Development of the Sector in Greece, in alignment with the radical developments occurring in the complex and uncertain era of multiple, sea-related, sectoral developments.

DIETARY SUPPLEMENTATION WITH *Agaricus bisporus*, *Agaricus bisporus Portobello* and *Pleurotus sp.* ON GROWTH PERFORMANCE, IMMUNOLOGY AND HISTOMORPHOLOGY OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Mushrooms contain several types of polysaccharides and other constituents that have proven antibacterial, antimicrobial, antioxidant and anti-inflammatory activities (Van Doan et al. 2019, Mohan et al. 2021). The genus *Agaricus* and *Pleurotus* are among the most popular and widely cultivated mushroom species for human consumption and recently have attracted the interest as potential natural prebiotics in aquafeeds having also immunostimulatory and growth-promoting effects (Van Doan et al. 2019). Despite the importance of gilthead seabream (*Sparus aurata*) in aquaculture, there is a lack of relevant information and thus this study aimed at evaluating the effects of dietary supplementation with mushrooms on growth, immunology and histomorphology of the species.

Materials and Methods

S. aurata juveniles of 3.36 g initial mean weight were stocked at 12 tanks (125L) in a closed seawater recirculation system. Fish in triplicate groups (22 fish/tank, 3 tanks/dietary group) were fed to satiety, twice a day for 75 days, each of the four isonitrogenous (50%), isoenergetic (21.5 MJ/Kg) and isolipidic (15%) diets that contained 0 (Control) or 2% dried powder of either *Agaricus bisporus* (AG), *Agaricus bisporus* Portobello (PO) and *Pleurotus sp.* (PL). At the end, fish were weighted to measure growth and feed utilization parameters. Blood samples from 18 fish per group were used for immunological analyses, while liver and foregut samples from 15 fish per group were used for histology.

Results and Discussion

The 2% supplementation with either mushroom species had not detrimental effects on growth performance and feed utilization, neither on feed palatability, but also had not any growth promoting effect in seabream (Table 1). A dietary supplementation with *A. bisporus* has been shown to promote growth in other fish species such as Nile tilapia (Dawood et al. 2020), common carp (Hoseinifar et al. 2019), koi carp (Safari & Sarkheil 2018) and sharptooth catfish (Harikrishnan et al. 2018), but not in rainbow trout (Amiri et al 2017). Also, *Pleurotus sp.* has promoted growth in Gibelion Catla (Sattanathan et al. 2018) and rainbow trout (Bilen et al. 2016), but not in cherry salmon (Oh et al. 2019). The histological analysis of seabream fed the experimental diets revealed a normal and similar histomorphological structure of liver and foregut among all dietary groups (results are not shown). Dietary supplementation with *P. ostreatus* significantly increased *E. coli* growth inhibition. On the one hand, the supplementation with both *Agaricus* species decreased ($P<0.05$) the bactericidal activity against *E. coli* and fish fed PO were significantly slower to assemble the complement complexe than control fish (Table 1). On the other hand, alkaline phosphatase was significantly increased in fish fed PO. Ceruloplasmin, nitric oxide concentration and trypsin inhibition were similar among the groups although the latter tended to be stronger in PO and PL fish. These results indicate that dietary supplementation with *Pleurotus* sp. significantly improved the antibacterial activity in fish serum through an increased complement. On the contrary, both *Agaricus* species had a detrimental effect on the antibacterial activity of the serum although the improved alkaline phosphatase activity by PO may show some positive effects on fish health. *Pleurotus* sp. has been shown to be an effective prebiotic for stimulating fish immunity by elevating either serum myeloperoxidase, phagocytic, lysozyme and bactericidal activities or developing immune resistance against infections in several fish species such as rainbow trout (Bilen et al. 2016), koi carp (Safari & Sarkheil 2018) and Gibelion Catla (Sattanathan et al. 2018). On the contrary to our results, *A. bisporus* has been proved to stimulate immune response in several fish species such as rainbow trout (Dawood et al. 2020) and sharptooth catfish (Harikrishnan et al. 2018). Future studies are required to enlighten the effectiveness of dietary supplementation with mushrooms on growth and immunology of *S. aurata*.

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Table 1. Growth, feed utilization and immunological parameters of *S. aurata* fed with the experimental diets.

Parameters / dietary groups	Control	AG	PO	PL
Growth performance and feed utilization				
Survival (%)	97.0±2.6	93.2±3.2	90.9±3.2	95.5±3.2
Feed intake (%/day)	2.96±0.07	3.10±0.23	3.27±0.39	3.40±0.24
Final weight (g/fish)	40.06±1.95	39.14±2.76	39.75±2.44	34.77±2.04
Weight gain (g/fish)	36.74±1.95	35.80±2.76	36.41±2.40	31.47±2.05
SGR (%/day)	3.32±0.06	3.28±0.12	3.30±0.07	3.14±0.08
FCR	1.01±0.02	0.99±0.05	0.97±0.07	1.08±0.06
PER	1.82±0.04	1.90±0.10	1.96±0.14	1.75±0.09
Immunological parameters				
E. coli growth inhibition (%)	64.43±3.99^{ab}	61.33±4.09^{a}	60.67±2.70^{a}	67.64±3.57^{b}
Complement assembly (min)	35.0±7.3^{ab}	31.0±3.9^{a}	38.3±7.9^{b}*	37.0±7.7^{b}
Nitric oxide concentration (μM)	0.25±0.09	0.23±0.10	0.21±0.07	0.23±0.09
Tryspin inhibition (%)	95.05±6.34	95.19±8.12	97.61±3.49	97.96±3.02
Ceruloplasmin activity (U/ml)	9.61±8.28	7.44±5.34	9.43±6.08	8.79±7.98
Alkaline phosphatase (U/ml)	7.13±1.24^{ab}	7.22±1.45^{ab}	8.20±2.29^{b*}	6.42±1.41^{a}

Note. Values represent means ± standard deviation. Values within each bearing a different superscript letter are significantly different (ANOVA, $P < 0.05$).

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GENETICS OF EARLY SEXUAL MATURITY IN RAINBOW TROUT, *Oncorhynchus mykiss*

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Introduction

When rainbow trout and the other salmonids become sexually mature, they experience reduced growth, poor carcass quality (external sex characters, reduced body fat and filet color), and high mortality if reared in seawater. Thus, early sexual maturity before the desired market size is a serious economic drawback for the producers. Limited number of estimates are available for genetic variation of this trait in rainbow trout, but evidence suggests the presence of significant genetic variation[1-4].

The aim of this study was to obtain reliable estimates of genetic variation for early sexual maturity at two years of age in rainbow trout, and to search for possible quantitative trait loci (QTL) for the trait that can be used for genomic (GS) and/or marker assisted selection (MAS).

Material and Methods

The population used in the current study originated from the breeding nucleus (year-class 2020) of Osland Genetics AS, comprising a total of 2054 fish, i.e., the offspring of 92 sires and 144 dams, with a median full-sib family size of 12 (ranging from 3-43). The fish were slaughtered at a mean body weight of 4.9 kg at about two years of age. The sexual maturity status (1=maturing; 0=immature) and the gender of each fish were recorded through the visual observation and palpation of gonads after the fish were killed and gutted. All the recorded individuals were genotyped using Illumina Infinium SNPs genotyping array carrying around ~22K SNPs.

Analyses: Estimates of genetic parameters were obtained using a linear mixed model(s) implemented in ***"ASREML, v4.2"*** with genomic and pedigree information. The GWAS analysis was performed with ***"GCTA, v1.94"*** program using the "--mlma-loco" function [5].

The fixed and the random effects in the applied statistical model are as follows:

where is the vector of the observed binary sexual maturity trait (0 immature and 1 maturing); is the overall mean; and are design matrices to relate the animal records to appropriate level of the fixed and the random genetic effects, respectively; is a vector of the fixed effect of gender, is the random animal genetic effect with , where is the genetic variance, is the genetic relationship matrix obtained using pedigree information, is a genomic relationship matrix computed using VanRaden's method 1; and is the vector of random residuals with . Additionally, estimates of genetic variation for early sexual maturity trait were also obtained on the underlying liability scales using a threshold model(s).

Results and Discussion

The incidence of early sexual maturing fish at two years of age was very low (2%); with males showing significantly higher incidence (2.3%) than females (1.7%).

The estimated genetic parameters revealed low but significant genetic variation for early sexual maturity with estimates of heritability ranging from ~0.06 to ~0.16 (Table 1) which varied across model (LM vs. TM) and source of information (pedigree vs. genomic).

The genome-wide association analysis revealed a strong signal of a quantitative trait loci (QTL) at chromosome 28 with 27 SNPs surpassing chromosome and/or genome-wide Bonferroni corrected significant threshold (Figure 1). In spite of the low frequency of early sexually maturing fish in the current data, the highly significant SNP at chromosome 28 explained ~31% of the total genetic variance computed using (Falconer and Mackay, 1996 [6]). The display of a single clear QTL at chromosome 28 together with some minor indications from the SNPs located at other chromosomes (18 and 30) directs that the trait may be affected by a few gene(s) with large effect and multiple other genes with smaller effect size. The results show that the selection through GS and/or MAS can be used to further reduce incidences of undesired early sexual maturity in this population of rainbow trout.

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Table 1: Estimates of variance components and heritability for early sexual maturity in rainbow trout.

Source	PEDIGREE		GENOMIC	
Comps/Models	LM	$TM_{sire-dam}$	LM	TM
$\sigma_u^2 \pm SE$	0.001±0.0004	0.044 ±0.0311	0.001±0.0004	0.184±0.1072
$\sigma_e^2 \pm SE$	0.018±0.0006	1.000±0.0000	0.018±0.0006	1.000±0.000
$h^2 \pm SE$	0.054±0.0227	0.162±0.1050	0.059±0.0205	0.156±0.0764

LM= Linear Animal Model; $TM_{sire-dam}$ = Threshold sire-dam model; TM = Threshold animal model; σ_u^2 = Genetic variance; σ_e^2 = Residual Variance; SE = standard error of estimates.

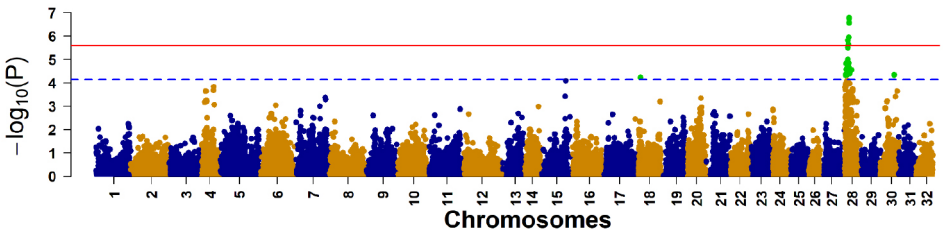

Figure. 1: Manhattan plot presenting association of SNPs with sexual maturity trait.

Similar parameter estimates will also be obtained based on data from a more recent year-class (2021). In addition, we will present estimates of accuracy of prediction using cross validation scheme(s) with different models (PBLUP, GBLUP, Bayesian, and MAS) to assess and compare the potential of genomic and/or marker assisted selection over classical pedigree information.

Acknowledgement
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GWAS REVEALS MULTIPLE QTLs FOR SEA LICE RESISTANCE IN ATLANTIC SALMON (*Salmo salar*)

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Introduction

Sea lice (*L. salmonis*) is an ecto-parasite that occurs naturally on wild and farmed Atlantic salmon. This parasite is one of the major threats for the farmed A. salmon industry in Norway, causing huge economic losses due to frequent treatment costs and increased mortality due to these treatments. The parasite load trait has shown low to moderate heritability with polygenic architecture of the trait reported in multiple studies [1-4]. There is a scarcity of studies showing significant signal(s) of quantitative trait loci (QTL) for lice count possibly due to lack of sufficient statistical power with low sample sizes and low lice counts.

The aim of the current study was to estimate genetic variation for lice counts obtained from three lice challenge tests belonging to the two year-classes, perform genome-wide association analyses of these data, and test the accuracy of genomic predictions from across and within population validation schemes.

Material and Methods

MOWI GENETICS routinely performs controlled challenge tests for lice on full- and half-sibs of breeding candidates and that smoltify at < one year (S0) and/or > one year (S1) of age. The recorded individuals were from two year-classes (YC); the parent YC-2018 (2825 "S1" individuals from 316 full-sib families) and the offspring YC-2022 (2329 "S0" and 2319 "S1" individuals representing 248 and 238 families, respectively) with family size ranging from 1 to 19. All 7473 individuals were genotyped using a custom developed Affymetrix axiom ~65K SNPs genotyping array. Moreover, tank (two or three per test), lice counter (3-6 for each of the three tests) and the body weight of the fish at the lice counting were also recorded. The phenotypic distribution of the lice count data was positively skewed and therefore the lice counts were log transformed to make them more normally distributed.

Analyses: The estimates of genetic parameters were obtained from the following linear mixed animal model(s) using genomic relationship matrix implemented in ASREML v4.2.

$$\boldsymbol{y} = \mu + \boldsymbol{Xb} + \boldsymbol{Zu} + \boldsymbol{e}$$

where $\boldsymbol{y}$ is the vector of log transformed lice count values $Log(LC)$; μ is the overall mean; $\boldsymbol{X}$ and $\boldsymbol{Z}$ are a design matrix to relate the animal records to fixed effects and genetic values, respectively; $\boldsymbol{b}$ is a vector of fixed effects of tank, lice counter and body weight of the fish as a covariate; $\boldsymbol{u}$ is a vector of random additive genetic effects $u \sim (0, \sigma_u^2 \mathrm{G})$, where σ_u^2 is the genetic variance, $\boldsymbol{G}$ is the genomic relationship matrix computed using VanRaden's method 1; and $\boldsymbol{e}$ is the vector of random residual effects with $e \sim N(0, I\sigma_e^2)$. The three datasets ("S0" of YC-2018; "S0" of YC-2022, and the "S1" data of YC-2022) were analyzed separately, and as a single trait by combining datasets, and with a bivariate model considering the two year-class specific $Log(LC)$ values as two different traits. Additional fixed effects of year-class and smolt type ("S0" and "S1") were also added when both year classes were analyzed together.

Genomic breeding values (GEBVs) were computed using two different $\boldsymbol{G}$ matrices derived from two sets of SNPs; ***G1)*** all SNPs regardless of linkage disequilibrium (LD) phase among parent and progeny year-classes, or ***G2)*** a subset of the SNPs selected based on consistent LD phase among the two year-classes. A fivefold cross validation scheme was designed and used to evaluate the accuracy of predictions across the year-classes with multiple scenarios i.e., across and within year-class predictions.

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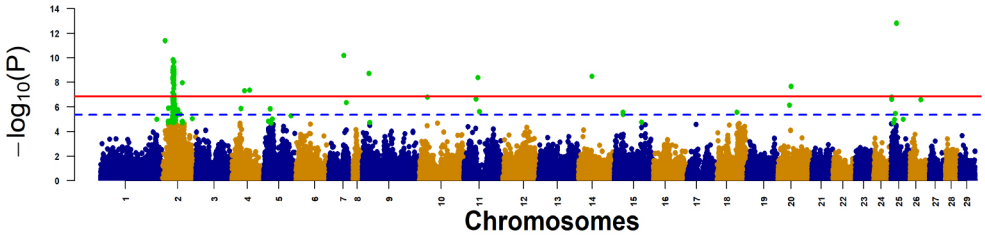

Figure 1: Manhattan plot presenting association of SNPs with the $Log(LC)$ trait. The red line is a genome-wide Bonferroni corrected significant threshold line while blue line is chromosome-wide Bonferroni corrected line.

The GWAS analysis was performed with the $Log(LC)$ of each dataset separately and by combining datasets from both year-classes as the same trait with GCTA software using the "*--mlma-loco*" function [5].

Results and Discussion

The heritability estimates for $Log(LC)$ was 0.25±0.03 (YC-2018) and 0.20±0.02 (YC-2022). The genetic correlation for $Log(LC)$ recorded on "S0" and "S1" populations of YC-2022 was high with estimates of 0.88±0.07 indicating possibility to analyze "S0" and "S1" of YC-2022 as a single trait. However, the genetic correlation of $Log(LC)$ $Log(LC)$ for the two year-classes was medium (0.69±0.06). The GWAS analysis revealed consistent strong signals of multiple QTLs across the two year-classes located at chromosomes 2, 5, 11 and 25. There were also inconsistent signals of QTLs detected, e.g., single SNP at chromosome 14 crossing significant line (Figure 1). Overall, low proportions of the genetic variances were captured by the different QTLs (calculated as $2p_iq_i\alpha_i^2$, Falconer and Mackay,1996[6]); e.g. the highest significant SNP at chromosome 2 explained 4.66% of the genetic variance.

The mean accuracy of the across year-class predictions of the GEBVs using either of the two types of G-matrices was generally low with magnitude of 0.22 and 0.42 using ***G1*** and ***G2***, respectively. The gain in accuracy for the across year-class prediction using ***G2*** was approximately doubled as compared to using ***G1***, possibly due to refining trait specific hidden relationship between the two year-classes. The mean accuracy of the within year-class prediction was 0.60 and 0.58 using ***G1*** and ***G2*** matrices, respectively. Hence, in the case of within year-class predictions the use of ***G2*** was not beneficial resulting in slightly lower accuracy of prediction as compared to the use of ***G1***.

In conclusion, lice count in A. salmon showed moderate heritability and a polygenic trait architecture, the accuracy of across-year class prediction was not advantageous compared to within year-class predictions (the latter of which is currently used in aquaculture breeding programs). GWAS revealed consistent signals of QTLs across the parent and the progeny year-classes.

Acknowledgement

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SUSTAINABLE PACKAGING OF FISH FILLETS USING A NOVEL CROSSLINKED EDIBLE FILM

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Introduction

Packaging materials represent the largest category of plastic waste, since almost 50% of global plastic waste comes from the packaging field. The compound annual growth rate (CARG) for global plastic waste is 5.4% from 2022 to 2030. Edible food packaging has been investigated as an alternative, environmentally friendly method to maintain food freshness and extend shelf-life (Galus et al., 2020).

However, packaging materials based on natural biopolymers often exhibit poor barriers and mechanical properties. For this reason, other components such as crosslinkers may be used to enhance barriers, and mechanical properties (Otoni et al., 2017). Crosslinking agents improve the mechanical and barrier properties of the films based on proteins and polysaccharides. When a crosslinking agent is added to the matrix of the polymer, a three-dimensional structure is synthesized by binding the polymer chains with covalent or non-covalent bonds, resulting in more hydrophobic films (Garavand et al., 2017).

Polysaccharides can be effectively used to synthesize biobased packaging materials, due to their biodegradability and biocompatibility. Among biopolymers, cellulose, and its derivates have been reported for their applicability in the packaging field, as cellulose-based films are odorless and tasteless. Carboxymethyl cellulose (CMC) is produced by adding carboxymethyl group (CH_2COONa) groups in cellulose molecules to obtain a water-soluble molecule. CMC-based films are biodegradable, but they exhibit poor barrier properties due to the hydrophilic nature of cellulose (Yildirim-Yalcin et al., 2022).

Plasma is an ionized gas containing electrons, photons, and atoms and it can be categorized as high temperature and low-temperature plasma. Cold Atmospheric Plasma (CAP) has been used in the packaging sector to improve the mechanical and barrier properties of polymers. Surface treatment of polysaccharide-based films using CAP may improve their functionality of the developed films (Zhu et al., 2021).

The objective of the study was the development of a novel polysaccharide-based and crosslinked, edible packaging film appropriate for fish and seafood. CAP was used as a surface modification method to enhance the barriers of the developed films. Fresh meagre fillets under refrigeration were selected as the case study.

Materials and methods

2% CMC-based films were produced according to the solvent casting method and glycerol was added as a plasticizer. Calcium cations (Ca^{+2}) were added as a crosslinking agent at the concentrations of 1% and 2%. The produced films were treated with CAP (kINPen® IND, neoplas GmbH, Germany) with continuous flow (argon, 4 L/min) for 5 and 10 min. Films without the crosslinking agent and plasma treatment were produced and tested as controls. Water Vapor Permeability (WVP) and Water Vapor Transmission Rate (WVTR) were evaluated according to ASTM E96/E96M. The hydrophilicity of the surface of the films characterized through contact angle with Theta Flow Optical Tensiometer (Biolin Scientific, Gothenburg, Sweden). The mechanical properties were measured according to ASTM D882 (ASTM, 2001), by Instron 3400 (Norwood, MA, USA) and load 50 N. The color changes of the films before and after plasma treatment were recorded. CMC-based films were applied on fresh meagre fillets (Argyrosomus regius) stored isothermally at 2°C for shelf-life evaluation, based on Total Viable Count (TVC) and Pseudomonas spp. growth and compared with the respective data obtained for conventional Polyvinyl chloride (PVC) films.

(Continued on next page)

Results

The results for the water barriers showed that by adding Ca^{+2} to CMC film forming solution the produced films had lower WVTR and WVP. The WVTR for CMC-based films was 1206.73±66.24 g/day× m^2 and reduced to 973.98±82.81 g/day× m^2 and 869.88±282.10 g/day× m^2 for films with 1% and 2% Ca^{+2}, respectively. All the produced films had higher water barriers after CAP treatment. The WVTR for the CMC-based films was 1206.73±66.24 g/day× m^2. After 5 minutes CAP the WVTR was 1107.01±127.61 g/day× m^2 and after 10 minutes of CAP treatment the WVTR was 1080.25±157.08 g/day× m^2. Similar trend was observed after CAP treatment for the films with the crosslinking agent. The contact angle of CMC based films was 60.25±8.04° represented hydrophilic materials. CAP treatment resulted in reduced contact angle (44.28±2.63° and 37.06±4.31° for 5 minutes and 10 minutes CAP, respectively). The addition of 1% Ca^{+2} led to increased contact angle of films (64.33±9.90°) but when 2% Ca^{+2} was added the contact angle was lower (47.69±3.81°). Films with Ca^{+2} had also lower contact angle after CAP treatment. The addition of Ca^{+2} into the CMC film-forming solutions led to more brittle films at higher concentrations (1.5 and 2% Ca^{+2}). When 1% of Ca^{+2} was added, the young's modulus of the films reduced from 3120.51±672.76 MPa to 2505±516.56 MPa for CMC-based films and CMC/1% Ca^{+2}-based films, respectively. The tensile strength and the elongation at break were not statically different for the two types of the films. The color of the produced films did not change by adding the crosslinking agent or after CAP treatment. The replacement of conventional PVC films with the developed CMC-based films did not affect the microbial growth of meagre fillets during refrigerated storage, resulting at shelf-life of 10 days at 2°C (same for all the tested conventional and alternative packaging films).

Discussion and conclusion

The results of the study show the potential of CMC-based, edible packaging films for the effective preservation of chilled fish, without affecting quality and shelf-life. The appropriate design of polysaccharide-based films and surface modification for the enhancement of the barriers and mechanical properties of the developed packaging systems will contribute to the sustainability of the aquaculture sector towards a zero-waste future.

Acknowledgment

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MINT (*Mentha spicata*) AFFECTED THE WATER QUALITY, GROWTH, AND MICROBIAL COMMUNITIES OF RAINBOW TROUT (*Oncorhynchus mykiss*) IN AQUAPONICS

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Introduction

Aquaponics combines recirculating aquaculture (RAS) with hydroponics, a soilless plant farming method. Plant choice in aquaponics may affect the growth and microbiome of fish because each plant has its specific microbiome, which may alter the microbes in aquaponics. Plant secrete root exudates, which may alter the microbes in the system. Mint is a plant known for its growth promoter effects on animals, and it may influence the fish growth when grown together with fish in aquaponics. On the other hand, fish in aquaponics excrete ammonia, which is toxic to fish and must be removed from circulating water. Ammonia is converted to nitrites and then to nitrates by microbial action during the process of nitrification. Nitrification is an essential process for the performance of RAS and maintains the water quality of aquaponics. The aims of this study were to investigate and the growth of fish and fatty acids contents of rainbow trout (*Oncorhynchus mykiss*) in aquaponics when grown with mint. The other aims were to investigate microbial communities in the mucous and gut of the rainbow trout and to compare start-up of nitrification in aquaponics and RAS.

Materials and methods

Seven-week experiment was performed with three replicated RAS and aquaponic systems. Twenty rainbow trout (initial weight 55 ± 1.06 g) were stocked in each fish tank for RAS and aquaponic systems. Thirty mint seedlings were transplanted in each deep water culture rafts. Change in biomass for fish was recorded at the end of the experiment. Microbial communities in fish mucous and gut were analysed at the start and end of the experiment. The fatty acids were extracted from fish muscle from the start (fingerlings) and end samples. Concentrations of total ammonia nitrogen, nitrite, nitrate were recorded during the experiment.

Results

The weight gains (aquaponics = 324.33 ± 31.87 g, RAS = 295.13, ± 9.70 g , p = 0.24) and specific growth rates (aquaponics = 2.11 ± 0.11 g, RAS = 2.00, ± 0.06 g, p = 0.21) of rainbow trout were similar in RAS and in the aquaponics'. Mint improved the fish growth through reduced feed conversion ratios (Figure 1). Mint reduced the feed consumption of the rainbow trout in aquaponics (Figure 1). However, fish in aquaponics maintained equal weight as fish reared in RAS despite of the lower feed consumption. The omega 3 fatty acids content of rainbow trout in aquaponics were slightly higher than the fish reared in RAS but without statistically significant difference. The retention of omega 3 fatty acids content was speculated in fish because fish maintained fatty acid composition similar to fish in RAS in spite of low feed consumption in aquaponics. Mint altered the microbial communities of rainbow trout in mucous and gut compared to fish reared in RAS. Nitrification start-up was faster in aquaponics than in RAS.

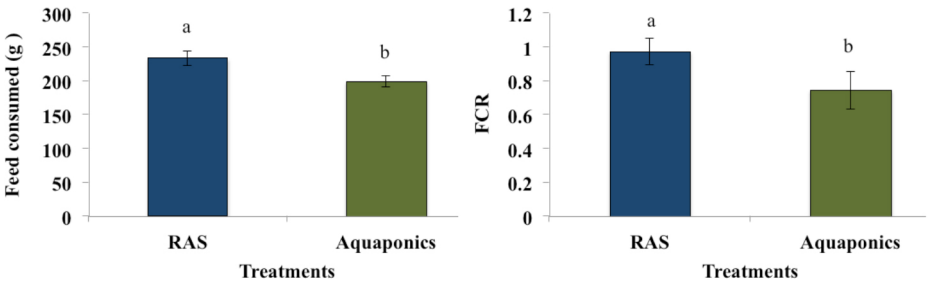

Figure 1: Feed consumed and feed conversion ratios (FCR) of rainbow trout in RAS and aquaponics for 7-week experiment. Independent samples t test, n = 3. Different letters differ statistically significantly. For the aquaponics treatment mint (*Mentha spicata*) was grown in a coupled aquaponics system together with rainbow trout (*Oncorhynchus mykiss*).

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Conclusions

Aquaponics with mint improved fish growth, water quality and initiated earlier start-up of nitrification as compared to RAS. Using mint in aquaponics may improve omega 3 fatty acids content of the fish. Growing fish together with mint in aquaponics may lower the feed consumption of fish compared to RAS which will reduce the cost related to fish feed and minimise the impact of aquaculture feed on aquatic environment.

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MOLECULAR PATTERNS OF A CHRONIC INFLAMMATORY RESPONSE IN EUROPEAN SEABASS (*Dicentrarchus labrax*) FED A TRYPTOPHAN-SUPPLEMENTED DIET

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Introduction

Despite the limited number of studies testing the effects of tryptophan on fish immune responses, data generally points out to immunosuppressive properties. Therefore, tryptophan poses as a potential regulator of an ongoing inflammatory process. This study main goal was to evaluate the effects of tryptophan dietary supplementation on immune and neuroendocrine responses of the European seabass, *Dicentrarchus labrax*, undergoing a chronic inflammatory response.

Materials & Methods

European seabass juveniles (34.55 ± 7.84 g) were distributed into twelve tanks of a recirculating seawater system. At first, fish were intraperitoneally injected with either Freund's Incomplete Adjuvant (FIA, inflamed group), or Hanks' Balanced Salt Solution (HBSS, control group). Within each group, fish were fed a control diet (CTRL) and a CTRL-based diet supplemented with tryptophan (0.3% DM basis; TRP) for 28 days. Samples of head-kidney were taken every week for neuroendocrine- and immune-related gene expression analysis.

Results & Discussion

The expression levels of *gr1* at the end of 1 week were lower in FIA-injected fish than in HBSS-injected fish, irrespective of dietary treatment. When TRP was provided to FIA-injected fish, *mcsfr* increased from 1 to 2 weeks, and remained high until the end of the experiment. CTRL-fed fish *mcsfr* mRNA levels also increased, but later, from 3 to 4 weeks. Moreover, *il34* expression at 1-week post FIA injection was higher in TRP-fed than in CTRL-fed fish. The canonical discriminant analysis clearly differentiated groups between those with the shortest inflammation period (one week) and those sampled later at four weeks (Fig. 1). Moreover, it showed how the feeding period seems to be critical in what tryptophan supplementation is concerned. Indeed, after one week, anti-inflammatory processes seemed to be favored in fish fed TRP (higher *gr1*, *il34* and *tgf*β*; lower il1*β). Later, at four weeks, the two dietary groups have more similar molecular patterns, both showing higher expression of T-cell and macrophages' related markers than fish at earlier stages of the response.

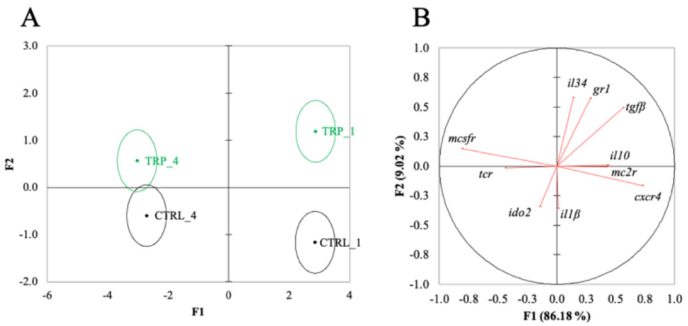

Figure 1. Canonical discriminant analysis of gene expression in the head-kidney of European seabass sampled at one or four weeks following intra-peritoneal injection with FIA. **A -** Canonical discriminant scores of each group. Groups centroids are marked by a small diamond, whereas circles indicate data distribution per group; **B -** Correlation variables/factors (factor loads) for two main discriminant functions (F1 and F2).

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Conclusion

Present results highlight tryptophan dietary supplementation potential in accelerating inflammation resolution, suggesting that its effects are mostly prominent at an early phase of the inflammatory response, dissipating towards the fourth week. It therefore provides important insights related to strategy of application.

Acknowledgements

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THE NEWRIFF PROJECT: NEW LIFE FOR RICE BY-PRODUCTS AND AGRICULTURAL WASTES: INSECTS BIOCONVERSION FOR FISH FEED PRODUCTION

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Introduction

The current meat and fish production systems are not sustainable, and the protein content of animal feed plays a key role in this regard. This holds true also for trout, a fish reared specie characterized by a high feed conversion rate, where the consumption of fishmeal is responsible for serious environmental issues (e.g., overfishing, loss of biodiversity) and economic concerns due to the constantly increasing price of these commodities. Meanwhile, the agriculture and agro-food industry generate a considerable amount of organic waste and by-products whose management is often problematic and expensive.

Through the insects rearing, these substrates can be locally re-utilized and valorized to produce alternative protein for the rearing of monogastric animals, reducing the impact on the environment related to animal feeding, organic waste, and by-products management. More in details, the use of insect meal as an alternative aquafeed protein source is an opportunity to exploit the efficient bioconversion by insects of agricultural by-products and other organic waste into an animal feed resource. In a circular perspective, this allows to reduce the use of traditional high-impacting protein sources and, at the same time, the impact of conventional management these matrices. In Lombardy, a region with high population densities, and intense agricultural activities and agri-food industries, a large amount of organic waste is available. Valuing this biomass by reusing it as a resource for an alternative feed appears to be an important opportunity for several actors involved in the supply chain. Rice (Oryza sativa) is a major crop in the region in terms of area, value of production and, above all, quantity of by-products resulting from processing.

The newRIFF project

newRIFF project aims at testing the suitability for recovery and enhancement of paddy rice processing by-products, and to use them, together with other organic waste, as a substrate for the cultivation of insects to be used in turn for aquafeed formulations. The tests of the use of the feed thus produced will be carried out with the rainbow trout (*Oncorhynchus mykiss*) as a pilot species, due to the importance that this fish has in the Italian aquaculture sector, particularly in Northern Italy.

The project aims to fill the following knowledge gaps: (i) to test the productivity of insects, in particular black soldier fly (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor*), raised on a mix of different matrices consisting of by-products of paddy rice processing and other organic by-products and wastes; (ii) to evaluate, by in vivo trials, different diets having increasing levels of insect meals (mix of meals produced from the two species)in substitution of conventional protein sources in rainbow trout farming. Fish performance and diets digestibility will be used to evaluate the effectiveness of the insect meal mix inclusion; (iii) to evaluate consumer acceptance and economic, environmental and social performance of trout farming by replacing traditional proteins (e.g. soybean meal and fish meal) with insect meal produced using rice by-products and other organic waste as insect rearing substrate; (iv) to identify best practices regarding the use of insect meal as a source of protein water feed and summarize all the information gathered during the project in order to develop guidelines and policy recommendations.

newRIFF involves different activities: (i) identification of the best substrate mix and optimal growing conditions for insects, (ii) insect meal production by rearing the two selected insect species, (iii) feeding trials on trout considering 3+1 diets: a control diet (the one usually utilized in commercial livestock farms) and alternative ones with different level of insect meal (i.e. 25, 50 100% of fish meal replacement), (iv) the evaluation of the nutritional, sensorial and food safety features of the produced trout in order to assess if the quality trout filet is affected (and if yes, how) using insect meal, (v) the sustainability assessment with a Life Cycle Thinking approach. The main benefits arising from the implementation of the newRIFF are schematized in fig.2.

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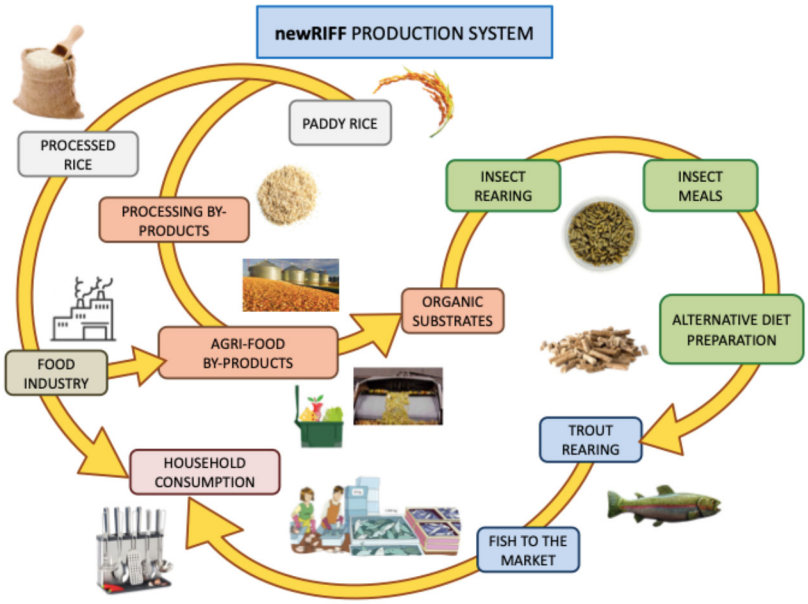

Fig 1. Production system that will be tested during the project

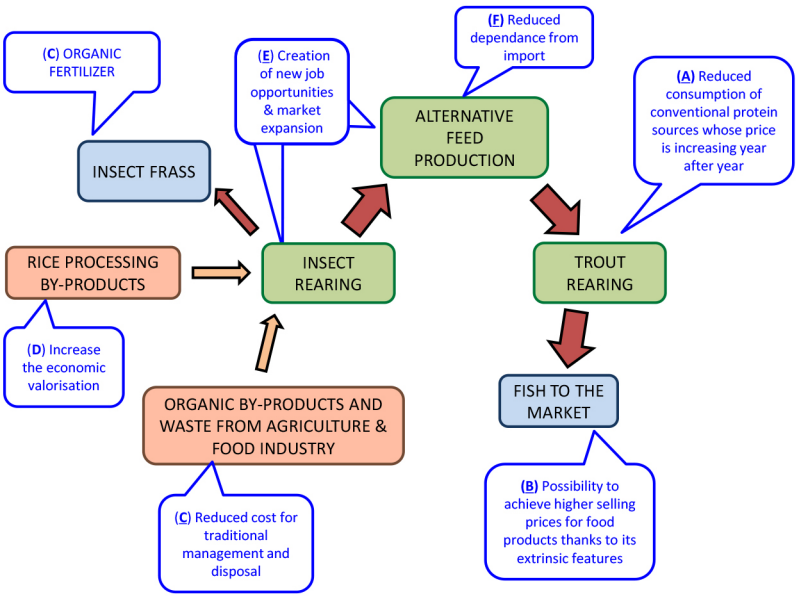

Fig 2 – Impacts on economic environment related to the newRIFF solution.

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COLD ATMOSPHERIC PLASMA AND PULSED ELECTRIC FIELDS AS DECONTAMINATION TECHNOLOGIES FOR RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

The development of new technologies for decontamination in Recirculating Aquaculture Systems (RAS) is a challenging task. Devices generating ozone or UV radiation are commonly used to reduce the amount of pathogens, to ensure an appropriate water quality and to guarantee the welfare of fish (Stiller, Kolarevic et al. 2020). Though these technologies are well established and well known, they have disadvantages concerning costs (Engle, Kumar et al. 2020). Previous studies confirmed the antimicrobial efficacy of cold atmospheric plasma (CAP) and pulsed electric fields (PEF) in water treatments (Banaschik, Burchhardt et al. 2016, Schmidt, Hahn et al. 2019). Our aim was to develop and test system models operated for the treatment of larger volumes on the on-site use.

Materials and methods

Experiments with the developed plasma source and PEF device were carried out using model aquaculture water with the addition of *Vibrio cholerae* DSM 100200 as fish pathogen. To determine the inactivation of *Vibrio cholerae*, treatment parameters as flow rate and treatment time were varied for the total volume of 90 L. Next to the detection of the antibacterial efficacy, water analyses were carried out by ion chromatography and determination of pH, conductivity as well as temperature.

Results

For the experimental setting an initial concentration of 10^5 colony-forming units/mL of *Vibrio cholerae*, a flow rate of 150 L/h and a total treatment time of 480 minutes was adjusted. A 270 minutes continuous treatment resulted in an inactivation to the detection limit for plasma, whereas an inactivation of 1.9 orders of magnitude was achieved with PEF. Furthermore, the low resulting increase of conductivity (plasma: +200 µS/cm, PEF: + 80 µS/cm) and temperature (plasma/PEF: 22°C up to 30°C) confirm the suitability of the system model for utilization in RAS and will probably not affect the fish welfare.

Conclusion

The decontamination results of the treatments by using this system models are promising for further research and development, especially for larger scale experiments. The system models will further be optimized regarding to the antimicrobial efficacy and energy consumption to establish plasma and PEF treatments as a sustainable decontamination technology in aquaculture.

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A HYDROXYTYROSOL-RICH EXTRACT FROM OLIVE JUICE POSITIVELY IMPACTS GROWTH POTENTIAL AND LIPID METABOLISM IN GILTHEAD SEA BREAM FED A HIGH-FAT DIET

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Introduction

In the last years, there has been a wider use of high-fat diets (HFD) in many farmed fish species. These diets aim to partially substitute the protein source, resulting in economic benefits, while also meeting the increasing demand for fish consumption. It has been demonstrated that HFD can enhance growth performance by improving the use of dietary protein (protein-sparing effect) (Sargent et al., 2002). However, an excess of lipids in the diet can lead to adverse effects, such as impaired mitochondrial and peroxisomal fatty acid β-oxidation efficiency and, consequently, depressed lipid catabolism and increased fat deposition in the liver (or others tissues) (Turchini et al., 2022). In this context, the inclusion of dietary feed additives that could mitigate these negative consequences is considered a promising strategy within the aquaculture sector. Hydroxytyrosol (HT) is a phenolic compound found in the olive tree (Olea europea L.), in leaves, fruit, olive oil, juice and their by-products. In mammals, HT has shown antioxidant, anti-inflammatory and anti-obesogenic properties, among others (Karković Marković et al., 2019). Nevertheless, research on its potential benefits as an additive in aquafeeds is still very limited.

Materials and methods

After one month of acclimation in the animal facilities of the Faculty of Biology (University of Barcelona), gilthead sea bream (*Sparus aurata*) juveniles (obtained from Piscimar fish farm in Burriana, Castellón) were distributed into eight tanks of 200 L (n=15 fish per tank) and four of 400 L (n=30 fish per tank) for the experimental setup. One week before the start of the experiment, fish were fed a HFD *ad libitum*. The identified level of satiation was then used as the standard ration in the experimental trial. The initial weight of the animals was 80.81 ± 1.43 g. The diet consisted of a high-lipid formulation (24% of dry matter) with 23 MJ/kg of digestible energy, where 50% of the total oils were derived from rapeseed oil. Furthermore, this diet was formulated either in the absence (HF) or presence (HF+HT) of a HT-rich extract from olive juice (HIDROX®, 0.52 g HT/kg feed) provided by Oliphenol LLC. (Hayward, CA, USA). The diets were administered daily using the determined standard (ST) ration or a restricted (RE) one (40% reduction) over a period of 8 weeks. Each experimental group was represented by three tanks, one of 400 L and two of 200 L. The daily feeding ration was adjusted every two weeks based on the fish's body weight. At the end of the trial, biometric data, plasma and tissue samples were collected. Plasma levels of insulin-like growth factor 1 (IGF-1) and metabolites were evaluated, while the expression of genes related to somatic growth and lipid metabolism was analyzed in the liver, white muscle, and/or bone. Additionally, lipid content and protein levels of the fatty acid transporter CD36 were measured in liver and white muscle.

Table 1. IGF-1 (ng/ml) and FFA (mg/dl) plasma levels of gilthead sea bream juveniles fed with a high-fat diet in the absence (HF) or presence (HF+HT) of hydroxytyrosol (0.52 g HT/kg feed), at a standard (ST) (3% biomass/tank) or restricted ration (RE) (40% daily reduction) for 8 weeks.

	HF_ST	HF_RE	HF+HT_ST	HF+HT_RE	D	R	D*R
IGF-1	3.88 ± 0.13^{a}	4.70 ± 0.12^{bc}	4.97 ± 0.10^{c}	4.44 ± 0.11^{b}	**0.001**	0.225	**<0.001**
FFA	6.42 ± 0.47^{a}	4.45 ± 0.42^{b}	4.28 ± 0.23^{b}	4.98 ± 0.38^{b}	**0.047**	0.109	**0.002**

Data are shown as Mean ± SEM (n=10). Statistical differences are indicated in 3 components: diet (D), ration (R) and interaction (D*R), using two-way ANOVA ($p < 0.05$, shown in bold). When the interaction between the two factors (D*R) was significant, comparisons among groups where analyzed by a Tukey's post-hoc test and significant differences are indicated by different letters ($p < 0.05$).

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Results and Discussion

After 8 weeks of experimental trial, the inclusion of the HT-rich extract to the diet did not change body weight or any of the other biometric parameters evaluated (body length, condition factor, and hepatosomatic and mesenteric fat indices), regardless of the feeding ration. However, plasma levels of IGF-1 and free fatty acids (FFA) were affected by diet and the interaction between factors (i.e., diet and feeding regime). Specifically, the highest circulating IGF-1 levels, along with the lowest FFA levels, were observed in fish fed the diet containing the extract (HF+HT) at the standard feeding regime compared to the other three groups, suggesting potential anabolic and fat-lowering effects (Table 1). Moreover, in line with this, although it was not statistically significant, it is worth noting that the same group of fish exhibited the lowest hepatic lipid percentage. In terms of gene expression, those same animals exhibited, in white muscle, higher mRNA levels of members of the GH-IGF axis (*igfbp-5b*, *ghr-1*, and *dock5*), in parallel with lower mRNA levels of fatty acid uptake and transport markers (*lpl*, *fatp1* and *fabp1*) in the liver, which agrees with an improved growth potential and a protective role under a lipid overload context by this polyphenol. Therefore, it could be hypothesized that a longer duration of the experimental treatment could have resulted in more pronounced effects. On the other hand, fish subjected to a 40% feed restriction showed a better feed conversion ratio and lower levels of HDL, LDL/VLDL, and triglycerides in their plasma than the ones that received the standard ration. Overall, HT appears to be a promising candidate for incorporation into functional diets for gilthead sea bream, as it shows the potential to positively impact lipid metabolism and enhance the growth capacity of the musculoskeletal system in this species.

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MOLECULAR SPECIES IDENTIFICATION OF ADRIATIC OYSTERS

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Introduction

The European flat oyster (*Ostrea edulis* Linnaeus, 1758), one of the most valuable bivalve species, is traditionally cultured in the Adriatic sea. Since 1970s, overharvesting, habitat loss and parasitic diseases (bonamiosis, marteiliosis) caused a rapid decrease in its production (Ezgeta-Balić et al., 2020; Šegvić-Bubić et al., 2016)under aquaculture conditions, feeding interactions between them were investigated in a highly productive environment of Lim Bay (Adriatic Sea. To compensate for the losses, the Pacific oyster (*Crassostrea gigas* Thunberg, 1793) was introduced to many European countries (Grize & Héra, 1991). The Pacific oyster has not been cultured in Croatia to date, but it has established self-sustaining wild populations with possible negative effects on the indigenous fauna (Diederich, 2005; Markert, Wehrmann, & Kröncke, 2010)southern German Bight. C. gigas settles predominantly on intertidal Mytilus-beds (M. edulis L.. The aim of this study was to find evidence of a putative hybridization between the two species in Croatia.

Materials and methods

European flat oyster samples were collected from three different locations in Croatia (Lim Channel n=36, Medulin Bay n= 33, Savudrija n= 35) and one from Ireland (n=12). Pacific oyster samples (n=12) were collected only from Ireland. Phenotypic and genotypic species identification was performed and the results were compared. Muscle tissue samples were collected and stored in 98% ethanol at -20 °C until DNA isolation, which was performed with an E.Z.N.A. Tissue DNA Kit following the manufacturer protocol. A multiplex PCR was used for the amplification of the 5S rDNA with four primers (ED1, ED2, CR1, CR2) published by Cross & Rebordinos (2006) using QIAGEN Multiplex PCR kit following the manufacturer protocol. The resulting PCR products were separated by agarose gel electrophoresis and the expected product size was 800bp and 400bp for *O. edulis* and *C. gigas*, respectively.

Results

We could not detect any deviations between the genotypic and phenotypic results. All *O. edulis* samples (n=116) amplified only one product with the expected size of 800bp and all *C. gigas* samples (n=12) amplified one product with the expected size of 400bp.

Discussion and conclusion

Our results do not support the hypothesis that hybridization took place between the two species in the Northern part of the Adriatic sea, near the Istrian peninsula, which might be explained by the differences in their reproductive cycle. We conclude that this novel multiplex PCR with the previously published primers might be suitable for DNA based species identification in oysters.

Acknowledgement

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THE ADAPTABILITY OF ATLANTIC SALMON (*Salmo salar*) TO NOVEL TURBULENT ENVIRONMENTS

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Introduction

Atlantic salmon (*Salmo salar*) is the most important farmed species globally. In Norway for example, exports generated USD 11 billion in 2020. The government predicted a doubling of production by 2030, however this may be restricted due to the lack of suitable farming sites and limitations to the number of licenses issued. Consequently, alternative sites are increasingly being considered by industries in Norway and other countries. One popular alternative includes the attractive 'offshore' locations in areas further from the coastline, exposed to high current speeds and strong wave action.

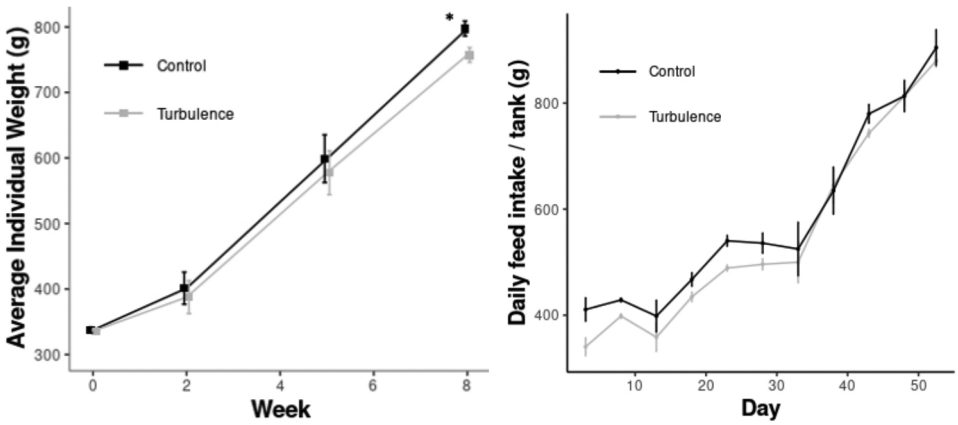
Yet, the effect that heavy wave action will have on fish reared within cages in these novel environments remain unknown, raising questions about the Atlantic salmon's coping ability and welfare.

The purpose of this original study was to investigate, in a fully controlled environment, the response of Atlantic salmon exposed to chronic water turbulence by measuring growth performance, behavioral conditioning, welfare scores, stress physiology, skeletal deformities, and swimming capability.

Materials and Methods

For 8 weeks, post-smolt Atlantic salmon were grown in three control and three treatment tanks at relevant farm stocking densities. Each treatment tank consisted of two tailor-made 100L buckets attached to a water pump, that when filled (approx. every 23s), tipped over back into the tank creating water turbulence. Appetite was monitored daily and various behavior traits were scored throughout the trial. At weeks 2, 5 and 8, a sub-sample of 48 fish were obtained to measure size, blood parameters and score welfare. At the completion of the trial, all fish were re-measured. Another sample of fish underwent a swim trial where the critical swimming speed (U_{crit}) was determined. A final sub-sample was analyzed with radiology to detect skeletal deformities.

Figure 1A. Left panel shows the average weight (g) of the Atlantic salmon. **Figure 2B.** Right panel shows the average of 5 daily recordings of feed intake (g of dry feed), averaged per group of three replicated tanks.



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Results

Atlantic salmon were able to cope and grow in constant turbulent conditions. A small 40g difference was observed between the groups (Fig.1A). The condition factor was similar; 1.27 in the turbulence treatment compared to 1.28 in the controls.

Appetite in the treatment tanks was 14% to 7% lower during the first 4 weeks of the trial, before reaching similar levels as the controls (Fig.2B). This variation coincides with a behavioral change observed. During the first 4 weeks of the trial, individuals in the treatment tanks swam less coherently (<90% standing in the current). However, after this period, the treatment and control groups were both swimming consistently (>90% standing in the current). No large difference in the total welfare score was observed.

Ongoing analysis of stress physiology, swimming performance and skeletal deformities will also be presented. These will provide further important insights into how well Atlantic salmon adapt, cope and develop in turbulent farm environments.

With the already analyzed data, both groups appear to have had similar coping, despite the slight differences observed during the first weeks of the trial. This suggests that Atlantic salmon have the capability to acclimatize to an environment with constant turbulence without compromising welfare or growth to a large extent. The difference in final size remains unexplained. It may have been caused by the difference in appetite at the start of the trial or by increased energy expenditure due to the efforts needed to swim in waves. Alternatively, if the trial had been running for longer, the weight differences might have disappeared due to compensatory growth.

This work is the first attempt to experimentally study the effect of chronic turbulent environments on a cultured fish species which provides valuable novel knowledge in the industry-wide efforts to establish responsible offshore aquaculture practices. However, as a land-based experiment, it does not fully mimic the true offshore conditions at a commercial production scale. Nevertheless, this work clearly demonstrate that Atlantic salmon are a robust species and have the capacity to adapt well in novel-high energy environments without suffering substantial reductions in welfare or long-term production performance.

MERCURY CONTENT IN EUROPEAN EEL (*Anguilla anguilla* Lineaus, 1758) FROM THE NERETVA RIVER DELTA

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Introduction

The European eel populations across Europe have been declining since the mid-20th century, and are now considered critically endangered (ICES, 2020). This species is a catadromous, migratory species and the success of migration largely depends on the physiological condition and sufficient reserves of fat that provide the energy for the journey (Righton and Metcalfe, 2011). One of the factors affecting the health of the migrating eels is the accumulation of contaminants such as mercury (Polak-Juszczak and Robak, 2015). Mercury is commonly used in household, industrial, and medical products (Ahmed et al., 2020). It can be transported into rivers where bacteria convert it into a soluble organomercury which enters the food web and accumulates in organisms over time (WHO, 2020). Since the eel has a long life cycle it can accumulate significant amount of mercury through feeding which lowers its physiological condition but also poses a health risk for human consumption (Polak-Juszczak and Robak, 2015). The aim of this research was to determine the mercury level in European eels caught in the Neretva river delta.

Materials and methods

The fish were sampled in the winter, spring, summer, and autumn of 2021 from Neretva river delta. Samples were weighed, total length was measured, and life stage was determined according to Acou et al. (2005). A portion of dorsal muscle without skin, was taken for the analysis of the mercury concentration that was determined using Direct Mercury Analyzer (DMO). Spearman`s correlation was used to test correlation between mercury concentrations and length or weight of the eels. The differences among groups for length, weight and mercury concentrations were tested using ANOVA. Post-hoc analysis with Tukey`s test was done to determine which groups statistically differ from each other.

Results

A total of 94 specimens of European eel were caught, 13 of which were silver and 81 yellow eels. Seasonal averages of weight and total length are given in Table 1. Seasonal length and weight differences among groups were determined to be statistically significant using ANOVA ($F=2.98$, $p=0.023$ and $F=3.51$, $p=0.01$). Using post-hoc test statistically significant differences for total length were found between yellow eels from the summer and silver eels ($p=0.021$), and yellow eels from the autumn and silver eels ($p=0.040$). Significant differences in weight were determined between yellow eels in the spring and silver eels ($p=0.023$), yellow eels in the summer and silver eels ($p=0.015$) and yellow eels in the autumn and silver eels ($p=0.018$).

The mean recorded mercury concentrations are given in Table 2. No statistically significant differences were found among groups using ANOVA test ($F=2.126$, $p=0.085$). No correlation was found between mercury concentrations and total length or weight of the eels.

Discussion and conclusion

Mercury in different forms (elementary, inorganic, and organic) can cause developmental and hormonal disorders such as lower growth rate and induced sex change (Crump and Trudeau, 2009). Polak-Juszczak and Robak (2015) stated that the negative effects of mercury in eels can be seen at concentrations above 0.5mg/l. In this research the highest concentration in a single specimen was 0.55mg/kg, while in the rest the concentrations was below 0.29mg/kg. No fish were found with the concentration above the maximal permitted level. Therefore, mercury was detected in only one specimen of eel in a concentration high enough to pose a danger for its health and all fish were safe for human consumption.

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Table 1. Total length and weight of the two life stages of eels from river Neretva delta throughout the seasons (n-number of specimens, SD-standard deviation)

Season	Life stage	n	Total length (cm)				Weight (g)			
			Min	Max	Mean	SD	Min	Max	Mean	SD
Winter	Yellow	16	31.90	58.70	40.92	7.59	50.00	340.00	127.50	73.61
Spring		38	33.50	60.30	41.24	5.81	57.00	394.00	130.21	68.22
Summer		13	29.40	51.80	38.31	5.32	47.00	267.00	101.00	56.79
Autumn		14	29.10	56.40	39.21	7.11	41.00	289.00	105.93	62.28
	Silver	13	36.20	73.00	47.18	11.69	73.00	861.00	235.08	235.12

Table 2. Mercury concentration throughout different seasons in the two life stages of eel from river Neretva delta

Season	Life stage	Mercury concentration (mg/kg)
		Mean ± SD
Winter	Yellow	0.14 ± 0.04
Spring		0.09 ± 0.06
Summer		0.13 ± 0.07
Autumn		0.12 ± 0.06
	Silver	0.16 ± 0.17

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MARINE BACTERIA FROM HATCHERY PHYTOPLAKTON CULTURES: FIRST APPROACH TO THE POPULATION IN THE HIGH TEMPERATURE SEASON

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Introduction
Phytoplankton cultures are essential in mollusc cultures, as they constitute the completing the diet in the complete cycle, i.e., from broodstock to seed. Different types of cultures were maintained to produce enough quantities to sustain production.

The bacterial load in microalgal cultures is high, and they are directly and continuously supplied to the bivalves. Therefore, microbiological knowledge should be useful for hatchery management.

In previous work, we demonstrated that microalgal cultures carry vibrios, including potential marine aquaculture pathogens, with some species adjusted to seasonal patterns, mainly associated with temperature. We also stated that the vibrio load is highly variable during the year, but also over the course of a week.

By other hand, we have also established that active cultures of different microalgal species are able to reduce the load of pathogenic vibrios, until very low levels or even under the detection threshold.

We now present the preliminary results of the study of marine bacteria in phytoplankton, focusing on the spring-summer season, when temperatures rise and vibrio populations in the food supplied to cultures are high in quantity and diversity.

Materials and methods
Phytoplankton is developed in Centro de Cultivos Mariños-CIMA (Ribadeo. Xunta de Galicia), following the routine protocols of the installation for the two types of cultures.

a) Small volumes, 6 l. flaks, kept in the isothermal chamber (19±1ºC) under constant illumination and CO_2-enriched aeration. Seawater is filtered and autoclaved, and enriched with Algal-1 medium.

b) The continuous culture system ("Sea Salter Continuous Algal Production Systems"), located in the greenhouse, consists of polyethylene bags with a volumetric capacity of 400 l. each. It is based on a constant supply of seawater with the nutrient medium (John Bayes Medium) and a constant harvest. The seawater is sterilized by pasteurization. The lighting is natural light supplemented with artificial light (leds), adjusted to a photoperiod of 18 h day:6 h night. There is continuous aeration in each of the bags and there is an addition of CO_2 that is controlled by measuring the pH. The inoculation of the bags is done with cultures from the isothermal chamber.

The cultivated microalgae are *Isochrysis*, *Diacronema*, *Tetraselmis* and *Chaetoceros*.
Microbiological samples were taken and immediately processed on site, spread on plates of Marine Agar (MA), for marine heterotrophic bacteria, by a standardized method developed in the course of this sampling, to obtain enough information with a simple processing, easy to execute by the installation personnel.

Bacterial isolation, preservation and identification of isolates were carried out by USC, following the methodologies described in Prado et al. (2014).

Results and Discussion
In this first approach, randomly selected flasks/bags of each microalgal species were sampled in June-July, as this is when *Vibrio* episodes begin in the continuous culture, acting as "asymptomatic carrier" of larval pathogens.

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Preliminary results, obtained until the time of writing, are mainly for the cultures in isothermal chamber. They showed the frequent presence of γ-Proteobacteria, mainly *Alteromonadaceae*. α-Proteobacteria were also detected, belonging to different families: *Maricaulaceae*, *Ahrensiaceae*, *Devosiaceae*, *Roseobacteraceae* and *Rhodobacteraceae*. And *Flavobacteriaceae* (Bacteriodota) were other important component of the microbiota in these cultures.

The first results of bacterial isolates from continuous cultures showed similar taxa, with presence of Alteromonadales (γ-Proteobacteria), Rhodobacterales (α-Proteobacteria) and Flavobacteriales (Bacteriodota).

These results are being completed, with isolates from both types of cultures, to obtain an overview of the marine bacteria present.

The microbiota of both systems will be compared to determine if there are differences that could explain the proliferation of vibrios in the CCS. In addition, the presence of potential algicide bacteria will also be studied.

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BIOFILM FORMATION BY PATHOGENIC VIBRIOS ON HATCHERY SURFACES

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Introduction
Episodes of mortalities caused by vibrios occur repeatedly in bivalve hatcheries, affecting all the cultured species. In our studies about the dynamic of these microorganisms in the installation, one important aspect is their persistence along the season and possible re-emergence. It is known that many microbes persist attached to surfaces with a structured biofilm ecosystem, where they function as an integral part of a population, obtaining ecological advantages.

In this work we demonstrated the ability of pathogenic vibrios of forming a biofilm in the surfaces of culture tanks.

Materials and methods
The assays were carried out at Centro de Cultivos Mariños-CIMA de Ribadeo (Consellería do Mar, Xunta de Galicia). The experiments were designed to optimize the obtaining of practical information in the hatchery, minimizing risks to the facility and enabling direct observation.

Pieces of used culture tanks were placed in containers with the seawater supplied to larval cultures and aeration. Each container was inoculated with a larval pathogen: P1 *Vibrio neptunius* 145.98, P2 *V. ostreicida* PP-203, P3 *V. europaeus* PP-638, P4 *V. bivalvicida* PP2-605 and P5 *V. splendidus*-like PP-58.

A protocol for scannning electron microscopy was developed and optimized. Samples were analyzed in FESEM Ultra Plus (ZEISS, Germany), at 3Kv, to obtain surface images using detectors SE/InLens (secondary electron detectors), depending on the information we needed. Samples were metallized with iridium to work in high vacuum.

Results and Discussion
All the pathogens formed biofilms on tanks surfaces under culture conditions.

Biofilms, assemblages of microorganisms attached to a surface, have ecological advantages versus individual cells (Davey and Toole, 2000): ability to acquire transmissible genetic elements at accelerated rates, acquisition of antibiotic resistance, virulence factors, and environmental survival capabilities. Biofilm-associated cells are more resistant to many toxic substances such as antibiotics, chlorine and detergents (Watnick and Kolter, 2000).

Therefore, biofilm formation should be taken into account as a pathogen resistance strategy in aquaculture facilities. In fact, *Vibrio ostreicida* PP-203 was isolated from tank surfaces in the nursery during recurrent mortality episodes when cultured bivalves reached post-larval stage (Prado, 2007), which could be explained by these structures and the re-emergence of the pathogen. The formation of vibrios biofilms has also demonstrated in phytoplancton tanks has also been demonstrated, recolonizing the microalgal mixture even after the source was eliminated (Prado et al., 2019). In the case of bivalve hatcheries, successive cultures of different species in tanks and the lack of specificity of larval pathogens, make the fact of persistence and re-emergence a high risk for production.

The ability of all the pathogens tested points out the need to consider these structures as a potential hazard in aquaculture production and, therefore, should be included as a target for cleaning protocols.

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CROSS-CULTURAL STUDY AND EVALUATION OF CONSUMER ACCEPTANCE OF GILTHEAD SEABREAM FILLETS (*Sparus aurata,* L.) AFTER MILD PROCESSING VIA OSMOTIC DEHYDRATION FOR SHELF-LIFE EXTENSION

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Introduction

Aquatic foods, increasingly recognized for their key role in food security and nutrition, have reached an all-time record of 214 million tonnes of production (EUMOFA, 2022). However, 30-35% of fish production is being wasted globally, mainly due to their perishability (FAO, 2002). There is a market demand for safe and healthy seafood products with longer shelf-life but also high sensory quality. Osmotic dehydration is a low-energy-consuming, mild treatment that has been proven to extend fish shelf-life (Tsironi et al., 2009; Alexi et al., 2020). The current study explored the effects of mild processing for shelf-life extension of gilthead seabream fillets stored at 2°C in two markets, Greece (GR) and Denmark (DK).

The study aims to evaluate consumer hedonic responses and associate those to the perceived sensory characteristics of the fillets from a consumer perspective to uncover country-specific hedonic drivers and barriers. Furthermore, it is being investigated whether consumers' background characteristics, such as sociodemographic, fish consumption habits and attitudes affect liking and sensory perception.

Materials and methods

The study was conducted in GR and DK, with approximately 80 consumers per country. The samples were prepared and served under the same conditions. The fillets sampled for the consumer study were for the Control fillets D1 and D6, and for the Treated (via osmotic dehydration) fillets D1, D6 and D9 of commercial shelf life (corresponding to 1, 6 and 9 days of chilled storage, respectively). Liking was evaluated for each sample via the 9-point hedonic scale (anchored "Dislike extremely" to "Like extremely", with a neutral middle point), subsequently the consumers were asked to provide quick profiling via the Check-All-That-Apply (CATA) sensory method. Besides the hedonic and sensory responses measured, at the end of the survey the consumers were also asked about sociodemographic as well as attitudinal and behavioural information regarding fish and shelf life. Additionally, the effect of mild processing on the fillet's quality was determined by microbial enumeration including total viable counts, *Pseudomonas* spp. and *Enterobacteriaceae*.

Results

Examining the results combing the profiling via CATA and hedonic responses, the consumers in the two countries showed different hedonic drivers and barriers, which were specifically associated with sensory characteristics that they perceived on a country basis in the fish fillets. On a country level, the Greek consumers did not discriminate their hedonic responses based on the treatment but mainly on the level of freshness. On the other hand, the Danish consumers preferred the osmotically treated fillets. Besides the differential liking responses, the consumers in the two countries used different attributes to describe the sensory characteristics of the samples, especially for the Control fillets as well as Treated D9 fillets. In specific, for the Greek consumers the Control fillets were associated with marine flavour and juicy texture whereas for Danish ones with bitter and metallic taste; in their turn these can be perceived as positive and negative characteristics acting as hedonic drivers and barriers, respectively.

Discussion

A clear hedonic driver for Greek participants is marine flavour which is perceived in the Control fillets only in Greece, whereas for the Danish participants, hedonic driver is the umami taste that is perceived mainly in the osmotically treated fillets, and only in Denmark. The observed cross-cultural differences can be associated with familiarity with the species as well as culinary traditions and fish consumption patterns and indicate a high potential for the treatment in the Danish market.

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DEVELOPMENT OF A BIODEGRADABLE PH-SENSITIVE INDICATOR FOR GILTHEAD SEABREAM (*Sparus aurata*) FRESHNESS MONITORING

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Introduction

Seafood products, increasingly recognized for their key role in food security and nutrition, have reached an all-time record of 214 million tonnes of production (EUMOFA, 2022). However, high perishability of fish and seafood products calls for novel processing and packaging methods (Tsironi et al., 2018). As safety and freshness is considered a priority for fish production and consumer acceptability (Chun et al., 2014), the development of novel technologies and systems intended to ensure safety and continuously monitor quality and shelf life at (Kerry et al., 2006). Smart packaging consists of systems capable of providing consumers and stakeholders with information regarding food quality from production up to the consumption (Mohebi et al., 2015). In such a way, freshness of fish can be monitored during transportation, retail display, and domestic storage (Kerry, 2012). The development of smart materials would also contribute to the seafood waste reduction which accounts for 30-35% of total fish production (FAO, 2022). The aim of the study was to design a biodegradable smart packaging system for the evaluation of fish freshness via a pH-sensitive colour response. The application of the developed smart system on gilthead seabream (*Sparus aurata*) fillets during storage at refrigerated conditions was evaluated.

Materials and methods

The pH-sensitive indicator was prepared by the incorporation of methyl red dye in a starch-cellulose paper. In order to evaluate the sensitivity of the fabricated indicator to the ammonia vapor, ammonia sensitivity test was performed according to Zwang et al. (2019) using NH_3 solutions with concentrations varying from 0.002 -1 M. This test was acquired to show the potential of color response toward the volatile nitrogen components such as total volatile basic nitrogen (TVB-N), which are produced in different amounts during fish spoilage. Color response performance of pH-sensitive indicator was evaluated at different pH values at a range of pH from 2 to 9. L,a,b values were measured and ΔE (total color difference) was calculated. With ΔE>3, the color change was visually observed (Sailer et al., 2014). Application of the indicator into the fish packaging was performed by sticking the indicator inside the headspace of sterilized packaging sealed bags containing 50 g of gilthead seabream fillet. The bags were stored in 4° C for 10 days for shelf life evaluation. Chemical and microbiological attributes of fillets and ΔE values of the indicators assessment followed. pH, TVB-N content, packaging gas composition and total viable count (TVC) enumeration were performed in duplicate on each day of storage.

Results

Examining the results of the NH_3 sensitivity test, the sensitivity of the fabricated indicator increased progressively with time (0-24 min) and reached the highest sensitivity value of 35.2% at 24 minutes of exposure to ammonia solution 0.02 M. Regarding the color response test, the indicator appeared red-dark pink at pH values 2-4, turned light pink at pH values 5-6 and finally changed to light orange-yellow at pH values 7-9, exhibiting its capability to identify changes in pH (ΔE>3). The initial TVC of fish samples was 3.6 $\log_{10}$ CFU/g (day 0) and the threshold TVC value of 7 log10 CFU/g was exceeded at day 7 of storage. The color of the indicator changed during storage at 4°C from red-dark pink (day 0) to light pink-light orange (day 7). ΔE (total color difference) values of the indicator increased and reached the value of 17 (ΔE>3) at day 7. Nitrogen concentration within the sealed package increased from 79.25% at day 0 to 89.05% at day 7. pH of fish flesh exhibited a decrease from 6.73 (day 0) to 6.37 (day 3) and subsequently increased to 6.66 at day 7.

Discussion

Preliminary results indicate that the fabricated smart indicator based on methyl red dye and starch-cellulose paper showed satisfactory change of color in contact with various pH buffer solutions, ammonia vapor concentrations and spoilage of fish. Further experimental procedures (e.g., smart film stability, morphology analyses etc.) would lead to the development of an integrated smart packaging system for continuous monitoring of fish freshness in the actual supply chain.

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Acknowledgment
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INSECT MEAL AS FISHMEAL REPLACEMENT IN DIETS FOR EUROPEAN SEA BASS (*Dicentrarchus labrax*): IMPACT ON INNATE IMMUNE STATUS AND OXIDATIVE STRESS

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Introduction

Insect meal (IM) has demonstrated an enormous potential as an alternative protein source to fishmeal (FM) in diets for European sea bass (*Dicentrarchus labrax*) (Basto et al., 2021 and 2022), one of the most important marine fish species in Mediterranean aquaculture. However, when IM is included at high levels to totally replace FM, alterations in the intermediary metabolism of sea bass are observed, which may compromise the health status of fish (Basto et al., 2023). In this connection, the impact of total FM replacement by an IM on the innate immune parameters and oxidative stress of European sea bass was assessed.

Material and methods

A FM-based diet with 47% of protein and 20% of fat was formulated and used as a control (CTRL). Two other isoproteic and isolipidic diets were formulated to replace 50 and 100% of FM by defatted *Tenebrio molitor* larvae meal (TM50 and TM100, respectively). Each diet was assigned to quadruplicate homogeneous groups of 15 fish (69 ± 5 g) that were fed until apparent satiation for 16 weeks. Fish were subjected to a 12-hour light/12-hour dark photoperiod regime and kept in a recirculating saltwater system (35‰, 22 ± 1 °C). By the end of the trial, 3 fish per tank (12 per dietary treatment) were sampled for analysis of health status-related parameters, and other 3 fish per tank were exposed to an acute stress episode consisting of 1 min air exposure followed by 1 h of recovery before sampling. Hematologic profile analysis (i.e., hemoglobin, hematocrit, and red and white blood cells), plasma metabolites (i.e., cortisol, lactate, and glucose) and immune parameters (i.e., total peroxidase, lysozyme, and alternative complement pathway activities), and hepatic oxidative stress (i.e., catalase, superoxide dismutase, and glutathione reductase, S-transferase and peroxidase) were evaluated in all fish sampled.

Results

A clear response to acute stress was observed, regardless of dietary treatment, by a significant decrease in hemoglobin, hematocrit and red blood cells, and a significant increase in almost all evaluated humoral parameters. On the other hand, the activity of total peroxidase and superoxide dismutase was significantly affected by the dietary treatments, being highest in fish fed TM100, but not affected by stress condition. Considering the canonical discriminant analysis, a clear separation between non-stressed and stressed fish was evidenced (Fig.1A). Based on the significant Mahalanobis distance of each group multivariate mean (centroid), it was possible to observe that both non-stressed and stressed fish fed TM100 were significantly different from those fed CTRL and TM50 (Fig. 1A). Non-stressed fish fed TM100 were positively loaded by hematocrit and red blood cells (Fig. 1B), whereas stressed fish fed the same dietary treatment were positively loaded by glucose and lactate levels, as well as by mean corpuscular volume (Fig. 1B).

Discussion and conclusions

The results of the present study demonstrated that the partial substitution of FM by IM did not affect innate immune parameters and oxidative stress of sea bass. Contrarily, total FM replacement by IM seems to compromise fish health status. The increased total peroxidase activity and superoxide dismutase, associated with a trend to higher glucose and lactate levels in fish fed TM100, indicate a counteraction to the excess of superoxide radicals and mobilization of fish energy reserves to overcome the stress condition. To better understand the impact of FM replacement by IM on fish immune response and oxidative stress it would be of high interest to deepen knowledge on their underlying mechanisms upon other challenging rearing conditions (e.g., pathogen exposure, chronic stress).

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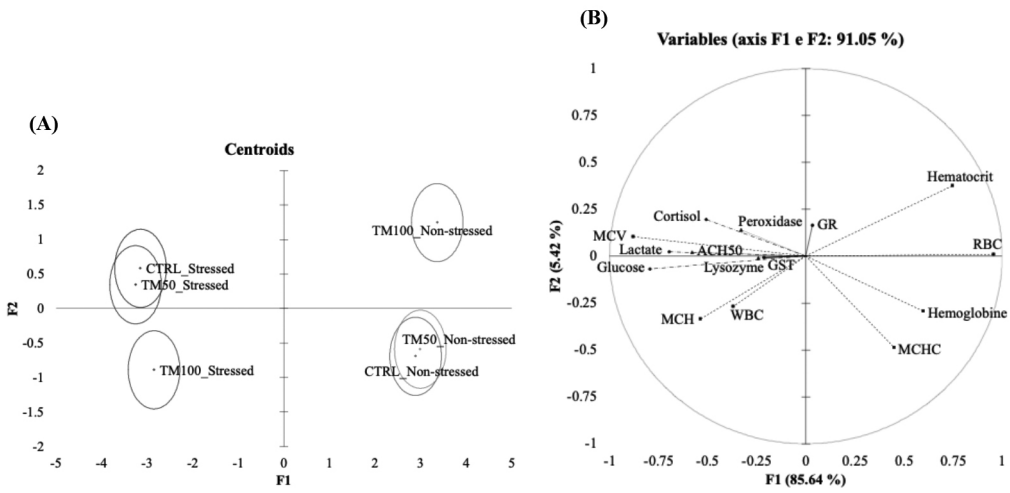

Fig 1. Canonical discriminant analysis of European sea bass fed experimental dietary treatments. Colored circles represent clusters and data distribution per group (A). Correlation variables/factors (factor loads) for two main discriminant functions (F1 and F2).

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COMPARATIVE GENOMIC REGULATION IN THE FARMED SALMONIDS ATLANTIC SALMON AND RAINBOW TROUT

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Introduction

The European project AQUA-FAANG (https://www.aqua-faang.eu/) has the primary goal of elucidating mechanisms of genomic regulation in the most commercially important fish species in European aquaculture. This includes two salmonids, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). We generated extensive standardized functional genomic data in both species, specifically RNA-Seq, ATAC-Seq, and ChIP-Seq spanning 14 developmental stages and 8 tissue types. This data was used to construct genome-wide regulatory annotations for both species, allowing us to explore both conserved and divergent genomic regulatory features, including in relation to the salmonid-specific whole genome duplication (ssWGD) event that occurred ~100 million years ago (Lien et al. 2016; Gundappa et al. 2022). Our results provide novel insights into the expression and transcriptional regulation of duplicated genes, transcription factor binding site (TFBS) usage and Hox gene cluster evolution.

Methods

We used ChIP-seq to detect histone profiles (H3K27ac, H3K4me1, H3K4me3, H3K27me3) and ATAC-seq to measure chromatin accessibility. We integrated the data from these assays with ChromHMM (Ernst and Kellis, 2012) to characterize chromatin regulatory states across the genome. From this, we annotated twelve chromatin states common between Atlantic salmon and rainbow trout and for developmental stages (late blastulation, mid gastrulation, early, mid and late somitogenesis) and adult tissues (brain, liver, muscle). By using gene tree and synteny information, we identified ohnologs (duplicates resulting from the ssWGD) and ortholog relationships between Atlantic salmon and rainbow trout. Streamlining this information to a list of 2:2 ohnologs genes (Atlantic salmon/rainbow trout tetrad, where both genomic copies of the same gene is conserved in each species), we investigated gene expression differences by clustering the gene expression of each tetrad. GimmeMotif (van Heeringen & Veenstra, 2011) was used to reveal enrichment in TFBS, targeting promoter and enhancer regions to compare the conservation of TFBS usage across ontogeny between the two species. To inform on developmental regulation in salmonid genomes, we curated the expression and regulation of the highly expanded Hox gene clusters in both species.

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Results

After characterizing chromatin states of Atlantic salmon and rainbow trout, we focused on promoter and enhancer regulatory elements. Enrichment of TFBS in these regions revealed that active enhancer regions exhibited 141 common TFBS across species, and 46/53 species-specific TFBS. Conserved vertebrate TFBSs including POU5F1, Sox and Hox were identified for both species during developmental stages, alongside conserved TFBS specific to particular tissues, e.g. NRF1 for brain, PPARG for liver or MEF2B for muscle. These findings illustrate an overall conservation of TFBS usage for both salmonids. Clustering of expression of ohnolog-tetrads revealed five distinct categories of genes. A subset of genes fell into the category where both copies of the same species is expressed differentially compared to the copies of the other species. Moreover, a group of ohnolog-tetrads displayed similar expression patterns between both species, with either both copies being co-regulated or either only one copy in each species. We anticipate that gene expression differences are associated with differences in regulatory activity of chromatin. By exploring the gene expression profiles of the different Hox genes cluster in both Atlantic salmon and rainbow trout, we observed that despite the duplication of all the clusters, the transcription of each of the copies were maintained, with the exception of the HoxA-b copy, which appeared to be lost in rainbow trout, and is evidently on the path to pseuodgenization in Atlantic salmon.

EDUCATION AND TRAINING IN AQUACULTURE: DEVELOPING SKILLS IN THE AUSTRIAN FISHERIES SECTOR

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Introduction

The aquaculture sector in Austria is relatively small compared to other sectors in Austrian agriculture, making it one of the smallest within the EU. In 2021, the total production of food fish in Austria reached 4,920 metric tons, as reported by Statistics Austria. This sector is primarily categorized into three major forms of production: salmonid farming, carp pond farming, and production in recirculating aquaculture systems (RAS). Despite its modest size, the Austrian aquaculture sector can rely on a fundamental basis of training and education, which is based on blended learning with a mix of face-to-face classes, practical lessons, and online and self-learning elements. The lectures are delivered by professionals from various fields, including economics, veterinary medicine, jurisprudence, and experienced practitioners. The vocational training in aquaculture primarily consists of three main components, offering comprehensive knowledge and skills to aspiring professionals in the field.

Skilled Worker in the Fishing Industries

The education is modular and can be completed within three years. The program covers topics such as salmonid aquaculture, carp pond farming and RAS, fish biology, lake management, freshwater ecology, fish health, and hydrology. The education in the special field of fisheries is conducted on behalf of the "Lehrlings- und Fachausbildungsstelle" of the Board of Agriculture for people throughout Austria. Subject-specific lessons take place at the institute in Scharfling, while general lessons and fish processing are conducted at the "abz Salzkammergut" in Altmünster. Similar to other professional guilds, the fisheries instruction follows a dual system, requiring apprentices to attend school in addition to their employment. The seven modules are conducted in rotation, allowing access to each module. The participants graduate after an exam by an examination board.

Master Craftsman – Master in Fisheries

On behalf of the Agricultural and Forestry Apprenticeship and Technical Training Center Upper Austria, fishery management master courses are centrally conducted for all of Austria at the Federal Office for Water Management. The 2.5-year training is modular, and entry is only possible for a complete module rotation. In addition to deepening fishing industry expertise, the training focuses on business and corporate management. Independent of the technical courses, the modules Instructor Course and Law & Agricultural Policy can be completed separately, with offerings available in all federal states of Austria. The individual modules are completed with an examination (written/oral). At the end of the training, there is a 5-hour written exam. A core element of the master craftsman training is the independent preparation of a master thesis in the specialist area of fisheries management, which is presented as part of a master craftsman examination before a commission.

Continuing Education and Basic Training

For individuals interested in aquaculture who do not wish to complete the skilled worker course, there is the option to join a basic training course. These courses consist of 40 hours and provide insights into the major concepts of fish production. The course is available for salmonid farming, carp pond farming, or RAS.

Further Information

For additional information on education and training in aquaculture in Austria, please visit https://www.baw.at/en/fish-and-water/education-and-training.html.

DEVELOPING A METHOD TO USE THE SETTLING VOLUME OF ZOOPLANKTON AS EASY CONDUCTIBLE MEASUREMENT FOR TJE NATURAL FOOD AVAILABILITY FOR COMMON CARP (*Cyprinus carpio*) IN PONDS

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Introduction

Common carp (*Cyprinus carpio* L.) bred in extensive carp pond farming in Austria rely on the natural food (zooplankton and benthic invertebrates) occurring in the ponds. The natural food, especially the zooplankton, provides the fish essential fatty acids and proteins (Dabrowski & Rusiecki 1983; Tocher 2003; Steffens 2011). In good managed carp ponds, the zooplankton genera evolve coherent with the life-stages of the carp. Carp fry mainly feeds on ciliates and rotifers, while adult carps feed on larger zooplankton genera, such as *Daphnia sp.* (Šusta 1887; Wunder 1968; Adámek et al. 2023). For good water quality and economically management, it is advisable to use additional feed, such as grains, as needed instead of following feeding plans given in the literature. As it is very difficult for carp pond farmers to assess the amount of zooplankton in the pond, we have been working on an easy way for measuring the zooplankton in situ..

The Settling Volume

To determine the right amount of additional grain feed it is essential for the carp farmers to measure the given amount of zooplankton (Schlott-Idl 1991). Thus, they can adjust their feeding strategy in such a way as the carp get a balanced diet and both, insufficient utilization and overexploitation of zooplankton are avoided. It is commonly known that fish has a major impact on the zooplankton populations in the pond (Hrbaček 1962). By balancing the amount of grain feed, predation on the zooplankton can be regulated (Steffens 2011). The first experiments to use the settling volume (SV) of large zooplankton (>1 mm) as measurement technique for the natural food amount for carps have been conducted in Austria in 1984. Daphnia can be sampled relatively easy, by using a 500 µm net. As they are especially important for adult carp diet, it makes sense to sample only larger zooplankton and disregard smaller ones.

To assess the SV, 5 L water samples on four different places at 0.5 m depth are taken from the pond with a Schindler trap. As already mentioned, we use a plankton net with a patch of 500 µm net on the bottom to catch only daphnia. After flushing the smaller plankton out, the daphnia are concentrated in the cod end. The zooplankton is killed with 20% formaldehyde and flushed into a volumetric tube with distilled water. After a short settling time the SV can be easily read from the scale on the tube. Schlott & Schlott (2001) showed that an abundance between 20 and 40 daphnia per liter can be considered as good amount for a stable population. Our research showed, that this abundance of daphnia has a SV between 0.2 and 0.55 ml. According to the SV, carp pond farmers can adjust their feeding strategy, what enables reduction of costs and needles nutrient input into the pond.

In contrary to the longsome commonly used way of counting zooplankton under the microscope, this method of measuring the amount of daphnia is an easy and practical way for every carp farmer to assess the stability of the zooplankton population. As the zooplankton is the major source of essential fatty acids and proteins (Dabrowski & Rusiecki 1983; Tocher 2003; Steffens 2011) and the additional grain feed provides the fish carbohydrates, a well-balanced diet containing these food sources guarantees healthy fish and in the end a high quality product.

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DEVELOPMENT AND HISTORY OF PONDS IN THE AUSTRIAN LANDSCAPE

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Introduction

Carp farming has been practiced in Austria since the 12th century, with a peak in the 16th century. Today it is concentrated in north-western Lower Austria (NUTS 3 region : Waldviertel) and southern and eastern Styria (NUTS 3 regions: Oststeiermark and Süd- und Weststeiermark). Ponds are important elements of the cultural landscape, but as the cultural landscape changes over the centuries, ponds disappear and reappear over time. Ponds play an important role in the landscape. They replace lost small water bodies, increase biodiversity and serve to retain and provide water in the landscape. With this in mind, it is worth looking back historically to explore the potential for restoring or revitalising old ponds.

Material and Methods

Digital georeferenced maps of the entire Austrian territory in WMTS format are available free of charge at www.basemap.de. Digital georeferenced historical maps in WMTS format (e.g. Franziszeiischer Kataster 19th century) are partly available free of charge via the federal provinces (e.g. Styria) or for a fee via the map service www.mapire.eu. On the basis of this map material, selected areas in the three NUTS 3 regions mentioned above were investigated with regard to their current and historical stock of ponds. In addition, one area in the NUTS 3 region Weinviertel was included in the study. The data were processed with the free open source software QGIS (www.qgis.org).

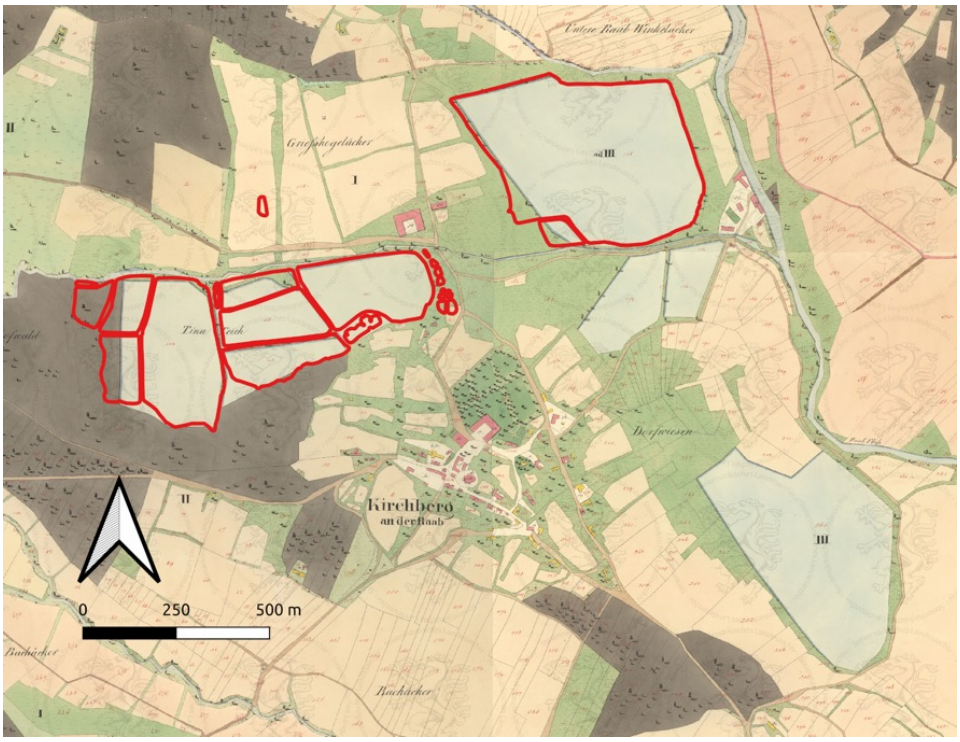

Figure 1: Historical map of Eastern Styria from the 19th century, supplemented by the current situation of the ponds (outlined in red). Data of the current ponds: Umweltbundesamt GmbH, CC-BY 4.0; Map source: Franziszeischer Kataster, Steiermärkisches Landesarchiv, CC-BY 4.0; Map created with QGis, BAW-IGF, 2023

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Results and Discussion

The evolution of the number of ponds can vary considerably from region to region. While in some parts of the NUTS 3 region Waldviertel the number of ponds has not decreased dramatically since the 19th century (e.g. municipality Litschau -3 ponds), the losses are higher elsewhere (e.g. municipality Schrems -12 ponds). In the municipality of Seefeld-Kadolz in the NUTS 3 region Weinviertel, not only have ponds been lost since the 18th century, but the remaining ponds have only a fraction of their original area. In most cases, the reason for the disappearance of ponds is a change in land use. For example, sheep or arable farming have replaced fish farming. In the NUTS 3 region of Eastern Styria there is a good example of how a still existing carp pond farm has changed since the 19th century (Fig. 1). In the 19th century the pond economy consisted of 8 larger ponds, today 3 of them have completely disappeared, while originally 2 ponds became a total of 6 ponds due to the construction of dams. In addition to showing the changes in the cultural landscape, the analysis of historical maps and current geography also allows the identification of former ponds that could be reconstructed, especially in the light of efforts to increase aquaculture production. In the Waldviertel region there are indeed examples of planned or realized re-establishment of ponds that have been abandoned since the 19th century.

PROACTIVE AND NON-INVASIVE PATHOGEN DIAGNOSTICS TO PREVENT THE SPREAD OF *Bonamia ostreae*

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The haplosporidian parasite *Bonamia ostreae* is one of the biggest issues facing European native oyster, *Ostrea edulis*, aquaculture and restoration. Measures to stop the spread of diseases in the United Kingdom have to date relied on the prevention of animal movement from disease positive to disease-free sites. However, these measures are not entirely successful and have seen recurrent failures in recent years, resulting in the gradual spread of *B. ostreae* across the UK. We tested the utility of protocols for pro-active pathogen diagnostics, combining a portable qPCR machine and field DNA extraction protocols with our experience detecting pathogens in disease challenge systems. We used a stepwise process of overnight incubation, sampling of substrates from the tank system, simplified DNA extraction and rapid diagnostic of the presence of DNA. Here we will present the concept of fully useable system that can be employed on the site of the shellfish farm. The process is now validated against a set of traditional diagnostic techniques including tissue qPCR and histopathology. This rapid, cheap and simple process allows native oyster farmers and restoration practitioners to make proactive decisions on whether to move animals, based on their up-to-date health status, and thus take full control of the risks associated with animal movement.

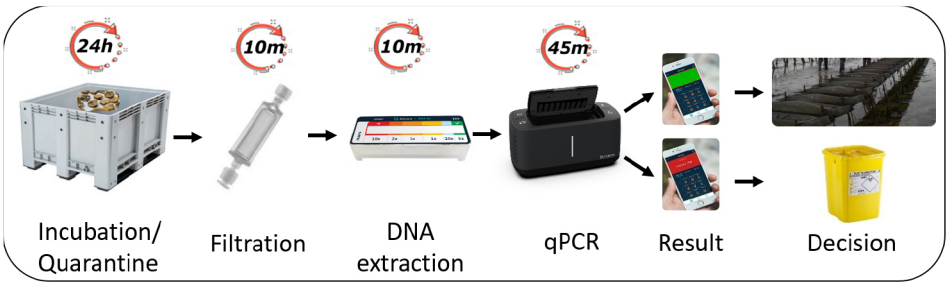

Figure 1. Schematic of protocol for non-invasive diagnostic process.

MariFish Inc: INCUBATOR AND LIVING LAB TO SUPPORT START-UPS IN SUSTAINABLE SEAFOOD PRODUCTION

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MariFish Inc. is a newly established living lab and incubator for start-ups in the fields of aquaculture and innovative fishery located in Ostend, Belgium. More specifically, MariFish Inc. brings together entrepreneurial expertise with the commercial support of the Flemish fish auctions. In addition, it is backed by several scientific institutions with a strong track record in aquaculture (Ghent University) and innovative fisheries techniques (ILVO). It offers dedicated, flexible, food safety compliant growing space for aquafarmers and supplies large quantities of good quality sea water, ready to use. In addition, there is the option of extensive lab support for the tenants and participants of the incubator. As such, this new initiative aims at lowering the entrepreneurial risk for newly established aquaculture ventures.

Initially, the incubator and living lab target the incubation of start-ups active in sustainable seafood production, for example:
* Onshore farming of seaweed, fish, crustaceans and shellfish
* Onshore processing of mariculture production at sea, e.g. making ready to sell
mussels/ oysters
* Watering of mariculture products
* Supporting technology to carry out the three previous activities
(quality monitoring, big data, sensor technology, intelligent packing)

In the longer term, the activity domain will be extended to the biorefining of marine products such as e.g. products or molecules refined from marine raw materials or aquaculture products (e.g. from seaweed).

LINKING CARDIAC MORPHOLOGY AND FUNCTION IN ATLANTIC SALMON (*Salmo salaR* L.)

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Introduction
The cardiac morphology of Atlantic salmon (*Salmo salar* L.) is known to exhibit phenotypic plasticity. Various factors, including environmental changes, production pressure, general health and life stage, contribute to altered morphology. The impact of such morphological changes on cardiac function, resulting from both biotic and abiotic factors, remains poorly understood.

Given the likely correlation between cardiac morphology and function, we investigated the impact of key morphological traits, such as ventricular shape and alignment of the bulbus arteriosus, on cardiac function.

Materials and methods
We employed *in vivo* echocardiography to assess cardiac function and utilized quantitative and qualitative morphological analyses to evaluate cardiac structures *ex vivo*. Cluster analyses and partial regression analyses were applied to examine the influence of distinct morphological traits on cardiac function.

Results
Initial qualitative assessment revealed large variability in the skewing of the bulbus relative to the long axis of the ventricle. Quantitative analysis indicated that a larger ventricular bulbus angle was linked to reduced systolic function, including lower cardiac output. Hearts with modest skewing of the bulbus presented with a lowered E/A ratio. Variable extension of the dorsal ventricular base was also noted and linked to systolic function. Indeed, greater extension of the ventricular base, measured as the angle of deviation from the heart's transverse axis, was associated with lower cardiac output.

Conclusion
In conclusion, we believe that the morphological variability of the Atlantic salmon heart is a key determinant of cardiac function and performance.

GENETICS DRIVES HEPATIC TRANSCRIPTOME AND DNA METHYLOME OF FARMED GILTHEAD SEA BREAM AFTER BROODSTOCK NUTRITIONAL PROGRAMMING

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Introduction

Broodstock nutritional programming improves the offspring utilization of plant-based diets in gilthead sea bream through changes in lipid metabolism. Attention was initially focused on fatty acid desaturase 2 (*fads2*) (the first and rate limiting step in the biosynthesis of n-3 long-chain polyunsaturated fatty acids, LC-PUFA), and selective breeding for enhanced *fads2* expression in broodstock fish improved the offspring utilization of plant-based diets (Xu et al., 2021). Otherwise, *de novo* fatty acid biosynthesis of mono-unsaturated fatty acids offers the possibility to mitigate the signs of deficiencies in n-3 LC-PUFA, and the broodstock nutritional programming with a diet rich in α-linolenic acid served to maintain regulated the enhanced expression of *scd1a* (stearoyl-coenzyme A desaturase) in the gilthead sea bream offspring through changes in DNA-methylation (Perera et al., 2020). How such regulatory processes can be driven by a different genetic background is hardly underlined, and the present study aimed to assess how broodstock nutrition affects differentially the offspring transcriptome and genome-wide DNA methylome of reference and genetically selected fish for growth.

Material and methods

Gilthead sea bream brood fish belonging to reference (REF) or genetically selected (GS) fish within the PROGENSA® selection program received a diet with low fish oil content during the stimulus phase. Two 5-month old offspring subsets of each genetic background were fed either a control (15% fish meal and 5.7-7.6% fish oil) or a FUTURE (7.5% fish meal and completely devoid of fish oil) diet for about 6 months (challenge phase). At the end of the trial, 6 juvenile fish per each experimental condition were anaesthetized and liver samples were taken for wide-analyses of gene expression (RNA-seq) and DNA methylation, using methyl-CpG-binding domain sequencing (MBD-seq) for a large coverage of the CpG methylome.

Results and discussion

The offspring of GS fish shared a better performance than those of REF animals during the challenge phase, and differences due to diet (with improved values with the control diet) tended to be lower in GS lineage. Data highlighted a different hepatic transcriptome (RNA-seq) and genome-wide DNA methylation (MBD-seq) pattern depending on the genetic background, which agrees with previous studies in fish (Liu et al., 2022). The number of differentially expressed transcripts (comparing control and FUTURE diets) following the challenge phase varied from 323 in REF fish to 2,009 in GS fish. The number of transcripts of discriminant value by multivariate analysis, and associated enriched functions (Gene Ontology-Biological Process, GO-BP, terms), were also markedly higher in GS fish. Moreover, after selecting differentially methylated (DM) regions with an opposite trend for DNA methylation and gene expression, correlation analysis depicted a hyper-methylated and down-regulated gene expression state in GS fish challenged with the FUTURE diet, whereas the opposite pattern was found in REF fish (Figure 1A). Thus, the resulting epigenetic clock of the latter animals might represent an older phenotype (Piferrer and Anastasiadi, 2023). Moreover, after filtering for functions with a high representation in GS fish, 115 genes were retrieved as epigenetic markers nutritionally regulated in this group of fish (Figure 1B). Among them, genes within the GO-BP term Lipid metabolic process (23) were the most reactive following ordering by gene expression fold-change, which rendered a final list of 10 top markers with a key role on hepatic lipogenesis and fatty acid metabolism (*cd36*, *pitpna*, *cidea*, *fasn*, *g6pd*, *lipt1*, *scd1a*, *acsbg2*, *acsl14*, *acsbg2*). These top 10 genes also showed a greater concentration of DM CpG sites in the promoter region. Down-regulation of most of those genes agrees with the initial statement that the epigenetic regulation of gene expression due to nutritional programming may preclude an over-expression of specific genes that might result counterproductive in a changing environment.

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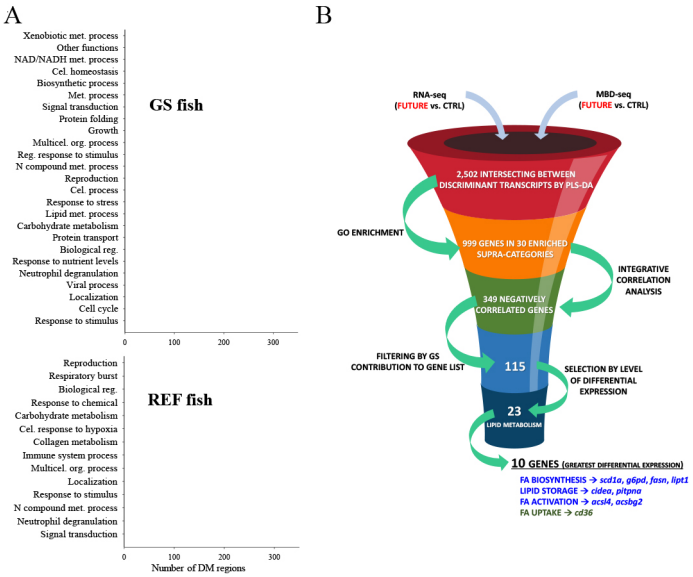

Figure 1. Enriched functions (GO-BP terms) with the number of DM regions showing an opposite trend for methylation and expression in GS and REF fish, being hyper-methylated regions shown in black and hypo-methylated in white (A). Filters applied in data from GS animals to select candidate epigenetic markers of nutritional programming (B).

Concluding remarks

Gene expression profiles and DNA methylation signatures following nutritional programming were clearly dependent on genetic background in our experimental model. Such assumption affected the magnitude, but also the type and direction of change. Accordingly, the resulting epigenetic clock of REF fish might depict an older phenotype with a lower DNA methylation for the epigenetically responsive genes with a negative methylation-expression pattern. That means that epigenetic markers will be specific of each genetic lineage, serving primarily the broodstock programming in our GS fish to prevent and mitigate later in life the risk of hepatic steatosis due to an exaggerated and/or poorly regulated hepatic lipogenesis when fish facing low fish oil diets.

Funding. This work was supported by H2020 Programme under grant agreement 818367 (AquaIMPACT).

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A HUNGARIAN SELECTION PROGRAM OF EUROPEAN CATFISH (*Silurus glanis* L. 1758) SUPPORTED BY GENETIC PLATFORMS AND TECHNOLOGICAL IMPROVEMENTS

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Introduction

Aquaculture production is a promising way to provide seafood for the rapidly increasing human population. Catfish species are becoming more popular in the Central and Eastern European region, as their bone-free, high-quality, lightly colored fillets represent an improved quality for the carp-dominant (*Cyprinus carpio* L. 1758) production structure of the region. According to experts, the production volume of the European catfish *(Silurus glanis* L. 1758*)* can be increased by orders of magnitude using improved lines and technological innovations.

Materials and methods

Our program – is based on collaboration by several Hungarian research institutions - aims to select elite European catfish lines with increased robustness and higher tolerance towards poor water quality or infectious diseases. This work is supported by genetic and genomic platforms, including parent-sibling identification by polymorphic microsatellite markers and will include comparative analysis of transcriptomes using high throughput sequencing.

Results

Currently, the F2 generation of the selected lines are being analyzed and preparing for off-seasonal artificial propagation to produce the F3 generation. Our results show that the average mass performance and the specific growth rate (SGR) both increased by 91% and 11.4%, respectively, when compared to unselected controls. Moreover, the coefficient of variation (CV %) and the feed conversation ratio (FCR) dropped in F2 by 19.5% and 14%, respectively.

In parallel with the selection process, we are also testing culture technologies tailored to the needs of faster growing selected lines.

Funding

The work was supported by the European Regional and Development Fund and the Government of Hungary within the project GINOP-2.3.2-15-2016-00025; and by the National Research, Development and Innovation Office of Hungary through its Frontline Research Excellence Grant (KKP 140353).

RAPID, COST-EFFECTIVE SNP GENOTYPING USING STANDARD BIOTOOLS SNPTYPE ASSAYS AND X9 REAL-TIME PCR SYSTEM

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The use of single nucleotide polymorphism (SNP) genotyping with non-model organisms—those whose genomes are yet to be sequenced—has increased in importance as it provides robust, comparative data sets that can be easily shared across organism communities for a variety of purposes. Non-model organisms have the added burden of low available genomic sequence information requiring custom SNP assay development. Until recently, SNP genotyping technologies have been prohibitive to these communities due to the high cost of developing and running quality SNP genotyping panels. Standard BioTools SNPtypeAssays and the X9 Real-Time PCR System have addressed these barriers with a custom assay design service, cost-effective and high qualitySNP assays, and a high-throughput workflow minimizing hands-on time. The salmon research community has been specifically hampered by the cost barriers, andwould benefit from the technology for conservation and management purposes. Using chum salmon (Oncorhynchus keta) as an example, we describe a simple workflow using Standard BioTools SNPtypeAssays, the X9 System, and 96.96 Dynamic Array™ Integrated Fluidic Circuits (IFCs) for Genotyping to achieve cost-effective and rapid development of a SNP genotyping panel. Moreover, SNPtypeAssays provide significant cost savings for high-throughput, routine testing post panel development.

COMPETITION FOR SPACE FOR OFFSHORE WIND AND AQUACULTURE WITH OTHER SECTORS AND MARINE PROTECTION IN THE NORWEGIAN ZONE

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Introduction

The coming era for the blue economy will be shaped by humanity's pressing need for sustainable energy and food, but any industrial expansion must take place in a safe, secure, and equitable manner. This requires careful study of the impact each industry has on its environment and on local communities, considering the cumulative effects from all industries combined. The MARine CO-existence scenario building (MARCO) toolbox will combine spatial and temporal analysis to capture this complexity, and the combined analysis address key uncertainties, barriers, and opportunities to deal with future spatial conflicts and to safeguard ocean health. The spatial analysis will utilise GIS (Geographic Information System) technology for mapping out plausible development trajectories in selected regions to link and explore implications to marine ecosystems and vice versa. The temporal analysis using system dynamics (SD) modelling links economic development with impact on nature through clearly devised causal relationships and feedback loops. To account for the lack of knowledge about key effects on ecosystems from e.g. offshore wind and/or offshore aquaculture development, consideration of uncertainty will be important and will contribute to a reduction of the trust gap among ocean stakeholders. Examples of scenario building and incorporation of ecosystem- wide and socio-economic information will be presented.

The Norwegian Government has recently opened two major areas in the Norwegian zone of the North Sea for offshore wind, Utsira Nord and Sørlige Nordsjø II. (Anon. 2023) Apart from providing more renewable energy, part of the rationale is to gain experience on the environmental impact and possible conflicts and synergies with other sectors. An auction for permits to establish offshore wind parks in this area is presently ongoing (Figure 1).

Even more recently, in April 2022. the Norwegian Water Resources and Energy Directorate has published a list of areas which could be suitable (Figure 1). However, in these areas, analysis of possible conflicts are probably still premature. The Government upholds a goal of installing 30 GW effect, equivalent to producing 100 TWh/year by 2040 from offshore wind in the Norwegian sector.

Furthermore, the Government has opened three areas for evaluation offshore aquaculture. Here, no auction has yet started, and the areas are to be further evaluated. There are still no plans for multi-use of areas in the Norwegian zone.

An extensive mapping of particularly vulnerable areas is presently ongoing (Eriksen et al. 2021). For instance, coral reefs and sponges has been mapped (Figure 2). Furthermore, data on spawning grounds and migration areas for juvenile fish has been mapped. These data, which are continuously updated, will contribute to the ongoing Marine Spatial Planning.

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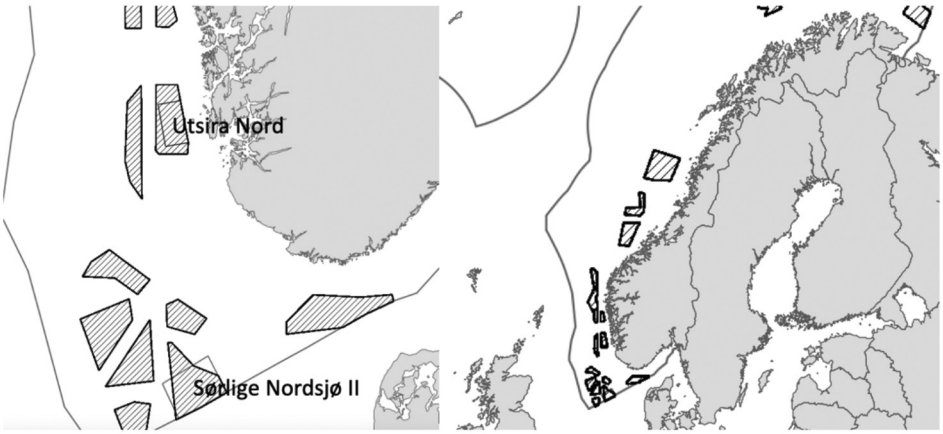

Figure 1 Areas to be evaluated for offshore wind in the Norwegian part of the North Sea (left) and in the Norwegian sector (right). The two areas where an auction process (Anon. 2023) has been started are marked in red (left)

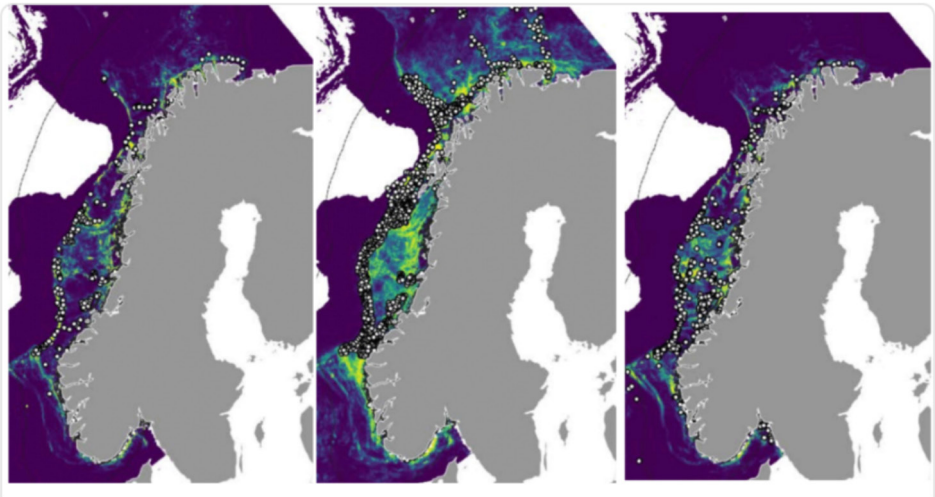

Figure 2 Horn coral reefs (left), coral reefs(centre) and swamp areas (right) on the continental shelf in the Norwegian zone. (from Eriksen et al. 2021)

A SMALL SCALE SMART OCEAN EXPERIMENTAL OCEAN OBSERVATORY FOR REAL-TIME ENVIRONMENTAL MONITORING AND WIRELESS UNDERWATER COMMUNICATION

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Introduction

The coming era for the blue and green economy will be shaped by humanity's pressing need for sustainable energy and food, but any industrial expansion must take place in a safe, secure, and equitable manner. Offshore activities in aquaculture, offshore wind, and existing fisheries, transport and oil&gas activities are expected to increase competition for ocean space, but collaboration on for example environmental monitoring could mean synergies. We are developing a small-scale smart ocean experimental ocean observatory where real-time environmental monitoring by a range of sensors, could be combined with wireless underwater communication. By realization of "an underwater internet of things" dramatically improved environmental monitoring can be foreseen, aiming to improve synergies among sectors, as well as environmental protection.

Details of the project

SFI Smart Ocean is a center for research-based innovation to enhance the ability of industry innovation and value creation through a greater focus on long-term research. The center is hosted by the University of Bergen, and consists of 20 partners from industry, public management and research, partly founded by the Research Council of Norway, running from 2020 to 2028.

The two sensor rigs have been in place at the facility since May 2022. They were from the outset equipped with an Anderaa Seabird SBE 37 Microcat and an Anderaa Sea Guard ADPC, the latter functions as the hub in the rig. In the surface unit, there are batteries and a GDSM sender unit, ensuring real-time communication. A third rig will be placed at the facility, adjacent to the aquaculture experimental sea cage facility. By these instruments, a range of physical and bio-physical parameters are monitored real-time, and made available to all the Centre´s partners as well as collaborators at the research station.

Development of the project

Development now is focused on underwater wireless communication means, testing a range of different acoustic modems. It is the intention of the Centre to develop the rigs to be increasingly autonomous. Whereas sensors in close vicinity to an aquaculture facility may easily be based on GSM or even cables, this will not be possible in deep-water environments of fjords or the open sea, or in areas such as for instance under the polar ice.

The research station will be used to develop such a multipurpose local-scale network of modular autonomous sensors, for monitoring of oceanographic environmental parameters, with candidate measurement parameters such as O_2, CO_2, gravity, gas leakage, pH, pressure, temperature, salinity, turbidity etc.

Key factors are highly cross-disciplinary: measurement parameters, flexibility and adaptive sampling in time and space, point measurements vs. monitoring over large areas, distributed measurements, measurement uncertainty and reliability, time history as input to big data analysis, machine learning, prediction and emergency response, data format aggregation and safety, increased up-time with predictive maintenance, combined with low power consumption and local sensor intelligence. Standardized interfaces will enable integration of a diversity of sensor types and communication measures, in particular wireless communication.

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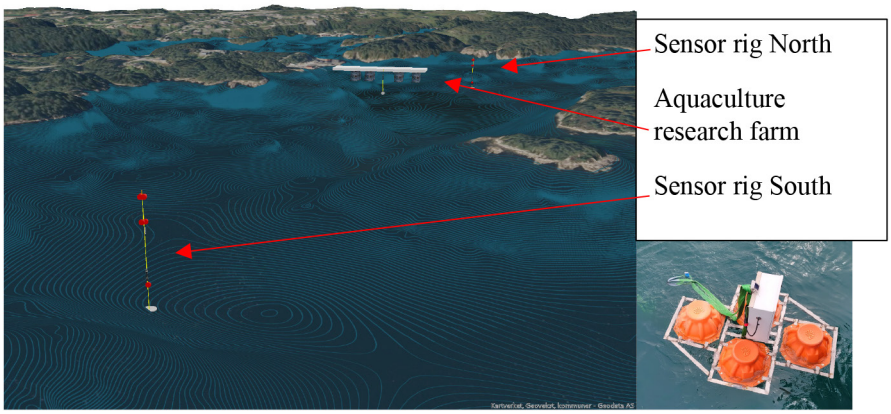

Fig. 1: A 3-D-view of the observatory and research facility at Austevoll, with floaters of an instrument/sensor rig (right)

Acknowledgements
This work is part of the SFI Smart Ocean a centre for Research-Based Innovation, partly funded by the Norwegian Research Council, project no. 309612.

HIGHLY DIGESTIBLE PROTEIN SOURCE FROM BLACK SOLDIER FLY LARVAE MEAL (HILUCIA™) IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

Aquaculture production has experienced rapid growth and is projected to continue expanding to meet the increasing demand for safe and healthy seafood. Nevertheless, the animal feed industry, particularly the aquafeed sector, faces challenges due to a shortage of conventional high-quality feed ingredients. To address this issue and meet the rising demand, sustainable sources of proteins and oils are necessary. Over the past decade, the Black Soldier Fly has emerged as a promising candidate in this regard.

An experiment was conducted to explore the viability of using black soldier fly larvae meal as a sustainable nutrient and energy source for European seabass (*Dicentrarchus labrax*). By assessing its digestibility in the fish, the researchers gained valuable insights into its nutritional value and potential advantages.

Material and methods:

An experiment was conducted to assess the Apparent Digestibility Coefficient (ADC) of nutrients and energy in black soldier fly larvae meal "Hilucia™" in European seabass. The trial included two types of diets: a reference diet (REF) that contained 0.05% yttrium oxide (Reis et al. 2008) as an inert digestibility marker, a TEST diet with 70% of the same basal mixture as the reference diet, and 30% of Hilucia™. Quadruplicate groups of 12 fish with a mean weight of 89 ± 5 g were kept in 45 L sub-square tanks at a constant temperature of 21 ± 1°C, and their feces were collected by decantation using the Guelph system.

Results and discussion:

It was found that the protein in Hilucia™ was highly digestible, with an ADC of 92%. This is in line with what was found in other studies (Magalhães et al. 2017). It provides a sustainable source of protein that can meet the nutritional requirements of European seabass. The study revealed that the fat and energy in Hilucia™ were also easily digestible, with an ADC higher than 93% and 89%, respectively. This means that BSF meal could potentially be used as an energy source in aquaculture feeds, in addition to its role as a protein source. The study found that the apparent digestibility of essential amino acids in Hilucia™ was high, with values exceeding 90% in most cases. This indicates that the protein fraction in BSF meal was highly digestible, apart from cysteine with ADC of 72%.

Conclusion:

Overall, the study concluded that Hilucia™ was a highly digestible source of protein, essential amino acids, fat, phosphorus, and energy for European seabass. The study provides important insights into the potential of BSF meal as a sustainable protein and energy source for aquaculture feeds. The high digestibility of nutrients and energy in BSF meal suggests that it could be an effective alternative to traditional feed ingredients, and further research could help to identify ways to optimize its use in aquaculture feeds.

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Table 1: Feed formula of experimental European seabass diets

Ingredients (%)*	REF	TEST
Fishmeal LT 70	30.0	21.0
Hilucia™		**30.0**
Soy protein concentrate	10.0	7.0
Corn gluten	10.0	7.0
Soybean meal 48	10.0	7.0
Wheat meal	10.5	7.4
Faba beans (low tannins)	10.5	7.4
Fish oil	9.0	6.3
Rapeseed oil	9.0	6.3
Vitamin & Mineral premix	1.0	0.7
Yttrium oxide	0.05	0.04

Table 2: Apparent digestibility of different nutrients in Hilucia™

Nutrients	Feed Composition*			ADC**
	Unit	REF	TEST	Hilucia™
Dry matter	%	94.69 ± 0.07	94.17 ± 0.02	-
Crude protein	% DM	43.27 ± 0.03	44.81 ± 0.05	91.8 ± 1.0
Crude fat	% DM	21.37 ± 0.04	19.80 ± 0.03	93.5 ± 1.9
Ash	% DM	10.84 ± 0.05	11.02 ± 0.04	-
Phosphorus	% DM	0.92 ± 0.04	0.90 ± 0.03	67.8 ± 1.4
Gross energy	kJ/g DM	20.47 ± 0.04	21.60 ± 0.02	90.7 ± 1.6
Yttrium oxide	mg/kg DM	526 ± 2	372 ± 5	-
Arginine	% DM	3.61 ± 0.05	3.37 ± 0.02	96.7 ± 0.5
Histidine	% DM	1.11 ± 0.02	1.17 ± 0.00	93.8 ± 1.3
Isoleucine	% DM	2.19 ± 0.01	2.20 ± 0.01	96.3 ± 1.4
Leucine	% DM	4.39 ± 0.02	4.03 ± 0.06	99.0 ± 0.9
Lysine	% DM	2.62 ± 0.02	2.90 ± 0.01	97.6 ± 0.8
Threonine	% DM	1.89 ± 0.01	1.98 ± 0.01	97.9 ± 1.5
Valine	% DM	2.51 ± 0.00	2.86 ± 0.07	96.1 ± 0.9
Methionine	% DM	1.15 ± 0.01	1.07 ± 0.01	95.1 ± 1.5
Cysteine	% DM	0.22 ± 0.01	0.21 ± 0.01	72.1 ± 3.8
Phenylalanine	% DM	2.59 ± 0.01	2.44 ± 0.02	98.2 ± 1.7
Tyrosine	% DM	2.43 ± 0.03	2.51± 0.02	96.3 ± 1.0

* Values are means ± standard deviation (n=2).

** Values are means ± standard deviation (n=4).

THE EFFECT OF TWO DIFFERENT EXPERIMENTAL REARING TEMPERATURES ON THE QUALITY AND THE LARGE-SCALE CRYOPRESERVATION OF EURASIAN PERCH (*Perca fluviatilis*) SPERM

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Introduction

Eurasian perch is an important fish species for European aquaculture diversification, but the quality of reproduction still remains one of the main limitations for further industry development. In particular, the optimal condition to obtain the best quality of sperm is poorly understood. Cryopreservation is an efficient technique for the long-term storage of fish sperm (Bernáth et al. 2017). The establishment of sperm banks can support aquaculture production and conservation programs (Cabrita et al. 2010, Martínez-Páramo et al. 2017). In Eurasian perch, the available techniques are usually limited by the small sperm capacity that can be preserved (0.5 mL straws). However, larger than 0.5 mL straws or cryotubes have not been tested for perch sperm.

Materials and methods

The aim of our study was to measure the possible effects of two experimental rearing temperatures (6 °C and the conventionally used 12 °C) and of hormonal stimulation, on the motility parameters (pMOT, VCL, VSL, LIN, ALH, BCF), osmolality and fertilizing capacity of Eurasian perch sperm at the end of the reproductive cycle. A prior untested, large-scale (5 mL cryotube and Polystyrene box) cryopreservation method was implemented using fresh sperm obtained from the two above mentioned temperature groups. Males were injected with 100 µg body weight kg^{-1} sGnRHa. For cryopreservation, an extender formerly adapted for perch sperm was applied (137 mM NaCl and 76.2 mM NaHCO3, Szabó et al. 2005) at a ratio 1:10 (Bernáth et al. 2015).

Results

No significant difference was recorded between the two rearing temperatures and between the saline control and sGnRHa treated groups on the different features of sperm quality. A similar fertilization rate was monitored in all sGnRHa treated (6 °C: 69±13%, 12 °C: 81±11%) and saline control groups (6 °C: 79±10%, 12 °C: 87±4%). Correspondingly, no significant difference in hatching rate was observed in the sGnRHa injected (6 °C: 27±9%, 12 °C: 40±20%) and saline control (6 °C: 35±18%, 12 °C: 36±7%) males. However, a notable negative effect of freezing process on sperm movement was observed following thawing in both temperature groups. No significant difference in the motility parameters was measured between the two temperature groups following large-scale cryopreservation. Furthermore, a similar result was observed in the fertilizing capacity (6 °C: 79±10%, 12 °C: 75±8) of thawed sperm as well as in the hatching rate (6 °C: 52±13%, 12 °C: 46±19%).

Discussion and conclusion

Our findings suggest that perch sperm quality and fertilizing capacity is not affected by the reduced rearing temperature (6 °C) following hormonal injection in Eurasian perch, using fresh or cryopreserved sperm. Despite a lower motility, sperm obtained from the reduced rearing temperature can be cryopreserved successfully using 5 mL cryotube. However, the freezing and thawing process needs to be improved using the adapted preservation method. Thawed perch sperm (cryopreserved in 5 mL cryotube) can be adapted for the fertilization of higher amounts of eggs in future experiments.

Acknowledgements

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EXPLORING EUROPEAN SEABASS (*Dicentrarchus labrax*) EICOSANOIDS METABOLISM WHEN FED DIETS WITH INCREASING INCLUSION OF FREE-CATCHES INGREDIENTS AFTER STRESS EXPOSURE

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Introduction

Inclusions of fish meal (FM) and oil (FO) from trimmings by-products and oil-rich microalgae (MIC) into aquafeed formulations, can advance sustainability by valorising industry waste streams and reducing the utilisation of marine wild resources. However, these ingredients may present differences in long-chain polyunsaturated fatty acids (LC-PUFA) (Sprague et al., 2016) and contain higher levels of bones and ashes with a lower availability of nutrients (Albrektsen et al., 2022), compared to marine wild one. LC-PUFA such as eicosapentaenoic (EPA), docosahexaenoic (DHA) and arachidonic (ARA) are precursors of eicosanoids, which are key protagonists as a mediators of inflammation and in the immune response modulation. Marine fish have a limited capacity to synthesise LC-PUFA from C-18 precursors, thus it is important to understand how "free-catches" diets may affect eicosanoid production, particularly in relation to a stressful situation. In this trial, the effects of five diets, formulated with increasing inclusions of fish meal and oil from trimmings by-products and microalgae, were assessed on the European seabass (*Dicentrarchus labrax*) eicosanoids production by exploring both molecular markers and the eicosanoids metabolites found in anterior kidney samples.

Material and Methods

Five diets (FMFO100-MIC0, FMFO50-MIC0, FMFO50-MIC50, FMFO0-MIC50 and FMFO0-MIC100), formulated to totally replace marine wild ingredients with increasing inclusion of FM and FO from trimmings by-products and MIC, were tested on triplicate groups of 45 fish (initial weight: 46.66 ± 0.04 g) reared in a RAS over a period of 88 days. At the end of the trial fish were exposed to a confinement challenge by increasing stocking density to 70 kg/m3 for 2 hours. Samples of anterior kidney were collected from 2 fish per tank (n = 6) at 0, 2 and 24 hours after the challenge. The expression of the selected genes involved in eicosanoids metabolism (EP2, Alox5, Alox12, Alox15 and P450) were evaluated on anterior kidney samples by quantitative real-time polymerase chain reaction method, as described in (Betancor et al., 2021). Eicosanoids metabolites were detected in anterior kidney samples by using HPLC-MS technique. Statistical analysis were performed by means of two-ways ANOVA ($P < 0.05$) in order to evidence significant differences among dietary treatments, time and their interaction. Starting from metabolite responses, explorative multivariate PCA were considered in order to discriminate between treatments and the role of each metabolite and their relationship.

Results and discussion

At the end of the trial no significant diet effect were detected on the growth performances among the dietary treatments (data not shown). Regardless of some differences in the fatty acids proportions of the diets, the markers evaluated to assess European seabass eicosanoids metabolism appeared similar among dietary treatments. With regards to the molecular markers evaluated in this trial, only the expression of the gene P450 at time 0 was found significantly different among the treatments. This was found higher in fish fed diets with higher inclusion of microalgae. No other significant difference in gene expression was observed between the diets at the other sampling times (time 2 and 24). The two-way ANOVA model reveals a significant influence of time on the relative expression level of EP2, Alox5, Alox12 and Alox15. After HPLC-MS analysis, 23 eicosanoids metabolites were found in European seabass anterior kidney. Explorative PCA analysis has highlighted some relationship between the metabolites and the diets.

Overall, these results suggest the potential of using completely free catches aquafeed formulations in the diet of European seabass. Moreover, modelling the eicosanoids metabolites and their LC-PUFA precursors can contribute to the deep characterisation of the biological pathways involved in the eicosanoids production.

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WHOLE-GENOME SEQUENCING TO CAPTURE GENOMIC VARIABILITY OF SEA CUCUMBER SPECIES

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Introduction

Holothuria is a genus of marine invertebrates commonly called sea cucumbers, that belongs to the phylum Echinodermata. *Holothuria* species are deposit feeders, ingesting sediment with oral tentacles, extracting and digesting the organic matter with associated microorganisms, and voiding the sand through the anus. In this way they bioturbate significant areas of the seabed, playing a central role in the benthic habitats where these species live. The interest in sea cucumber aquaculture began in Asia, where many species are considered luxury seafood with a high market value, and soon reached the European market, making these species promising candidates to establish novel aquaculture production systems worldwide.

The genus counts more than 160 species, many of which are uncharacterised at the genome level. The only reference genomes so far available are those of the *H. leucos*pilota (HL; Chen et al. 2023) assembled at a chromosome level and *H. glaberrima (HG;* Medina-Feliciano et al. 2021*)* and *H. scabra* (HS; Luo et al. 2022) assembled at a scaffold level.

In this study, we provided a preliminary comparative genome analysis among five sea cucumber species.

Material and methods

Whole genome sequencing samples of HG, HS (n=3) and HL were retrieved from the Sequence Read Archive of NCBI (https://www.ncbi.nlm.nih.gov/sra). Samples from the Mediterranean *H. tubulosa* (HT; n=4), *H. polii* (HP; n=3) were generated in-house with at least 20X of genome coverage and added to the dataset. These data were aligned to the three reference genomes (HG, HL and HS), with standard options using BWA mem (Li and Durbin, 2009). For each of the alignments, the genome coverage was evaluated. Variant calling was performed utilising the reference with the best output among the three references with bcftools (Danecek et al. 2021). A multidimensional scaling was performed to test the ability of the SNPs to discriminate the five species. Pairwise F_{ST} statistics were also performed for HS, HT and HP.

Results

The genome coverage using the three different reference genomes is reported in Table 1. The HG genome showed the best results, especially for the Mediterranean HP and HT and therefore was used for variant calling.

Variant calling performed utilising the HG-based alignment retrieved more than 2 million SNPs that showed a good power of discrimination among the five species (Figure 1), with HT and HP being close to each other as sympatric species. F_{ST} analyses was in line with the multidimensional scaling, showing a lower value (F_{ST}=0.25) between HP and HT and a higher value (F_{ST}=0.67) between HS with HP and HT.

Conclusion

HG reference genome provides the most suitable template for genomic analyses, especially for the Mediterranean HT and HP species. Nevertheless, there is the need to increase the efforts on expanding the genomic resources for holothurians.

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Samples	Reference genome		
	HG (%)	HL (%)	HS (%)
HG	99.61	31.58	16.08
HL	43.86	97.16	20.47
HS	11.84 ±0.56	23.35 ±16.45	85.59 ±1
HP	60.37 ±3.21	43.39 ±3.73	23.68 ±5.6
HT	65.79 ±1.54	48.32 ±1.22	26.36 ±3.03

Table 1. Genome coverage of the five species using the three reference genomes available

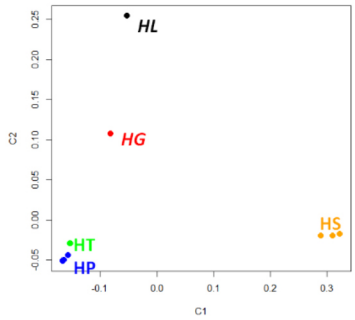

Figure 1. Multi-dimensional scaling of the five species included in the analyses.

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AN R-BASED PIPELINE FOR GENOTYPE CALLING AND PARENTAGE ASSIGNMENT OF TRIPLOID OFFSPRING TO THEIR DIPLOID PARENTS

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Introduction

Triploids, which are individuals bearing three sets of chromosomes, are commonly used in fish breeding. Triploids are sterile which allow (1) higher growth after maturity, (2) better flesh quality and (3) lower risk of genetic introgression into wild populations. In rainbow trout where triploids account for a significant proportion of production, selective breeding programs are all performed on diploid lines expecting transfert of the genetic progress to their triploids progenies. However, evaluating only diploid performance is suboptimal as genetic correlations for the same trait between diploids and triploids may differ from unity. Hence, maximizing genetic gain in triploids requires the evaluation of breeding values on triploid sibs of the diploid candidates. In mixed-family designs of breeding programs, this implies correctly genotyping triploids and recovering their pedigree, which is not currently possible with open source tools. Here, we present a pipeline to genotype and assign triploids obtained by second polar body retention after fertilization (the technique applied in salmonids) to their diploid parents based on a newly developed R package, GenoTriplo and an adaptation of APIS (Griot et al., 2020).

Material and Methods

We genotyped 1,232 triploid offspring obtained from 190 diploid dams and 98 diploid sires of rainbow trout on a Thermo Fisher SNP chip array with 57,501 SNPs, from which we kept the 38,033 highest quality markers. Allele signals were obtained via Axiom Analysis Suite software as the luminescence of probesets A and B (S_A and S_B) for each marker and individual. GenoTriplo was designed in two steps, clustering and genotype calling. For the clustering, each individual was represented by a couple of coordinates (x the contrast and y the signal strength) for each marker.

$$x = \text{Contrast} = \log2\left(\frac{S_A}{S_B}\right)$$

$$y = \text{Signal Strength} = \frac{\log2(S_A) + \log2(S_B)}{2}$$

Rmixmod R package was used to find clusters of individuals sharing the same genotype for a given marker. In the iterative algorithm, the initial number of clusters was set to 8, i.e. twice to the maximum number of possible genotypes. Clusters with close contrast values were merged to keep only four or less clusters. Five iterations were realized and we kept the iteration with the highest likelihood value. For the genotype calling, we identified the more extreme cluster based on its contrast (x) mean value and set it as homozygous (AAA if mean(x)>0 or BBB if mean(x)<0). Then, the remaining clusters were ordered by contrasts. Three main criteria were involved to improve cluster precision and to discriminate low-quality markers. (1) Genotype was declared as missing if the probability of the individual to belong to the cluster was below 0.85. (2) Genotype was also set missing if the distance between the cluster center and the individual exceeds $2.8*SD_{cluster}$. (3) Genotypes of all individuals of the same cluster were all set missing if the standard deviation of the cluster exceed $0.28*(1+0.5*abs(Mean_{cluster}))$. There were three categories of markers: PolyHighRes markers with 3 alleles observed and 4 genotypes, NoMinorHom markers with one homozygous genotype missing and CRbelowThreshold markers with call rate below 90%. Finally, we adapted APIS to enable parentage assignment of triploids. To do so, we created three new likelihood tables, one for each possible offspring genotype (AAA, ABB, ABC – this latter being relevant only for microsatellite markers). Similar tables were produced to perform assignment by exclusion. To test whether this pipeline was efficient, we used APIS to assign the 1,232 triploid offspring genotyped with GenoTriplo. The assignment was done using the exclusion method with the 1,000 best markers selected on the highest values for minor allele frequency and call rate.

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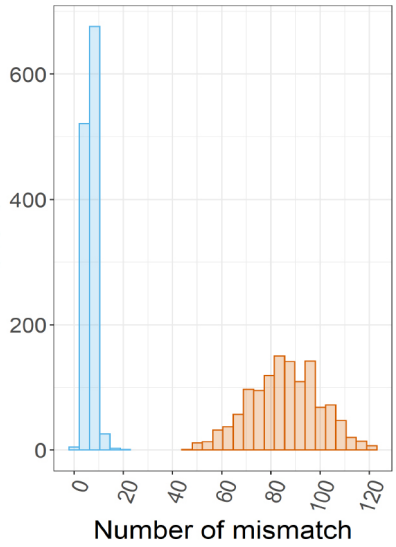

Figure 1. Number of offspring as a function mismatches for the best parental couple (blue) and the second-best parental couple (red).

Results

Thanks to the 8 clusters initially targeted, most of the rare genotypes were detected. This resulted in minimizing the proportion of CRbelowThreshold markers (4,734 markers ≈ 12.4%) and maximizing the proportion of informative NoMinorHom and PolyHighRes markers (27,948 markers ≈ 73.5%). Furthermore, all offspring were assigned to their parents. The best couple category had a maximum of 19 mismatches for 1,000 markers and a mean number of mismatches of 6.9 which is low (<1% of mismatch). The second-best couple had a minimum of 47 mismatches for a mean of 85.6 (Figure 1).

Discussion

Initially targeting eight clusters instead of four is a major difference between GenoTriplo and the method proposed by Grashei et al., (2020)for example, hexaploidy in wheat, octaploidy in strawberries, and diploidy, triploidy, tetraploidy, and pseudo-tetraploidy (partly tetraploid. It enabled the algorithm to identify clusters with few individuals that would have been included in a bigger cluster and which would have then been discarded due their distance to the cluster's center. Consequently, GenoTriplo maximizes the number of informative PolyHighRes markers found. Meanwhile, we showed that the accuracy of GenoTriplo allowed to assign all our triploid offsprings to their diploids parents. Even though the true parents were unknown the difference in the numbers of mismatches between the best and the second-best couple was large enough to confirm the success of the assignment. Hence, this pipeline is a major breakthrough towards the selection of diploid lines to improve performances of their triploid sibs.

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HEMP BY-PRODUCTS AS A PROTEIN SOURCE FOR ATLANTIC SALMON, *Salmo salar*

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Introduction

Animal protein demand will double by 2050 with aquaculture expected to fill this gap given that it is the most efficient animal production system, with feed conversion ratios of approximately 1.1, lower than both poultry (1.7) and cattle (6.8). The rapid development of aquaculture will result in higher protein demands that cannot be fulfilled by soy protein (Gatlin et al., 2007), the main protein in aquafeeds as it leads to deforestation and carries a high CO_2 footprint both from farming and international shipping. Alternatives of locally produced protein sources, or derived by-products, are required within the circular economy/zero waste concept. Hemp provides a competitive protein source to soy, being up to 5 times more effective than trees at sequestering CO_2 (Madden et al., 2022). Rare Earth Global (REG) have developed a UK model for supplying locally produced and sustainable hemp protein, which avoids deforestation through fitting into UK farmers' crop rotation schedules. This means that through full plant utilisation of stalks for primary uses (construction/energy), there can be a negative CO_2 impact on the use of the seeds as a protein source. In contrast to other protein alternatives (e.g. microbial protein, insect meal), the scale needed for commercial use can be met through UK offtakes for the stalk (30K mT). The present study investigated the suitability of UK produced hemp seed by-product as a protein source for the Scottish Atlantic salmon industry. The first step to validate the use of hemp protein as an ingredient for aquafeeds is to characterize its nutritional attributes and assess its digestibility value.

Materials and Methods

Three different types of hempseed meal (HM) were nutritionally characterized (moisture, oil, protein ash, minerals and amino acid and fatty acid profiles) and antinutritional factors (phytic acid and glycosylates) analysed. The two HM with the highest protein content (HM1-42 and HM2-46%) were chosen for in vivo and in vitro digestibility studies. A trial with three experimental feeds, one control and two feeds including a 30% of either HM1 or HM2 was carried out (Fig. 1). A total of 360 Atlantic salmon smolts, with an average body weight of 802.0±15.9 g and 40.3±4.5 cm were distributed into 9 tanks (3 per treatment) and fed the experimental feeds for two weeks. At the end of the trial all fish in each tank were euthanized at ~6h after feeding and measured for weight, length and operational welfare indicators. Next, faecal samples were collected by stripping based on the methods reported by Glencross et al. (2005) for digestibility and short fatty acid analysis. Faeces were collected as two pools of 10 fish each per tank (total sample size n = 18). In addition, 4 fish per tank were dissected and anterior and posterior intestine as well as liver collected, fixed in 4 % buffered formalin for histopathological evaluation of the tissues. The bioaccessibility and the bioavailability of nutrients of the two HM selected based on nutritional characterization were evaluated on an artificial salmon gut (www.salmosim.co.uk) in order to complement the results obtained in vivo.

Results and Discussion

The protein content of the three analysed HM ranged from 35.6-46.0%, while the fat content oscillated between 8.6-10.5%. The two HM that were more protein and energy dense (HM1 and HM2) also displayed the lowest carbohydrate levels. The three HM included high levels of aspartic acid, glutamic acid, phenylalanine, histidine and arginine comparable to those of fish meal. Levels of methionine are approximately half to those present in fish meal, similar to soy protein concentrate. No glycosylates were detected and the phytic acid contents were 3.9%, similar to other terrestrial vegetable meals.

At the end of the feeding period, fish fed the diet containing HM2, displayed the highest weight gain. The protein digestibility of the test diets proved to be good at 84 and 87% for HM1 and HM2, respectively. The protein and amino acid digestibility of the two test ingredients was also generally high and in many cases was 100%. This proves that the digestibility of both HM was excellent and therefore may have the potential to be utilized as ingredient in hot extruded feeds for Atlantic salmon. These results were corroborated by the in vitro trial, indicating the potential high digestibility of this ingredient in cold extruded or compressed pellets. The histology assessment of liver showed the lowest degree of intracytoplasmic lipid vacuolization in fish from HM2 treatment, albeit not different to Control. The only morphological alteration found in intestine was a higher density of goblet cells in the posterior intestine of fish fed HM1, when compared to those fed the control feed, indicating greater mucus coverage. The results suggest that hempseed meal may be a good novel ingredient for Atlantic salmon aquafeeds. Long term trials are now needed to evaluate the impact that commercial inclusions of these ingredients can have of salmon performance and stress resistance.

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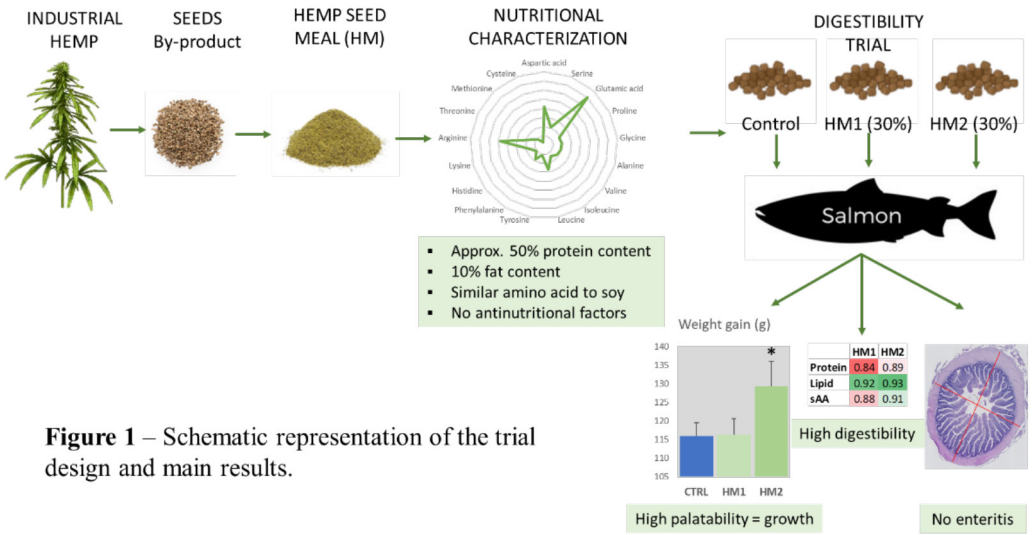

Figure 1 – Schematic representation of the trial design and main results.

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GENETIC DIVERSITY AND HETEROZYGOSITY OF EUROPEAN GRAYLING *Thymallus thymallus* (LINNAEUS, 1758) FROM FISHERY STOCK IN UKRAINE

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Introduction

The European grayling *Thymallus thymallus* (Linnaeus, 1758) is listed in the Red Book of Ukraine and has the status of a vulnerable species. Therefore, the protection and reproduction of this indigenous salmonid species for Ukraine is an important task. The work in this field was initiated by specialists of the Institute of Fisheries in 2006 at the farm of the Synevyr National Nature Park from individuals caught in the Chorna River (a tributary of the Tereblya River), and since 2014 the work continues at the Lopushno trout farm (Chernivtsi region, Ukraine) (Kucheruk et al., 2018). This fish farm participates in the program of the National Academy of Sciences of Ukraine, aimed at creating a brood stock of grayling with increased productivity and its further use for stocking the river network of the Western region of Ukraine to preserve the species diversity of local ichthyocenoses. Comprehensive work with this species includes both the classical assessment of morphological and productive parameters of the obtained generations of fish on the farm, monitoring of infectious and invasive fish diseases, and also genetic analyses. A set of molecular tools allows studying allelic diversity, assessing stock heterozygosity, and determining phylogenetic origin or relationships with other populations, and thus contributing to the effective management of the genetic resources of graylings on farms and their further use for the preservation of ichthyocenoses in rivers. This work aimed to analyze grayling generations from the Ukrainian fishery using microsatellites and mitochondrial DNA analysis.

Materials and methods

For molecular genetic studies, fish fin clips were collected from individuals of the first generation of grayling from 2017 and the second generation from 2021 of the Lopushno trout farm (Ukraine) with the aim to analyze and monitor changes in the genetic structure of the stock. The study of the genetic diversity was carried out using microsatellite loci (BFRO 005, BFRO 006, BFRO 010, BFRO011) with data processing in Genalex 6.5 software and related programs to obtain basic genetic parameters. Phylogenetic analysis of the existing stock was carried out using the gene of the control region of mitochondrial DNA. The obtained results of the analysis of mtDNA fragments were edited in Sequencher v5.1 and analysed together with reference sequences from the NCBI, BOLD databases in MEGA X, DNAsp and PopART.

Results

The number of alleles for all loci varied from 4 to 6, with values of the effective number of alleles per locus from 2.985 to 5.128. The average value of the allelic richness index (Ar) in the first generation was 4.7 ± 0.5 less than in the second generation 5.7 ± 0.5. Shannon's diversity index ranged from 1.192 to 1.543 in the 2017 generation and 1.392 to 1.692 in the 2021 generation. In the first generation, the observed heterozygosity (Ho) = 0.600 ± 0.147 and the expected (He) = 0.729 ± 0.025, while in the second generation Ho = 0.767 ± 0.053 and He =0.778 ± 0.021. The average value of the fixation index (F) was lower in the second generation (0.014) than in the first (0.184). Phylogenetic analysis based on sequenced fragments of the control region suggested that the Ukrainian samples belong to the Mixed Central Europe clade defined by Weiss et al., 2002. This clade corresponds to lineage II of European graylings according to the separation proposed by Gum et al., 2009.

Discussion

In the comparative analysis of two grayling generations from the fish farm, it can be stated that the genetic diversity did not decrease in the second generation when taking into account indicators such as the number of alleles (Na), the effective number of alleles (Ne), allelic richness (Ar) and Shannon's biodiversity index (I). However, further monitoring is needed to increase the number of monitoring points and to assess changes in effective population size. The predominance of expected heterozygosity (He) over observed heterozygosity (Ho) in both groups indicates the presence of inbreeding. However, the values of the fixation index (F) suggest, that the inbreeding decreased in the second generation.

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Based on the assessment and comparison of the morphological and productive parameters of generations at the stage of young-of-the-year (0 +), the most characteristic features observed were that the yield was at the level of 30% in the group of individuals of 2017 generation during the growing period from May to September, which is less than that obtained in the 2021 generation. However, the second generation was characterized by a lower average weight (4.6 g) than the first (9.73 g). Considering the principles of the identity of growing conditions, this fact may be related to technological shortcomings. To fully characterize the differences in the data of the two generations further studies of the 2021 generation are planned after the onset of puberty (for example, estimation of males and females, their fertility), as well as a comprehensive analysis of the variability of morphometric parameters. The study of the genetic features of the obtained generations of graylings is planned to be used in the future to preserve the species diversity of the ichthyocenoses of the river network. Phylogenetic studies of grayling allow establishing the belonging of the Ukrainian samples to the Mixed Central Europe clade and lineage II, which corresponds to previous generally presented schemes and, as indicated, is a mandatory step in the planning of conservation and stocking programs (Gum et al., 2009).

Conclusions

The results of the molecular genetic analysis will allow creating a basis for monitoring and controlling changes in the genetic structure and further selection work with these briding stock to maintain genetic biodiversity and rational economic management of them. Knowledge of the phylogenetic origin of the material will help avoid negative consequences during the stocking of rivers.

Acknowledgements

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DEVELOPMENT OF A MODULAR AND SEMI-QUANTITATIVE SCORING SYSTEM TO ASSESS THE GILL HEALTH STATUS IN GILTHEAD SEABREAM (*Sparus aurata*) FED WITH FUNCTIONAL FEEDS FOR SPARICOTYLOSIS MITIGATION

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Introduction

Sparicotyle chrysophrii is a gill hematophagous monogenean of gilthead sea bream (*Sparus aurata*) causing sparicotylosis, currently considered one of the most relevant health issues in sea bream farming in the Mediterranean mariculture. This parasite can be responsible for significant worsening in growth performance, serious gill pathologies even at moderate infection intensity up to mortality at massive infections in the most susceptible fish, i.e. juveniles. Since currently there are no effective, safe and environmentally friendly therapies authorized in European aquaculture, in recent years the implementation of mitigation strategies based on specific functional feeds has been pursued as an optimal option, being able to combine direct antiparasitic effects with positive effects on host immunity and growth, always when administered in the context of good health management practices. In previous studies, the parameters used to evaluate *in vivo* the efficacy of different functional feeds have been mainly related to the quantitative parasitology (prevalence, intensity and abundance), and biometric/growth and haematological indices of the host. In order to have an additional tool for the analysis of the results of feeding trials and the comparison between the experimental groups, a semi-quantitative histological scoring system with a modular structure - following the key principles suggested by Gibson-Corley et al. (2013) - has been developed to perform a more objective evaluation of the gill health status in caged sea bream fed with different functional feeds for Sparicotylosis mitigation.

Materials and methods

In the context of a 10-month trial based on the administration of two different functional feeds to prevent/mitigate sparicotylosis in cage-reared gilthead seabream (Musmanno et al., 2022), a histological study was performed in order to develop a semi-quantitative scoring system useful for assessing the gill health status during the whole trial but also in wider contexts. For this purpose, the four gill arches from the left side of 10 fish for each cage were monthly sampled (6 experimental cages×10 fish/cage/month×10 months=600 fish in total), fixed in 10% neutral buffered formalin, then processed following standard lab procedures. All the sections were 5µm thick and stained with Hematoxylin-Eosin (HE). The criteria of the system were identified and defined on the basis of a common consensus reached by three operators (fish pathologists) before the observation of the histological preparations.

Results and Discussion

Two modules were chosen for the development of the scoring system. In the first module, non-specific criteria were taken into consideration, chosen as independent stereotyped and generic reactions of the branchial tissue and grouped into 4 macro-categories: tissue adaptations, circulatory anomalies, tissue degeneration and inflammatory infiltration. In the second module, 5 specific criteria were taken into consideration, relating to the presence and intensity of pathogens such as primarily *S. chrysophrii*, associated or not with *Furnestinia echeneis*, Aporocotylidae eggs or other parasites, Epitheliocystis and the presence of bacterial aggregates linked to gill alterations. The score attributed to the overall evaluation was obtained from the sum of the scores obtained in the two modules. The range of the final score of the system, from 0 to 24, was divided into 4 levels of gill condition: excellent (0-6), good (6.001-12), poor (12.001-18), bad (18.001-24).

A good correspondence between the scores obtained in the different experimental groups and the quantitative parasitological parameters was highlighted by the first round of histological observations completed, although the accessibility and robustness of the system are being tested using Cohen's kappa coefficient in a simple blind survey on 600 gilthead sea bream, aimed at assessing the concordance between the scores recorded by the pathologists, chosen so as to provide each one with a different level of competence (intermediate, advanced and expert).

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Conclusion

The histological scoring system applied to the gills of gilthead sea breams examined during the feeding trial appears to be a suitable tool to characterize and support the promising results given by the quantitative analyses obtained so far. Strength of this scoring system would be the overall approach combining generic alterations of the gill environment and the presence of *S. chrysophrii* and other transmissible pathogens for characterising gill conditions and evaluating the pathology under study, providing fish pathologists and researchers with a standardised tool to interpret, monitor and compare gill disease trends and severity in various experimental and farming condition, even on a large scale. Furthermore, the modular nature of the system is suitable for any fish species, expanding the range of applications available compared to traditional systems, as the second module can be easily adapted to most aetiological agents responsible for gill pathology in aquaculture. The ongoing comparison of blind histological observations performed by three pathologists at different level of expertise will give further robustness to the scoring system.

Acknowledgements

This research was undertaken under the NewTechAqua (New technologies Tools and Strategies for a Sustainable, Resilient and Innovative European Aquaculture) project, which has received funding from the European Union's Horizon 2020 Programme under grant agreement No 862658 (https://www.newtechaqua.eu/).

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ADOPTION OF DIFFERENT CULTURE SYSTEMS AND IDENTIFICATION OF SUITABLE FEED FOR FRESHWATER MUD EEL (*Monopterus cuchia*)

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Introduction

Freshwater Mud Eel (FME), *Monopterus cuchia,* is a highly nutritious and medicinally beneficial fish and it contribute to fulfill nutritional requirement and socioeconomic well-being in Bangladesh. Even in few years back, this eel fish was abundant in the natural bodies of Bangladesh, but now the abundance has declined drastically due to indiscriminate capture from natural habitat and insufficient knowledge of proper culture methods. FME population health is highly dependent on natural reproduction, and still no suitable method implies for its culture and feed development. Considering these issues, this study aims to develop appropriate aquaculture technique and suitable feed for mud eel.

Material and Methods

In order to identify suitable culture methods FME were stocked in three different habitat namely earthen ponds (T_1), plastic tanks (T_2) and cement tanks (T_3) with three different stocking densities (2, 4, 6/m^2) for each culture methods. Another experiment was designed with applying three different feed *viz*. trash fish (F_1), live worms (F_2), and commercial feed (F_3) in order to identify suitable feed.

Results

After three months of culture period, highest eel body weight was observed in T_1 with the stocking of 2/m^2 followed by T_3 and T_2. Although, higher fish body weight was observed at 2/m^2, but overall production found to be higher at the stocking of 4/m^2 which is more economical. On the other hand comparatively faster growth was observed in eel fish fed to trash fish (F_1) followed by live worms (F_2). Both the trash fish and live worm treated groups showed nearly identical growth patterns. In conclusion, the result suggested that, earthen pond system with 2-4 stocking density and both trash and live feed could be suitable for mud eel aquaculture. Although further field trial is necessary in order to disseminate this technology.

Acknowledgement: Khulna University research cell for financial support

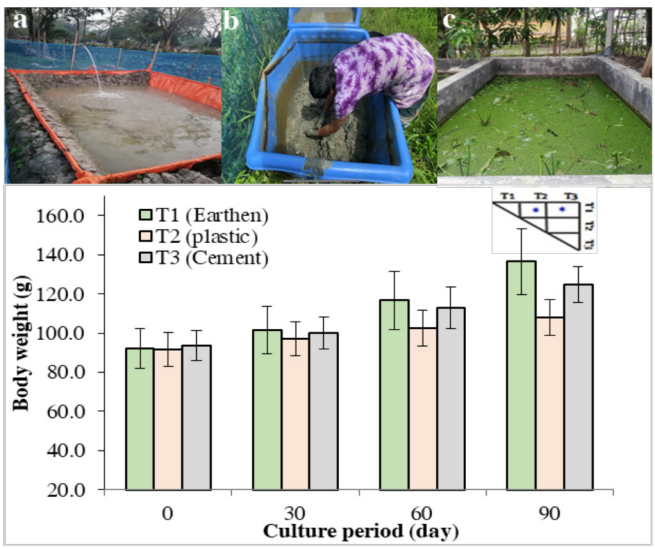

Fig. Different culture system of mud eel, a) earthen ponds, b) plastic tanks, c) cement tanks. Graph represents the growth of mud eel at different culture system. Star (*) in the box indicate significant different among the treatment ($p < 0.05$)

IN VITRO LIFE CYCLE AND FECUNDITY OF *Anisakis pegreffii*

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Introduction

Marine nematodes from the genus *Anisakis* are gaining more research spotlight as a result of an increase in the number of anisakiasis cases (*i.e.*, the clinical condition in humans caused by *Anisakis* spp.) in Europe. In the natural environment, free-swimming second-stage larvae (L2) hatch from the eggs released in the seawater via the feces of the final hosts (marine mammals). The larvae are preyed upon by crustaceans (mainly euphausiids) and possibly small fish (intermediate hosts), moulting into third-stage larvae (L3). Intermediate hosts are consumed by larger fish (paratenic hosts) in which L3 migrate in the visceral cavity and remain in parathenesis until final hosts prey upon, and digest the infected paratenic host. In the final hosts, L3 moult through fourth (L4) and fifth (L5) juvenile stages into reproductively active adults of separate sex.

This research aimed to obtain *in vivo* the adult stage of *Anisakis pegreffii* from L3 isolated from fish, and to assess the production and hatchability of eggs over the lifetime of the adult specimens. The reproductive traits of this zoonotic nematode, such as fertility and life span will be helpful for the epidemiological modelling and risk assessment estimations.

Materials and Methods

Anisakis spp. type I larvae (identified as *A. pegreffii* following genotyping) were isolated from naturally infected blue whiting *Micromesistius poutassou* in the Adriatic Sea (Croatia). Isolated L3 larvae were cultured in Schneider's *Drosophila* media supplemented by 10% chicken serum (n=30 in triplicate; B1, B2 and B3) to reach the adult stage using previously published protocol (Mladineo et al., 2023). The development of the adults from isolated L3 and the production of the eggs expelled in the medium was recorded every second day. Eggs collected were incubated in Sea Salt Solution at 19°C and the time of hatching was recorded. *Anisakis* fecundity was expressed as the daily expelled number of eggs according to Moratal et al. (2023). Additionally, the number, sex, and date of adults removed from the culture were recorded to calculate the sex ratio (expressed as a percentage of females) at each count. A graph was plotted depicting the number of eggs per day and per reproductive female.

Results and Discussion

Larvae begin to reach fourth-stage from 4th day in the medium, and another shedding of the cuticle marking the moulting into L5 was observed after 15 days. The adult stage, marked by the first shedding of eggs appeared after 17 days. However, the growth rate of the worms was asynchronous as some developed faster than others. The eggs were observed in the medium from 17 to 133 days post-incubation. The first hatching of eggs (*i.e.*, first fertilisation) appeared 44 days post-incubation. For the following 51 days (*i.e.*, 95 days post-incubation) eggs were fertilised and hatched into L2 larvae. Hatching occurred 5-7 days post-incubation of eggs. The average fecundity peaked on day 100 post-incubation, ranging from 80,000 to 266,000 eggs per day per female counted in triplicates. B1 encompassed 19 females, 9 males, 2 L5; B2 contained 22 females, 7 males, 1 L5; and B3 contained 19 females, 7 males, 4 L5 (L5 died before reaching the adult stage). Thus, a sex ratio of 1:2 was calculated for B1, and 1:3 for both B2 and B3. The number of dead worms was recorded throughout the experiment and the cumulative mortality is shown in the Figure 1.

At the end of the experiment, cumulative mortality was 59.99, 49.99, and 53.26% for B1, B2, and B3, respectively. Most of the dead worms recovered from the media were smaller in size compared to the others; indicative of underdevelopment and their inability to compete for nutrients with the others. Other biological factors could also contribute to their mortality.

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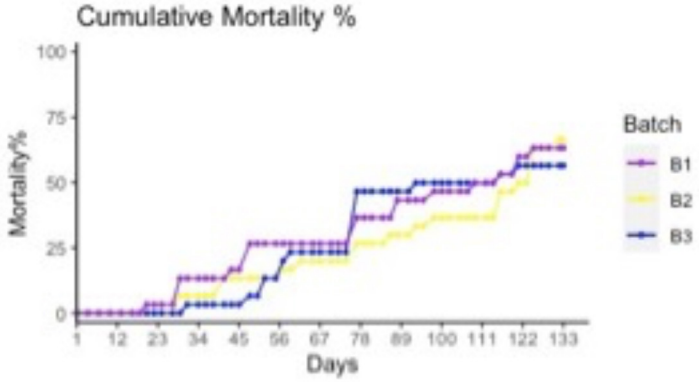

Figure 1: Cumulative mortality (%) of *Anisakis pegreffii* adults kept in Schneider's *Drosophila* medium enriched with 10% chicken serum.

Conclusion

Anisakis pegreffii is able to survive, grow and reproduce in insect medium for as long as four months. However, the fertilisation of eggs and thus hatching of L2 for downstream experiments is feasible during a limited time (till 95 days post-incubation), similar to what has been reported by Moratal et al. (2023). Nonetheless, the observed cumulative mortality of adults, if maintained in enough replicates, guarantees the harvest of fertilised eggs satisfactory for robust downstream experiments.

Funding

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EFFECT OF DIFFERENT DIETARY SUPPLEMENTATIONS OF *Nannochloropsis gaditana* ON THE PRODUCTION TRAITS OF TWO-YEARS OLD COMMON CARP *(Cyprinus carpio)* STOCK

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Introduction

Diets containing microalgae as an alternative protein source have a growing importance in the feeding of intensively reared fish species. Microalgae are characterized by high protein content of 30-55% (López *et al.*, 2010) and a favourable essential amino acid content (Brown *et al.*, 1997). Their lipid content ranges between 2-50% (López *et al.*, 2010). Microalgae are rich in highly unsaturated fatty acids, carotenoids, vitamins and minerals (Idenyi *et al.,* 2022). Microalgae positively affect the immune status of fish and have a beneficial effect on their growth and health (Ayala *et al.*, 2023). The aim of the study was to determine how different amounts of *Nannochloropsis gaditana* supplementations affect the production traits of two-years old common carps (*Cyprinus carpio*) in a closed fish rearing system.

Materials and Methods

A six-week experiment was carried out at the Research Center of Aquaculture and Fisheries, Hungarian University of Agriculture and Life Sciences, using a recirculation aquaculture system (RAS). A total of 144 two-years old common carp individuals with an average initial weight of 720 ± 77 g were randomly distributed into 9 tanks in triplicate (16 fish per tank of volume 2 m^3). A complete randomized design was used to set up the experiment. Experimental diets were formulated to contain *Nannochloropsis gaditana* supplementations in two different amounts. The control diet (Control) contained no algae, while the experimental diets contained 10 % (Algae 10) or 20 % (Algae 20) algae supplementation. Experimental feeds were fed at 2 % of biomass during the six weeks of the experiment. All diets were fed in triplicates. Fillet samples were collected from 9 individuals at the beginning of the experiment. At the end of the experiment fillet samples were taken from 45 individuals (5 fish/tank).

Results and conclusions

Growth rate of common carp fingerlings is shown in Figure 1. No significant differences were found in the mean weight between the groups at the two-weeks sampling times. Data of growth parameters and production traits determined at the end of the experiment are summarized in Table 1. After six weeks of feeding no significant differences were found between the groups either in terms of growth (weight gain, SGR) or feed conversation ratio, protein efficiency ratio, and fillet yield. At the same time some tendency can be seen towards a better performance of Algae10 group. Overall, it can be stated, that the fish utilized the *Nannochloropsis gaditana* containing feed well, without compromising the production and nutrient utilization parameters during the experimental period.

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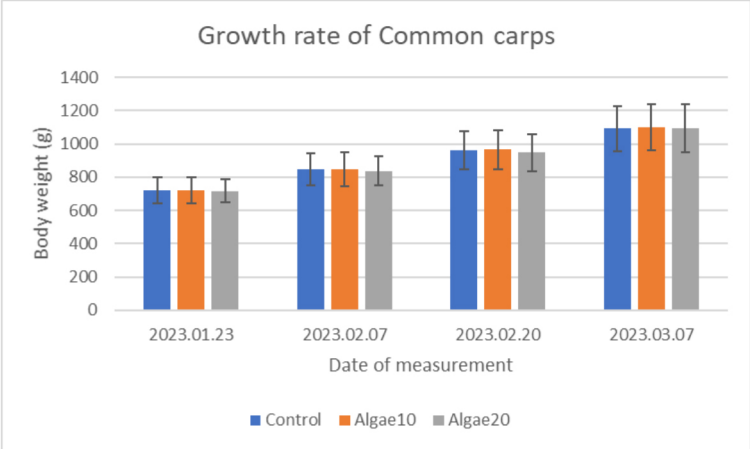

Figure 1. Growth rate of Common carps during the 6-week feeding experiment

Table 1. Effect of different diets on the production traits of two years old carp fingerlings

Production trait	Diet			P-value
	Control	Algae10	Algae20	
Initial body weight (g)	722±11.5	721.3±21.7	718±15.1	0.954
Final body weight (g)	1093.3±19.7	1100.7±24.8	1095.3±68.9	0.977
Weight gain (g/day)	8.84±0.29	9.03±0.08	8.98±1.34	0.963
SGR (%/day)	0.99±0.03	1.01±0.02	0.99±0.12	0.955
FCR (kg/kg)	1.37±0.03	1.34±0.02	1.37±0.16	0.917
PER (kg/kg)	2.15±0.06	2.20±0.04	2.18±0.28	0.931
Filleting yield (%)	34.17±0.67	35.47±0.29	35.50±1.08	0.062
Survival (%)	100±0.0	100±0.0	97.92±3.61	0.483

Acknowledgement

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EFFECT OF SHARK BY-CATCH MEAL AS AN ALTERNATIVE OF FISHMEAL ON GROWTH AND PHOSPHORUS LOADING IN JUVENILE YELLOWTAIL, *Seriola quinqueradiata*

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Introduction

Although fish meal (FM) has been considered the most preferred protein source in aquafeeds, intense focus has been given on the reduction and/or elimination of FM protein over the past several decades due to its high price and insufficient supply. Shark by-catch muscle meal (SM), which has been otherwise wasted away, was used to replace fishmeal (FM) in the diet of juvenile Japanese yellowtail, *Seriola quinqueradiata* to investigate its effect on growth and phosphorus (P) loading to the ecosystem.

Materials and methods

The control diet (C) was composed of FM as protein source, and 25%, 50%, 75% and 100% of FM protein from diet C was replaced by SM to formulate diets SM25, SM50, SM75 and SM100, respectively. A group of 25 fish (mean weight 18.01 ± 0.09 g) were stocked into each 500 L tank in triplicate for each treatment, fed two times daily until apparent satiation for 6 weeks. Feces collection trial was carried out by using chromic oxide as an inert marker after finishing the growth trial. The photoperiod was set to 12 h of light (07:00-19.00) and 12 h of dark during the whole rearing period. The UV-treated filtered seawater was supplied at 7 L/min per tank, and water temperature and dissolved oxygen levels during the test period were 26.1 ± 1.0°C and 6.4 ± 0.7 mg/l, respectively. During the test period, the bottom was cleaned with a siphon every day at 11:00, and dead fish were counted and weighed if mortalities were observed.

Results and conclusion

There were no significant differences in growth parameters among fish fed with diets C, SM25, SM50 and SM75 ($P < 0.05$, Tukey's test), though SM100 produced significantly lower performance compared to all diets ($P > 0.05$). Moreover, there was a strong negative ($R^2 = 0.964$) and positive ($R^2 = 0.904$) linear correlation between daily feeding rate and SM, and between feed efficiency and SM levels in diets, respectively. Although there were no significant differences in protein and fat productive value among the treatments, P productive value was significantly increased with increasing levels of SM in diets, resulting in a significant lower P loading from SM-based diets ($P < 0.05$). There were no significant differences in plasma levels of total protein, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and total cholesterol ($P > 0.05$); however, plasma levels of triglyceride, blood urea nitrogen and ammonia were significantly increased in fish fed with diet SM100 compared to the control group ($P < 0.05$). In conclusion, the results suggest that 75% of FM protein can be replaced by SM without compromising the growth performance and health condition, and that a significant ecological benefit can be achieved by reducing P loading from SM-based diet. From an economic standpoint of view, the application of SM would help to reduce dependency on high cost and scarce FM.

PHOTO-OXIDATION: A PROMISING SOLUTION FOR THE BIOSECURITY OF AQUACULTURE WATERS

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The pollution of seawater by both biotic (bacteria, viruses, toxic algae etc.) and abiotic contaminants (toxin, pesticide, pharmaceutical residues, etc.) frequently leads to economic losses in aquaculture activities. The methods of water treatment commonly used in aquaculture (UVC, filtration...) do not allow to eliminate both biotic and abiotic contaminants. Advanced Oxidation Processes (AOPs) such as heterogeneous photocatalysis allow the removal of all organic contaminants present in water and therefore could reduce production losses in land-based farms (closed facilities). If this process has already been widely studied for the abiotic decontamination, its application for biotic disinfection is still overlooked (especially on viruses). Over the past decade the production of the Pacific oyster *Crassostrea gigas* has been regularly affected by massive mortalities due to the Pacific Oyster Mortality Syndrome (POMS). This syndrome is a complex and polymicrobial disease involving an initial viral infection by the *Ostreid Herpes Virus 1* (*OsHV-1 µVar*) followed by multiple bacterial infections. In this context, we investigated seawater disinfection by the heterogeneous photocatalysis (UV/TiO_2) method in the context of POMS by addressing both the impact of the treatment on a single opportunistic pathogenic bacterium (*Vibrio harveyi*) and on a complex microbial community refection a natural POMS event (*OsHV-1 µVar* virus and opportunistic pathogenic bacteria consortium). The viral inactivation has been monitored using experimental infections to see if viral particles were still infectious after UV/TiO_2 treatment. Moreover, changes on the total seawater bacterial community have been investigated comparing UV/TiO_2 treatment with UV-irradiated seawater and non-treated seawater. This study gave promising results for UV/TiO_2 seawater disinfection. Both oyster's pathogens tested were efficiently inactivated in a few hours of treatment. Even if treatment impacted transiently the total bacteria community, the seawater microbiota shift toward untreated seawater few days after the end of the treatment. Altogether, these results revealed that heterogeneous photocatalysis could be an interesting alternative process for the disinfection of land-based oyster farm seawater to prevent vibriosis and viral diseases.

UTILIZING ELECTROLYZED WATER TREATMENT TO MANAGE VIBRIOSIS CAUSED BY *Vibrio harveyi* IN AQUACULTURE SETTINGS

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Introduction

Vibrio harveyi (*Vh*) is a Gram-negative halophilic bacterium widely distributed in marine environments that can cause disease in numerous aquatic animals, including cultured species. In fact, the bacterium has been recognized as one of the major causes of vibriosis (Amaro *et al.,* 2020). Nowadays, epizootics and outbreaks caused by *Vh* are increasing due to its wider distribution because of global warming. Antibiotics have used to control *Vh*-vibriosis in aquaculture for years. However, resistant strains could pose a major threat to this industry, urging the need of developing alternative methods. Electrolyzed water (EW) is a powerful and environmentally friendly disinfectant. Its generation, through electrolysis, from a saltwater solution, results in the separation into two streams, alkaline and acidic. The acidic stream (anolyte) contains hypochlorous acid (HOCl) which is a potent bactericide, disrupting the cell membranes and leading to their fast destruction (Wang *et al.,* 2020).

The objectives of this study were to determine the bactericidal effect of EW against *Vh* and assess its potential as a complementary method to antibiotic therapy for the control of *Vh*-vibriosis outbreaks in aquaculture settings.

Material and Methods

V. harveyi strains, isolated from diseased fish, were grown in Luria Bertani plus 5 g/L NaCl broth (LB-1) at 28 ºC for 24 h with shaking (100 rpm). Electrolyzed Water was generated from a saltwater solution at different pH (3.5, 5, 6.5 and 7.5), salinity (0.5, 1.5 and 3%) and available chlorine (from 5 to 125 ppm) conditions. EW samples were inoculated with *Vh* cultures (1:10 ratio) and incubated up to 15 min. Bacterial viability in EW samples was measured by drop plate onto Tryptic Soy Agar with 1% NaCl (TSA-1). All experiments were performed in triplicate.

Results and discussion

High concentrations of available chlorine (125 ppm) in EW had a strong effect against *Vh* strains, independently on the salinity and pH conditions. EW at 25ppm with salinity of 1.5 % and acidic pH (5) was highly bactericidal, leading to survival of less than 0.01% (*Vh* initial population: 10^6 cfu/ml) in 10 min, equivalent to more than 4 log unit reduction. This effect probably resides in the combined effect of free available chlorine concentration and all reactive oxygen species (ROS) generated in the electrochemical preparation. Under these conditions excepting for pH, EW was less efficient reducing the bacterial population (25% and 0.1% at pH 6.5 and 7.5, respectively).

At 25ppm and salinity of 3% (mimicking sea conditions), the effect of pH was clear: at pH 7.5 a reduction of three log units was observed in the bacterial concentration in less than 15 min (Fig. 1). The other test strains showed the same survival tendency under the same conditions (Fig. 2)

Conclussions

EW technology was highly effective reducing the *Vh* population, thus it could help to minimize the antibiotic treatments during vibriosis outbreaks. Moreover, implementation of EW in aquaculture facilities as a preventive strategy during stressing periods would probably contribute to reduce the load of pathogenic bacteria in farm seawater.

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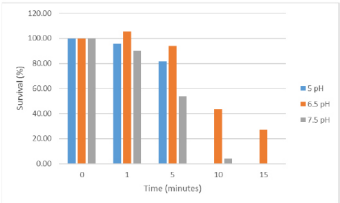

Figure 1. Survival of *Vibrio harveyi* (strain C2) in electrolyzed water (3% salinity and 25ppm of chlorine) under different pH

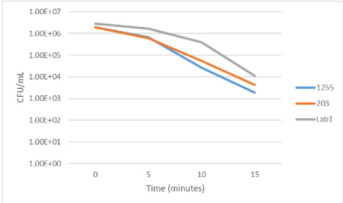

Figure 2. Survival of *Vibrio harveyi* strains (1255, 203 and Lab1) in electrolyzed water at 3 % salinity, 25 ppm of chlorine and pH 7.5.

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CONNECTIVITY BETWEEN AQUACULTURE SITES - RISK OF PARTICLE TRANSPORT AND ASSOCIATED SPREAD OF DISEASE

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Introduction

Mortality in Norwegian salmon aquaculture averages at around 15% during time at sea, with gill disease being a major cause of mortality [1]. Gill health is impacted by many factors including pathogens, fish handling, and sea lice treatments, as well as waterborne particles such as harmful algae, jellyfish, and biofouling fragments [1, 2]. Biofouling assemblages occlude pen nets and are commonly removed via regular (up to weekly) in-situ net cleaning operations [3, 4]. During this process, cleaning waste is released into the water column and able to disperse within the pen and farm site. Contact with hydroid particles can cause gill injuries, lasting for up to 7 days [5]. In addition, hydroids and other biofouling organisms can harbour pathogens [6, 7], and dispersal of their fragments via currents may contribute to the spread of disease between pens or farm sites [8].

The study used a hydrodynamic particle tracking model to examine the spread of biofouling particles arising from in-situ net cleaning operations at a case-study farm located in mid-Norway. We determined in particular the potential transport of these particles to neighbouring farms.

Material and methods

The particle distribution model was set up for two similar sized farms in close vicinity (4 km apart) located off the coast of mid-Norway [9]. Input variables to the model were (i) hydroid particle abundance, (ii) hydroid particle size distribution and (iii) hydroid particle sinking rates. Abundance and particle size distribution were estimated based on an assessment of biofouling present before and after cleaning, and analysis of cleaning waste concentration and composition measured in situ. Particle sinking rates were measured in a laboratory study. Results are presented as particle concentrations per square meter accumulated for the upper 35 m of the water column (i.e. average depth of a sea cage) for individual pens, as well as for surrounding waters.

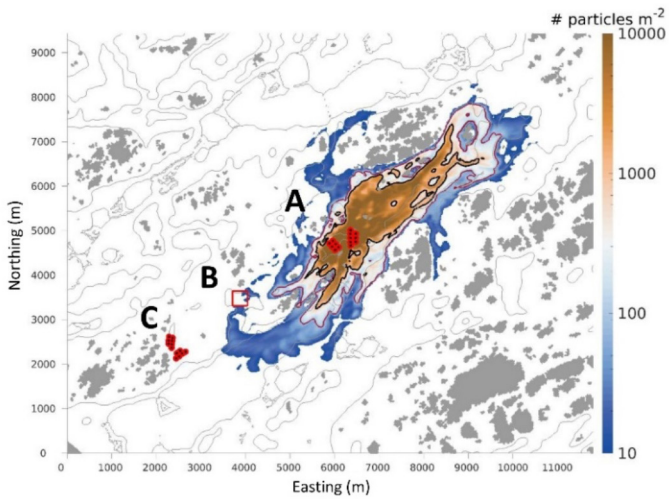

Figure 1: Model results showing maximum hydroid particle concentration after net cleaning on farm site A. Concentrations (particle m^{-2}) are shown accumulated for the upper 35 meters of the water column. Areas surround by blue contours have at least 10 particle m^{-2}, areas with red contours exceed concentrations of 100 particles m^{-2} and concentrations in areas within black contours are above 1,000 particles m^{-2}.

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To put the dispersal of hydroid particles from our case-study model farms into a broader spatial context, we quantified the distance of 936 salmon farms along the Norwegian coast to the salmon farm nearest to it. We then determined the proportion of nearest-neighbour farm distances around Norway's entire salmon aquaculture industry that was equal to or smaller than the dispersal distance of hydroid material quantified by our model.

Results and discussion

Hydroid particles released during simulated net cleaning at the two model farms resulted in a maximum concentration of 958,135 particles m^{-2} (integrated across the upper 35m of the water column) within individual pens. Particles released from one pen were transported into adjacent pens, leading to small 'peaks' in particle concentration even in pens that were not being cleaned. As a consequence, fish in individual pens were exposed to hydroid particles multiple times while they, or surrounding pens were being cleaned, increasing the risk for encountering harmful particles and gill injury. With recovery times after one-time exposure taking up to 1 week [5], repeated exposure may contribute to worsening gill conditions in salmon during time at sea.

Maximum dispersal of biofouling material released via cleaning occurred 48 hrs following onset of net cleaning operations (Figure 1). The largest unidirectional dispersal distance for hydroid particles was > 4.8 km. Of the 936 salmon farms along the Norwegian coast, 63% have at least one nearest neighbour farm within this distance. While our study was restricted to a case study location, these results suggest that inter-farm dispersal of biofouling material may also take place elsewhere.

Net cleaning may facilitate the dispersal of gill damage inducing particles between farm sites, including particles that are known to harbour pathogens. Novel cleaning tools that avoid the release of biofouling particles via containment or more regular cleaning (grooming) of nets [4] have the potential to reduce gill damage arising from biofouling maintenance operations.

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EFFECTS OF A CHRONIC EXPOSURE TO POLYSTYRENE NANOPLASTICS IN THE GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Nanoplastics (NPs), characterized as measuring below 1000 nm, represent a major part of plastic pollution, and are now considered ubiquitous in aquatic ecosystems (Sharma et *al.*, 2021). NPs have been detected and quantified in most environmental and urbanised matrices, with polystyrene (PS) being a commonly detected polymer (Dong et *al*., 2021). Their nanoparticle properties translate into the ability to travel with blood through an organism, and to cross biological barriers, such as the blood/brain barrier (Ma et *al.*, 2021). Although plastic contamination has been given increasing consideration over the past decades, little is still known on the effects of prolonged exposures to such pollutants in living organisms. The present study aimed to investigate the response of the commonly farmed gilthead seabream (*Sparus aurata*) to a waterborne exposure to PS-NPs of 42 nm diameter over a period of 28 days by investigating health and welfare parameters such as haematology and behaviour. In addition, accumulation of PS-NPs in different organs was investigated.

Material and methods

Juvenile seabream (9.15 ± 0.75 cm total length and 9.09 ± 1.73 g total weight) were randomly allocated into 9 experimental aquaria, each of which represented a replicate of either of 3 experimental conditions: Control (0µg/L); low concentration of NPs (100 µg/L) and high concentration of NPs (1000µg/L). Each aquarium contained 5 fish, and following an acclimation period in the experimental aquaria, the treatments were applied. Behaviour was recorded over a period of 10 minutes (2 minutes before feeding, while feeding and up to 8 minutes after feeding) on the first day of the challenge, and subsequently every 7 days. Following the 28-day exposure period, fish were randomly selected, and blood was extracted through caudal puncture using heparinized syringes. Samples were stored at 4 ºC and analysed using the automated haematological analyser SYSMEX XN-1000V adjusted for fish blood. Following blood extraction, fish were euthanised by spinal rupture, and gills, liver, gut, muscle, and brain were excised and immediately snap-frozen in liquid nitrogen. The video recordings were analysed using ImageJ (Mattson et *al.*, 2015), taking into consideration feeding time, distance travelled during swimming, and exploratory behaviour after feeding. Quantification of PS-NPs in tissue was performed by size exclusion chromatography (SEC) coupled to high-resolution mass spectrometry (HRMS), equipped with an atmospheric pressure photoionization (APPI) working under negative conditions.

Results and discussion

The haematological parameters considered were white blood cell count (WBC), red blood cell count (RBC), haematocrit (HCT), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and platelet count (PLT), amongst others. No significant differences were found in any of these parameters, which is in accordance with a previously published study investigating the effects of this polymer in the model organisms *Carassius auratus*. (Brandts et *al*., 2022). Gills, gut and liver were selected for PS-NPs quantification as they may represent a principal portal of entry for fish during waterborne exposure. Brain was also selected for this analysis, as NPs have been shown to cross the blood-brain barrier, and accumulation in this organ is likely to have strong deleterious effects on the health and welfare of fish, which will be potentially reflected by changes in behaviour. On the other hand, muscle was sampled for PS-NPs quantification as it represents a potential source of exposure to this contaminant for humans. Results will include both quantification of PS-NPs and behavioural analyses. At the overall functional level results reveal that variables are not initially affected by NPs, but at molecular and genetic level NP do induce alterations.

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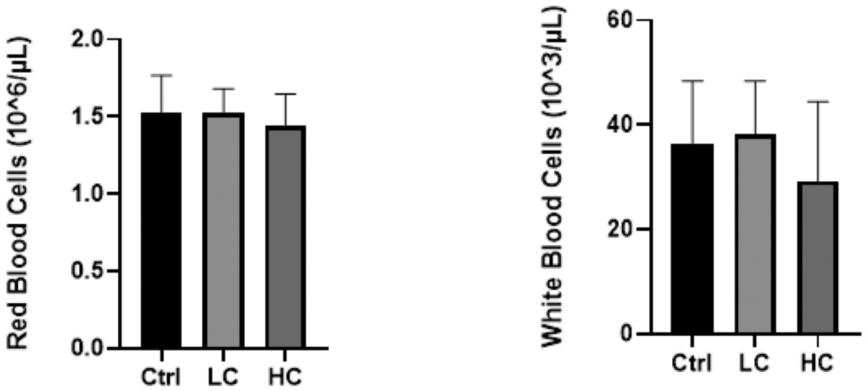

Figure 1: Erythrocyte (10^6/µL) and lymphocyte (10^3/µL) count obtained from haematological analysis with the SYSMEX XN-1000V following a 28-day waterborne exposure to PS-NPs (Ctrl: control group, exposed to 0.00µg/L PS-NPs; LC: low concentration group, exposed to 100µg/L PS-NPs; HC: high concentration group, exposed to 1000µg/L PS-NPs).

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GILL BIOPSY-BASED SORTING OF EUROPEAN CATFISH (*Silurus glanis* L. 1758) ALLOWS FOR REDUCED VARIATION OF PARASITE COUNT WITHIN EXPERIMENTAL GROUPS

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Introduction

European catfish (*Silurus glanis* L. 1758) is the largest predator in the freshwater bodies of Europe, located at the top of the food chain in most of the aquatic habitats. This species has remarkable somatic growth potential, robustness and high quality bone-less fillet positioning it as the next great candidate for recirculated aquaculture systems (RAS). The volume of aquaculture production is often reduced by infectious diseases. Among these, the European catfish-specific monogenean gill fluke (*Thaparocleidus vistulensis,* S. 1932) is a major pathological agent threatening farmed stocks, especially those kept in RAS facilities. Systematic studies on parasite-host interaction require regular access to diseased catfish. However, parasite loads could vary widely among hosts, which adversely affects the reliability of comparative studies. Furthermore, the daily management of gill parasite infected catfish stock on a laboratory scale is a challenge.

Materials and methods

We established a *T. vistulensis* parasite culture by cohabitating infected catfish individuals with healthy ones. Daily operation and maintenance over a prolonged period was achieved by regular water exchange (30%) combined with optimal water parameters (25±0.5 °C; pH of 7.13 ± 0.5; 80% O_2) for catfish.

At Experimental Location 1 (EL1), catfish were anaesthetized, and a 3x3 mm biopsy was cut from both filaments of the first gill arch within the gill chamber. The total parasite load of the individual was estimated with the parasite count obtained from the biopsy. Hosts with similar loads could then be paired, and the two members of the pair were eventually divided between the two experimental populations to test whether using this method would result in a catfish population with a more homogeneous overall parasite burden at EL1 (Figure 1.).

Individual fish were collected randomly at Experimental Location 2 (EL2) to analyze the parasite burden differences among hosts (Figure 1.).

Results

Randomly assembled groups of catfish hosts were analyzed for parasitic load by counting the gill fluke under the microscope at EL2. The assumption of equal variances has been violated for total parasite numbers between EL2 experimental catfish groups ($p=0.001$) resulting in a high variance in total parasite numbers.

No preference of *T. vistulensis* was observed between the two sides of the gill ($p=0.683$) however, the first and second arches showed significantly ($p=<0.05$ for both) higher load of parasites of the hosts, compared to the others.

Minimally invasive gill biopsy is proven to be a reliable tool (correlation: $p<0.001$; Spearman $r=0.7913$) to estimate the parasite load of a given individual in the size range of the examined catfish (48–84g). Biopsy-based pairing yielded reduced variation (equal population variances, $p=0.350$) in parasite counts within experimental groups (EL1) as compared to randomly assembled groups (EL2).

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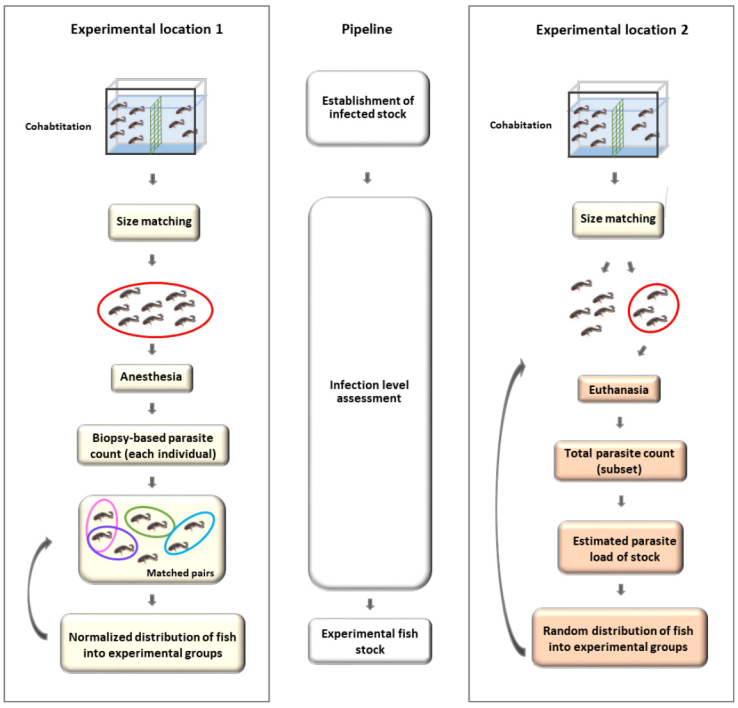

Figure 1. Generation of experimental groups of catfish infected by cohabitation, using a biopsy-based estimation of parasite load (EL1) and random collection (EL2).

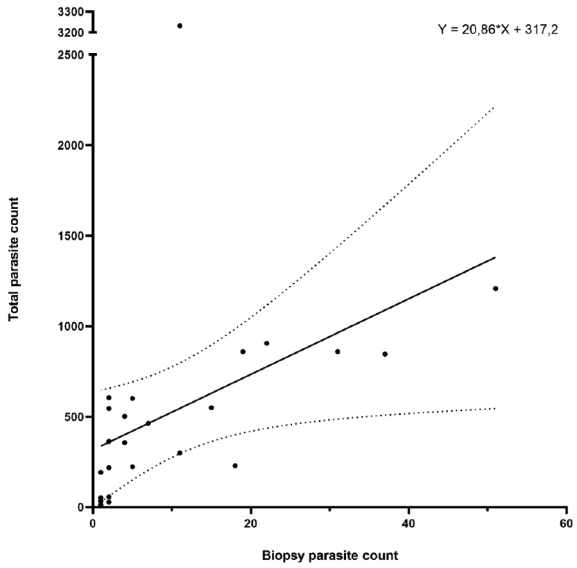

Figure 2. Gill biopsy-based parasite counts show statistically significant correlation with total parasite counts from the same individual.

Conclusions

We believe that our findings are novel and significant, and that our work will contribute to a better experimental grouping of catfish, infected with gill flukes. Furthermore, the method can be potentially adapted to other gill parasitizing monogeneans and other fish species as well.

Funding

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CULTIVATION OF GIANT GROUPER (*Epinephelus lanceolatus*) IN RECIRCULATING AQUACULTURE SYSTEMS (RAS) IN EUROPE

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Introduction:
Giant grouper, *Epinephelus lanceolatus***,** a species already successfully farmed in aquaculture in the Pacific region, appears ideally suited for RAS aquaculture in Europe because of its good growth performance, known reproduction and market potential. However, for successful cultivation in RAS, reliable and site-adapted data must be available. Preliminary studies were carried out for future commercial cultivation in closed facilities and economic calculations for the species were performed for Germany. An important aspect is the identification of formulated feeds that meet the requirements of the species and can be purchased in Europe. In addition, it is necessary to determine an optimal rearing density at which the animals can grow in an economically viable manner while optimizing animal welfare.

Material and methods:
For the feed trial, commercially available feeds in Germany were tested. 3 basically suitable feeds with different protein/fat ratios were selected for the trial. Feed 1: 42/13, Feed 2: 54/14, Feed 3: 55/17. Juvenile grouper (61.5 ± 6.9 g) were fed ad libitum in replicate (4) four times daily for 35 days. At the end of the experiment, growth- and blood parameters and body composition were determined. In a second experiment, juvenile grouper (437.4 ± 40.3 g) were kept in quadruplicate tanks for 8 weeks at different stocking densities - low: 60 kg/m³, medium: 80 kg/m³, high: 100 kg/m³. Growth, stress parameters and external appearance were determined regularly.

Results and conclusion:
Results showed that 2 of the 3 tested diets, appear suitable in principle for giant grouper (table 1). Growth was comparable to previous studies using optimized diets Lin / Yeh (2022). However, insufficient protein content has a negative effect on the animals. In addition, it was shown that the tested rearing densities had no effect on growth, stress and external appearance in the trial. These data, together with production data of existing giant grouper aquacultures and site-specific financial and market data, confirm that the giant grouper appears suitable for cultivation in RAS in Europe.

Table 1. Growth performance of juvenile Giant Grouper fed diets with different protein/fat ratios. N = 12, Mean ± SD.

Growth parameter	Feed 1	Feed 2	Feed 3
Individual weight$_{Start}$	61.25 ± 7.36	61.72 ± 7.10	61.47 ± 6.32
Individual weight$_{End}$	151.67 ± 17.46	172.54 ± 16.83	179.97 ± 17.05
Ind. weight gain	90.42 ± 1.45	110.83 ± 1.59	118.51 ± 4.92
Condition factor$_{Start}$	1.97 ± 0.14	1.95 ± 0.15	1.96 ± 0.14
Condition factor$_{End}$	2.20 ± 0.17	2.11 ± 0.13	2.27 ± 0.14
SGR (% daily weight gain)	2.59 ± 0.03	2.94 ± 0.03	3.07 ± 0.08
DFI (% body weight per day)	2.05 ± 0.02	1.98 ± 0.03	2.05 ± 0.09
FCR	0.84 ± 0.01	0.73 ± 0.00	0.73 ± 0.03

Lin, Y.-H., & Yeh, C.-C. (2022). Effects of dietary lipid levels on growth, lipid deposition, oxidative stress and hepatic morphological changes in giant grouper, *Epinephelus lanceolatus*. *Aquaculture Research*, 53, 2431–2438. https://doi.org/10.1111/are.15760

Calanus finmarchicus HYDROLYSATE IMPROVES GROWTH PERFORMANCE, REDUCES OXIDATIVE STRESS, AND PROMOTES BETTER INTESTINAL HEALTH OF EUROPEAN SEA BASS JUVENILES

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Introduction

The world will be dependent on the development of novel feed ingredients from renewable sources to ensure sustainable growth of the aquaculture industry. A more balanced harvesting approach across the ecosystems can maintain ecological diversity in the oceans and make alternative marine resources available as future feed ingredients for aquaculture. Zooplankton like *Calanus finmarchicus* are viable raw material candidates, as they have optimal nutrient profiles for aquatic animals and may be sustainably harvested in large volumes. In this study, the aim was to investigate if a novel protein hydrolysate of *C. finmarchicus* could influence growth performance, oxidative stress, and intestinal health of European sea bass (*Dicentrarchus labrax*) juveniles in a feeding trial lasting 84 days.

Material and methods

The effect of dietary inclusion of hydrolysates was tested in a feeding trial with European sea bass juveniles, benchmarking calanus hydrolysate (CH) against other commercial hydrolysates of sardine, tuna, and salmon at 5 % inclusion. Fish were group weighed at day 0, 30, 67, and 84. Three fish per replicate tank were sampled for health assessments; the liver for analysis of oxidative stress (hepatic protein carbonyls and antioxidant enzymes), and faecal content for intestinal status (calprotectin and mucins). Data were subjected to a one-way analysis of variance (ANOVA), and means were compared with the Tukey method. Statistical significance was tested at a 0.05 probability level (n =3).

Results

CH improved growth performance of European sea bass juveniles, showing higher body weight and lower FCR compared to diets with other marine hydrolysates (Fig. 1).

The health assessment results at the end of the trial showed significantly lower oxidative stress (Fig.2A) and lower intestinal inflammation (Fig. 2B) in European sea bass juveniles fed diets with CH inclusion.

Conclusions

Dietary inclusion of CH at 5 % led to increased growth performance of European sea bass juveniles in the feeding trial. CH was also associated with significantly lower oxidative stress and less inflammation in the intestines, highlighting the potential of the hydrolysate as a functional feed ingredient for improved growth and health.

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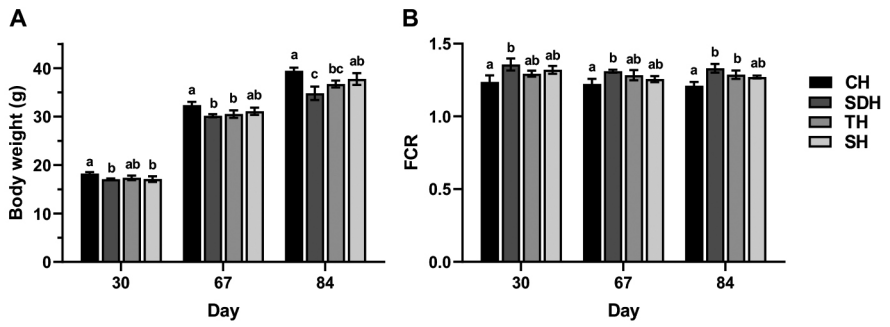

Fig. 1. Benchmarking growth performance of European sea bass juveniles fed diets with marine hydrolysates at 5 % inclusion. A: Body weight. B: Feed conversion ratio (FCR). CH: Calanus hydrolysate. SDH: Sardine hydrolysate. TH: Tuna fish hydrolysate. SH: Salmon hydrolysate. Lower case letters (a, b, c) denote statistical differences at $p < 0.05$ (n = 3).

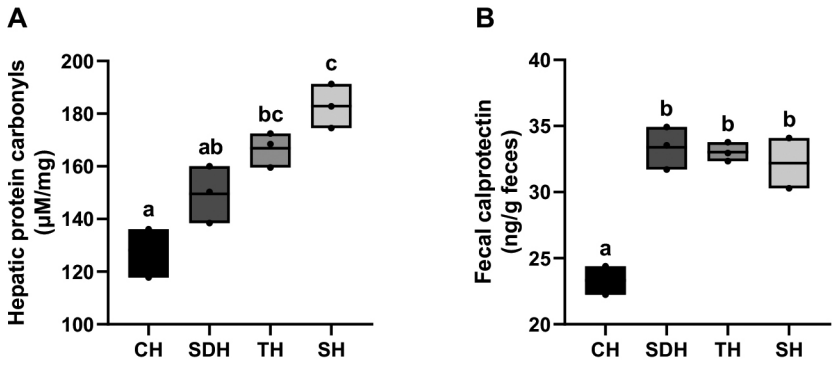

Fig. 2. Benchmarking oxidative stress and intestinal inflammation in European sea bass juveniles fed diets with marine hydrolysates at 5 % inclusion. A: Hepatic protein carbonyls as a biomarker of oxidative stress. B: Fecal calprotectin as a biomarker for intestinal inflammation. CH: Calanus hydrolysate. SDH: Sardine hydrolysate. TH: Tuna fish hydrolysate. SH: Salmon hydrolysate. Lower case letters (a, b, c) denote statistically significant differences at $p < 0.05$ (n =3).

IMTA IN ACTION: TWO DECADES OF GROWING THE GREEN SEAWEED *ULVA* IN INTEGRATED LAND-BASED SYSTEMS ON SOUTH AFRICAN ABALONE FARMS

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Introduction

The first commercial system using abalone effluent to cultivate the green seaweed *Ulva* was built on an abalone farm in South Africa in 2002. Over 1000 tonnes fresh weight of *Ulva* was produced in South Africa in 2007, grown predominantly in abalone effluent, and the main reasons for this seaweed production was as additional feed for the abalone, as well as to provide bioremediation of the effluent to enable partial water recirculation on a section of a single farm. Bolton et al. (2009) carried out a SWOT analysis of the use of these abalone/IMTA systems. After a further 15 years the aim of this contribution is to re-examine the findings of the analysis of Bolton et al. (2009) in the light of the subsequent development of the abalone/*Ulva* IMTA commercial operations in South Africa.

Progress and strengths

The strengths previously documented are largely still applicable, and so we could say that the development of the South African abalone aquaculture industry, and the role of IMTA, has developed as predicted with no major changes. The *Ulva* produced on abalone farms is not sold but is primarily utilised on-farm as feed addition. Thus, official government figures, the latest being 2718.10 tonnes in 2020, are an estimate. There are 5 farms (of the total of 14 commercial abalone farms in South Africa) which operate ca. 30m-long paddle raceways to cultivate *Ulva*. Four of these grow the *Ulva* in abalone effluent, and the other grows only in fertilised seawater. Thus production has increased by almost threefold in the last 15 years. Also in 2007 there was a newly built section of a single farm operating on partial recirculation using *Ulva*, and since 2014 two entire new farms are operating on this principle. The success of these integrated operations is evidenced by the continuous fully commercial operation in various forms for more than 20 years. The integrated system warms the water for abalone cultivation resulting in increased growth rates, and there are demonstrated improvements in the microbiome and other aspects of system and animal health.

Problems and weaknesses

Apart from the COVID-19 pandemic, the major setback has been a large and unusual dinoflagellate bloom (HAB) in January 2017 resulting in losses of >250 tonnes of abalone at three farms. At least one other farm successfully made use of the predicted potential of the IMTA system to switch to 100% recirculation. This can be done for at least 3 days without adverse effects on integrated farms. Lack of knowledge of the *Ulva* crop has been largely remedied, and most of the predicted weaknesses are well managed.

Opportunities

These systems have indeed assisted with the continued expansion of the local abalone industry, and integration is becoming widely acknowledged as a potential benefit both locally and internationally. Much work is being conducted on the benefits of *Ulva* as a component in formulated aquafeeds, as predicted. The stated benefits to sustainability of this system are becoming better recognised but are still far from being fully realised.

Threats

Diseases and grazers have thus far proved amenable to successful management. The predicted two major threats to IMTA expansion still exist. The cost of using space to grow *Ulva* rather than profitable abalone was borne out by a farm which was designed as an IMTA system but was changed to a pure abalone cultivation shortly before construction. A main reason cited was potential for biosecurity risks with recirculation of water and/or seaweed through the system. Nevertheless, area under IMTA has considerably increased although some producers are still reluctant to integrate their operations due to these thus far unsubstantiated risks.

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Conclusions

These South African abalone/*Ulva* integrated aquaculture systems are slowly becoming recognised as a leading example of IMTA in action. The benefits to the system with respect to biosecurity, both in water integration and seaweed as feed component, are increasingly being demonstrated. Nevertheless there is still the perception of recirculation as a disease transmission threat, which could slow further development.

Aspects of these systems have been replicated in several other world regions, including in Europe and Australia. There are three main reasons for growing *Ulva* in these land-based systems: 1: as a product (feed, feed component, biomaterial, human food etc.), 2: to enable partial recirculation saving pumping costs, 3: to bioremediate effluent for environmental benefits. In different regions/IMTA systems one or more of these benefits may dominate the reasons for integration. It seems very likely that the application of land-based, seaweed/invertebrate integrated aquaculture will continue to be developed as a successful example of marine IMTA in South Africa and around the world.

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VERIFICATION OF THE ORIGIN OF STURGEON PRODUCTS DOWN TO FARM LEVEL USING STABLE ISOTOPIC METHODS – PROOF OF CONCEPT

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Sturgeons are the world's most critically endangered group of species. One reason for this dramatic situation is the long-time unsustainable and often intransparent or even illegal sourcing and trading of the fish or its products, such as caviar. For sturgeon conservation, it is fundamentally important that products of poached sturgeons cannot infiltrate the legal market and that sturgeon aquaculture and trade are fully trustworthy. Furthermore, it is in the interest of caviar producers and traders to increase consumer confidence and to prove that their products and their business conduct are fully correct and conforming to the law at every stage of the supply chain.

The quantitative measurement of stable isotopes has the potential to determine the regional provenance of food commodities as it has been established for various agricultural and animal products, such as beef, dairy products, honey, and beverages. To increase transparency and traceability further, it is also necessary to track the origin of products back to farm level. That technology was already demonstrated in the BLE-funded Watermark project (2015, FKZ-28-1-91.024-13). It was shown that tracing pigs and cattle to the farm is possible within a probability context.

Traceability possibilities have not been tested for sturgeon yet. Therefore, a proof of concept has been launched to verify the potentials to generate individual signature patterns of sturgeon farms and their products (caviar, meat) based on stable isotope profiles

The focus is to verify how robust the isotope signatures are at the sturgeon farm over a longer rearing period. As a rule, the animals' tissue and tissue water reflect the isotopic signatures of the local water and feed. However, isotopic profiles of local water source and feed can change over time due to seasons. In addition, there is a risk that processing steps (extraneous water, salts) can also influence traceability.

Accordingly, in the Transparent-Sturgeon project, caviar, water, sturgeon and feed samples are taken from different European farms over a longer period of time and the robustness of the farm signature is tested. In a first step it could be shown that isotope signatures from 50 caviar samples across three different farm locations in Belgium, Germany and Italy were constant over a period of at least 3 to 6 months.

These results are promising to enhance the dataset with additional farms and extended sample periods. Thus, a standardized protocol could be established of tracking the origin of traded caviar back to the farm level which will help to increase transparency in the caviar market.

INFLUENCE OF STRESS, SEX AND SEXUAL MATURITY ON SCALE CORTISOL AND DHEA CONCENTRATIONS IN RAINBOW TROUT (*Oncorhynchus mykiss)*

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Introduction

Fish welfare is traditionally assessed by measuring cortisol levels, which is the quintessential stress hormone in these animals. However, the best practice to assess animal welfare is to utilize multiple indicators in order to provide a more comprehensive portrait of the animal condition, especially when assessing long-term stress. Among the physiological indicators, dehydroepiandrosterone (DHEA), a precursory androgen with anti-stress effects, seems to be promising in diagnosing mammalian chronic stress through the cortisol:DHEA ratio [1] besides DHEA has recently been demonstrated to increase in fish exposed to a chronic stress [2]. However, while DHEA in mammals has been shown to act in opposition to cortisol in many physiological pathways and to exhibit antioxidant, neuroprotective and immuno-protective characteristics [3], still very little is known about its physiological meaning and possible implication in the stress response in fish. For this reason, the present study aimed to investigate how the levels of this steroid are affected not only by a common stressful aquaculture practice, but also by sex and degree of sexual maturity in rainbow trout (*Oncorhynchus mykiss*) scales, a promising medium for multi-hormone stress analyses [4].

Materials and methods

The experiment was performed at a commercial farm where four groups of rainbow trout (n=96) were sampled: mature females (990 ± 28.5 g) and males (1005 ± 40.7 g), immature females (1350 ± 48.8 g) and males (1394 ± 33.3 g). Half fish of each group were subjected to 30 minutes confinement stress and the other half were controls. Prior to sampling, fish were sacrificed with an excess of anesthetic (MS222 Sigma-Aldrich) and subsequent cut of the spinal cord. Scales were collected after removing excess mucus by scraping the side of the fish with a small plastic rod and subsequently stored at -20°C until the analysis.

Cortisol and DHEA were quantified in the scales using a specific microtitre radioimmunoassay (RIA). Different washing protocols were tested and the RIA protocol was adapted and validated. All the data are expressed as mean ± standard error and were previously evaluated for normal distribution. Differences between treatments were analyzed using a general linear model (GLM) using *sex*, *maturity* and *stress* as main factors. The level of statistical significance was set at $p < 0.05$.

Results and discussion

Among the three factors studied, the degree of sexual maturity was the major factor influencing cortisol concentration, with mature fish having significantly higher cortisol levels than the immature ($p<0.01$). This result aligns with previous studies conducted on rainbow trout, in which mature individuals, both males and females, showed higher plasma cortisol levels than immature [5]. Differently, sex affected DHEA concentration, with males showing significantly higher levels than females ($p<0.05$). To our knowledge, DHEA has not been previously quantified in relation to sex in fish and thus there is currently no information available to compare our findings. However, there is evidence of higher DHEA concentrations in males than females both in humans and other mammals [6,7]. Results did not show stress-related difference in both cortisol and DHEA levels. This could be linked to the kind of stress applied in this study, i.e. acute, and to the bony nature of the scales, that slowly accumulate hormones. Nevertheless, the cortisol:DHEA ratio, resulted higher in the stressed fish compared to the controls, although not significantly ($p=0.058$). At present, knowledge about the involvement of DHEA in fish stress response is scarce. However, considering that it has been shown to counteract cortisol in mammals, this ratio might better describe an animal's stress status than either hormone alone. Indeed, in humans and other vertebrates, high ratios of cortisol to DHEA have been considered indicative of chronic stress [8]. Finally, the meaning of the interactions between the three factors studied (sex, maturity and stress) is still under investigation.

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Conclusions

In the present study, both cortisol and DHEA levels were successfully quantified in the scales of rainbow trout. Furthermore, the obtained levels are perfectly in line with those reported in the only other study currently present on rainbow trout exposed to chronic stress [2], attesting the applicability of the RIA method developed in this study in quantifying these hormones in fish scales. To our knowledge, this is the first time DHEA has been evaluated with respect to sex and degree of sexual maturity in fish. Nevertheless, further investigation is required to better understand the role of this hormone in fish physiology. Moreover, these results need to be implemented by assessing a longer-term stress in order to test the suitability of DHEA as an alternative physiological indicator to be used alongside cortisol in the assessment of chronic stress in fish.

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FURTHER STEPS IN SPERM HANDLING IN AQUACULTURE

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Introduction

The most apparent aquaculture benefit of the reproduction of external fertilizers, such as fish, is that their gametes which are naturally released into the environment where fertilization occurs, could be collected before fertilization and used in artificial breeding measures. Thus the gametes handling becomes almost an indispensable part of any artificial reproduction in most fish species. It allows optimization of fertilization rates, producing large numbers of healthy offspring, and applying selective breeding programs, increasing the quality and efficiency of artificial reproduction.

Compared to artificial reproduction programs in other species, including mammals, which generally focus on acquiring a relatively small number of individuals, fishes produce a huge quantity of gametes in both sexes and rely on a large number of offspring. In such a situation, preserving the high quality of maximal numbers of eggs and spermatozoa before fertilization is essential. At the same time, it is important to keep enough high genetic variability to avoid inbreeding and potential homogeneity of progeny.

In this report, we are summarising our recent results on sperm handling and suggesting the most promising steps for optimizing and improving sperm handling methods in the future.

Results

Sperm cryopreservation. Currently, cryopreservation protocols cover the vast majority of commercially important fish species with high preservation of sperm motility and fertilisability after thawing, using different cryoprotectants and freezing techniques. Moreover, sperm cryopreservation still requires the next steps in technology development, probably by cost-cutting and simplifying the methods, to become widely used in aquaculture.

In the past few years, we (1) optimized the protocols for sturgeon and carp sperm cryopreservation by adjustment of sperm concentration before freezing (Nascimento et al., 2021; Sotnikov et al., 2023); (2) tested using hypertonic cryopreservation media for cryopreservation of large volumes of carp and sterlet spermatozoa required for fisheries practice (unpublished data); (3) optimized two most common methods of uncontrolled sperm cooling by floating raft in Styrofoam box and dry shipper container (Horokhovatskyi et al., 2017).

Short-term sperm storage. Similarly to cryopreservation, many protocols for short-term storage exist for fish spermatozoa. In contrast to cryopreservation, short-term storage accumulates damage in the spermatozoa slowly, and the last storage stages are commonly associated with increased bacterial growth, suggesting using antibacterial storage media. Therefore understanding the changes in the spermatozoa during storage is the critical element for further improvement of this handling method.

Recently we tested the effect of hypothermic storage: (1) on the epigenetics of sperm and the resulting embryos (Cheng et al., 2023); (2) on spermatozoa's ability to tolerate temperature (Zhang et al., 2023) and osmotic shock (unpublished data); (3) on spermatozoa metabolome (unpublished data) and (4) bacterial contamination and apply polyphenolic extracts as an alternative to antibiotics (unpublished data).

Sperm separation. Unavoidable damage (due to ice crystals growing in cryopreservation or sperm aging during short-term storage) results in the appearance of a nonvaluable and semi-valuable population of spermatozoa (which still could be involved in fertilization). Thus, developing spermatozoa separation methods is the most promising next step for improving fertilization outcomes by eliminating damaged sperm populations. At the same time, these methods may allow us to study the separated populations more deeply to understand their specific physiological differences and possibly resistance to different types of damage.

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Currently, we are developing and testing methods for spermatozoa separation in different fish species by ultrasound (unpublished data) and Percoll gradient (Horokhovatskyi et al., 2018). At the same time, we are searching for the metabolic markers of individual sperm samples which can predict their storage and freezing capacity to maximize the outcomes.

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EX SITU BIODIVERSITY CONSERVATION*:* IMPORTANCE, ISSUES AND STRATEGIES

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Introduction

Sturgeon species and populations have experienced a dramatic collapse mainly due to a progressive disruption of habitat suitability in their distribution range and overexploitation for commercial purposes (He et al., 2017). This has pushed many of the species to the brink of extinction (Congiu et al., 2023). Consequently, massive efforts have been implemented in the past and others will be needed in the future, to rescue these threatened species.

In this context, *ex situ* conservation is a cornerstone of protection, maintenance and propagation of species outside their natural habitats and should guarantee needed conditions for secure life and optimal breeding so that releases can be put in place in the future (Overton et al., 2023). For sturgeons, *ex situ* conservation is entrusted, in a relevant part, to aquaculture facilities where they are farmed mainly for commercial interest, opening a heated debate about the use of aquaculture stocks for conservation purposes and about which features they should have to ensure their management success from establishment to propagation (Froehlich et al., 2017).

Results and Discussion

High long-term performances are achieved if the *ex situ* strategy aims at maintaining genetic diversity of the stocks in order to minimize adaptation to captivity and genetic erosion and maximise adaptive potential after release. This is complicated in sturgeons by their complex genomes, long life cycles, late sexual maturity and large size as well as by the ease interspecific hybridisation with production of fertile progeny.

Models, principles and *ex situ* strategies for an informed biodiversity conservation of aquaculture stocks are presented and discussed on the bases of case studies on different sturgeon species (Barca et al., 2022; Boscari et al. 2121; 2022). Important conservation topics that will be covered are: the role of the identification of conservation units or geographic structured populations, the importance of stocks' pureness, the principles underlying the selection of target stocks for conservation such as the assessment of relatedness and the reconstruction of pedigree information, and useful strategies to set-up specie-specific breeding plan.

Conclusion

The aim of this presentation is to stimulate the discussion on the importance of a shared approach among different stakeholders. Recognition of this need will require adoption of a new paradigm for sturgeon *ex situ* conservation, in which on the one hand aquaculture makes an additional effort to preserve diversity while on the other hand conservation projects and competent authorities support the extra costs of farming-for-release. The protection of the most endangered group of animal species would certainly benefit from this change of perspective.

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CULTIVATION OF HIGH-ADDED VALUE SEAWEED SPECIES IN A ON-LAND OYSTER-SEAWEED IMTA SYSTEM: THE CASES OF *Codium tomentosum* AND *Chondrus crispus*

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Introduction

Based on the association of different species connected by trophic interactions, the concept of Integrated Multi-Trophic Aquaculture (IMTA), performed in-land, aims to improve water quality of aquaculture systems and this diversification would ensure more stable incomes for producers. Oyster farmers usually perform mono-specific cultures, leading to a high vulnerability of economic spinoffs linked periodically to poor water quality and population declines, highlight a need for securing the sector's future. Therefore, IMTA including seaweeds in oyster systems should be tested to evaluate potential of seaweeds 1) to improve water quality and 2) to add economic value to oysters' farmers. In this context, we performed pilot IMTA experiments with Pacific cupped oysters and two high-added value seaweed species, *Codium tomentosum* and *Chondrus crispus*. In this study, we particularly explore:

- the impact of oysters' effluents on growth and composition of both seaweeds
- the potential of seaweed nutrients uptakes

Materials and Methods

Specimens of *C. tomentosum* and *C. crispus* were collected in Brittany (France) from longlines on the CEVA concession and from mid-littoral tide pools at Pors Rand respectively. Half-raising oysters *Magallana gigas* were provided by a local oyster farmer. For each seaweed species, the experiment was designed with the following conditions:

- a seaweed monoculture in 340L tanks at a density of *ca*. 1.2 g/L, used as a control of the oyster-seaweed experiments
- a co-culture of seaweed in 340L tanks at a density of *ca*. 1.2 g/L, in a recirculating system with 340L tanks containing oysters at a density of *ca*.5 g/L
- (Only for *C. crispus*), a seaweed monoculture in 340L tank at a density of *ca*. 1.2 g/L, enriched with F/2 culture medium, used as a positive control of "classical" on-land seaweed production.

Except the F/2 culture medium condition which was run in duplicate, all conditions were run in triplicate. Oysters were fed every 2 days with a Shellfish Diet following the manufacturer recommendations. Seawater of each tank was renewed weekly and was sampled before and after renewal to assess ammonium, nitrates, and phosphate contents. The whole biomass of each seaweed tank was weighed every two weeks to estimate the Specific Growth Rate (SGR). In addition, visual appearance of seaweeds and epiphyte development were qualitatively recorded during the experiment. At the end of each experiment, fresh biomass of each replicate was weighted and dried to evaluate the dry matter content and components of economic interests were quantified, i.e. glucuronic acid and sulphated polysaccharides for *C. tomentosum* and carrageenan for *C. crispus*.

Results & Discussion

Whatever the seaweed species, the recirculating system with oysters rearing showed a positive effect on SGR and fresh biomass of seaweeds. Indeed, SGR was significantly higher in the co-culture condition with SGR values between 1.5 and 4 times higher compared to the control. This difference was particularly marked during the first weeks of cultivation for *C. tomentosum* and after 4 weeks for *C. crispus*. However, due to the high nutrients input from oysters in the co-culture condition, a high development of epiphytic organisms has been reported some weeks after the beginning of the experiment pointing out the need of pure initial biomass. By opposite, a discoloration of specimens of both seaweed species in the control condition was observed which suggests a nutrients/minerals/vitamins deficiency (Fig. 1 for *C. crispus*). However, for both seaweed species, almost no significant differences have been observed between the co-culture and the control condition in the components analysed, suggesting that oyster-seaweed IMTA systems are likely to produced seaweed of valuable commercial interest. Interestingly, *C. crispus* cultivated in seawater enriched with F/2 medium displayed the highest values of SGR during the first weeks of the experiment and the highest final biomass. Moreover, *C. crispus* specimens showed a high-volume development compared to both other conditions (Fig. 1). Therefore, even if oysters provide enough nutrients to increase biomass gain of seaweeds, the F/2 medium remains more efficient.

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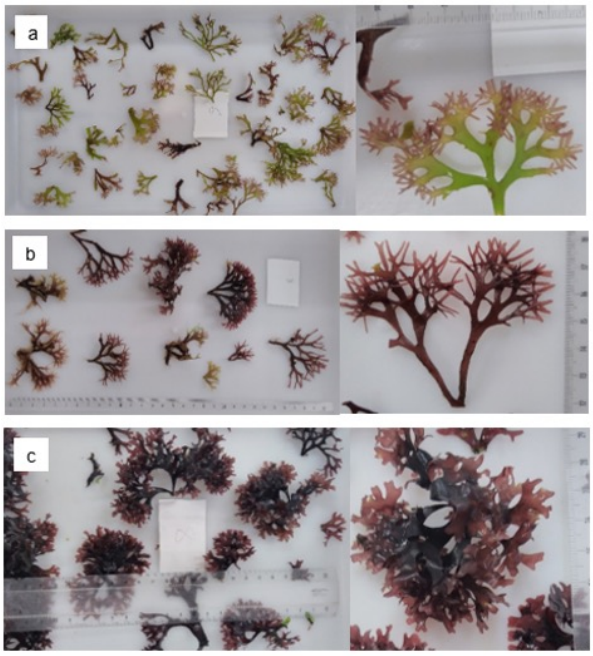

Fig. 1. Visual appearance of Chondrus crispus after 12 weeks in recirculating system with three different culture media (a: seawater only, b: seawater with oysters, c: seawater with F/2 medium)

Despite high concentrations in renewal water, nitrates are almost completely consumed by both seaweed species in all conditions, suggesting that *C. tomentosum* and *C. crispus* are efficient to recycle nitrogen inputs from either natural environment or oysters' excretion. For phosphate concentrations, higher values were reported in the co-culture condition compared to the control for *C. tomentosum* while similar values were observed for *C. crispus*, suggesting that *C. crispus* is more efficient than *C. tomentosum* in recycling phosphates from oyster effluents. No clear pattern has been highlighted for ammonium.

Altogether, this study provides first insights into the potential of IMTA with oysters to growth economic high-added value seaweed species. Beside the economic interest, the two seaweed species studied, but particularly *C. crispus*, could be considered as new biofilters to extract effluents from various on-land rearing such as fish or shrimp ponds.

REUSE OF WATER FROM BFT SYSTEMS THROUGH DENITRIFICATION PROCESS FOR *Penaeus vannamei* FARMING

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Introduction

Nitrification-dominated bioflocs are a common challenge in aquaculture systems, where nitrate production becomes constant and accumulates in the system. The build-up of nitrate over several cycles can harm farmed animals, causing reduced growth and survival. In order to overcome this production bottleneck and treat the water, strategies such as the biological denitrification process must be adopted to make the water suitable for culture or disposal. This study aimed to evaluate the performance of *Penaeus vannamei* and the water quality after being subjected to a biological anaerobic denitrification process using water from a biofloc technology (BFT) system. By assessing the effects of the denitrified water on the growth and survival of the shrimp, as well as on water quality parameters, this study provides valuable insights into the potential benefits of using denitrified water in BFT systems, ultimately contributing to the sustainable development of aquaculture.

Material and Methods

A 63-day experiment was carried out at the Marine Station of Aquaculture of the Federal University of Rio Grande. *P. vannamei* juveniles (1.30 ± 0.48 g) were stocked in 150-liter tanks at a density of 500 animals/m^3. The experiment consisted of comparing denitrified water and natural seawater (never used for shrimp cultivation) under different biofloc formation strategies: with or without mature biofloc inoculum. Thus, the treatments were: DWI – denitrified water with inoculum; DWF – denitrified water without inoculum; SWI – sea water with inoculum; and SWF – seawater without inoculum, with three replicas each. In the treatments with biofloc inoculum, 15 L of water from a mature biofloc system was, added to the tanks (10% of the working volume). In the treatments without inoculum, the bioflocs formation was stimulated from zero through the manipulation of the C/N ratio. Temperature, salinity, dissolved oxygen, pH, ammonia, nitrite, nitrate, alkalinity, and total suspended solids in the water were monitored. To assess growth and health conditions, 30 animals were randomly collected each week from each unit, weighed individually, and then returned to the tanks. At the end of the experiment, survival, final weight, weekly growth, and productivity were evaluated.

Table 1 - Water quality parameters of *P. vannamei* rearing in a biofloc system with water subjected to anaerobic denitrification. Different letters on the same line indicate a significant difference ($p<0.05$) between means.

	SWF	SWI	DWI
Dissolved oxygen	6,13 ± 0,35	6,19 ± 0,40	6,11 ± 0,41
Temperature (°C)	28,48 ± 1,64	28,36 ± 1,69	28,65 ± 2,08
pH	8,02 ± 0,18^b	7,97 ± 0,17^b	8,21 ± 0,23^a
Salinity (g/L)	30,90 ± 1,00	30,99 ± 1,00	30,47 ± 1,28
TAN (mg/L)	2,61 ± 2,37^a	0,33 ± 0,26^b	0,23 ± 0,14^b
Nitrite (mg/L)	12,32 ± 10,84^a	2,02 ± 3,17^b	1,43 ± 3,13^b
Water exchange (%)	173,00 ± 83,27	0 ± 0	0 ± 0

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Results

Animals exposed to denitrified water treatment without biofloc inoculum (DWF) died after one week of cultivation. The 100% mortality found in the treatment using denitrified water without biofloc inoculum could have been caused by various factors such as the colonization of opportunistic microorganisms in the environment, leading to diseases and ultimately death of the shrimps; the formation of nitrogenous disinfection by-products, which are known to be toxic to aquatic organisms; some unidentified metabolite or compound produced during the denitrification process, leading to adverse effects on the shrimps. Further investigation is necessary to identify the exact cause of the mortality and prevent future occurrences. Either way, no differences in survival (<89%) were found in the treatments using denitrified seawater with inoculum and natural seawater treatments, which indicates that the biofloc inoculum served as a biological treatment in denitrified seawater, readjusting the water for cultivation. Differences in the water quality parameters were found in ammonia, nitrite, nitrate concentrations, as well as in water exchange volume, organic carbon, and alkalizing agents between treatments. Total ammonia concentrations were highest in the treatment without biofloc inoculum, and nitrite concentrations were higher in the same treatment. Final nitrate concentrations were highest in the denitrified water treatment and lowest in the seawater treatment without inoculum. The treatment with denitrified water had higher pH values, and no significant difference was found in alkalinity concentrations between treatments. The treatments with inoculum had no water exchanges and organic carbon supplementation. The results indicate that it is possible to use denitrified seawater from a biofloc system for shrimp farming. However, alternatives for water treatment after the denitrification process need to be investigated.

Conclusion

The findings suggest that water from a biofloc system can be recycled by undergoing anaerobic biological denitrification. The denitrification process used is straightforward and can be performed in sedimentation basins or the same tanks used for cultivation once the animals are removed. This reduces the requirement for equipment and operational procedures. However, alternatives for further treatment of denitrified water are still necessary to ensure its suitability for cultivating *P. vannamei*, irrespective of the use of biofloc inoculum.

DO COMMON STUNNING METHODS RENDER AFRICAN SHARPTOOTH CATFISH (*Clarias gariepinus)* INSENSIBLE PRIOR TO SLAUGHTER?

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Introduction

Farmed fish are a critical source of food worldwide, and aquaculture is expected to provide nearly two-thirds of the world's fish supply by 2030. However, there is growing concern about the welfare of farmed fish, as many slaughter procedures have the potential to cause substantial suffering for prolonged periods of time. To address this issue, it is crucial to conduct comprehensive evaluations of potentially more humane slaughter procedures.

Humane slaughter requires that fish are stunned prior to killing (*i.e.*, rendered insensible) and remain so until death without experiencing avoidable fear, anxiety, pain, suffering, or distress. To assess insensibility following stunning, behavioral indicators such as the ability to maintain equilibrium, reactions to painful stimuli, the vestibulo-occular or 'eye roll' reflex, and ventilatory reflexes have traditionally been used. However, it has become increasingly clear that these indicators alone are insufficient, and that neurophysiological evidence of insensibility must be obtained to ascertain the effectiveness of stunning.

A relatively robust approach for gauging the state of sensibility in fish is by assessing the presence or absence of visually evoked responses (VERs) from electroencephalographic (EEG) measurements. VERs are measurable changes in brain electrical potential in response to a visual stimulus (*i.e.*, a flashing light) that produce a distinct waveform in EEG recordings milliseconds after the stimulus. This makes it a powerful tool for evaluating the effectiveness of stunning procedures, as the abolition of VERs has been confirmed to be an objective and unequivocal indicator of insensibility in numerous species of mammals, birds and fish.

Thus, here we utilized a non-invasive electroencephalographic (EEG) method to monitor changes in the state of sensibility of African sharptooth catfish (*Clarias gariepinus*) to assess the effectiveness of various stunning procedures (Brijs *et al*., 2021).

Materials and methods

A custom-made suction cup fitted with electrodes was attached externally to the head of the catfish (Fig. 1A-B). The EEG of catfish was then continuously measured in response to 150 ms light flashes at 2 Hz from a strobe-light in a dark room. EEG signals were subsequently filtered and analysed for VERs for 10 min prior to stunning (Fig. 1C).

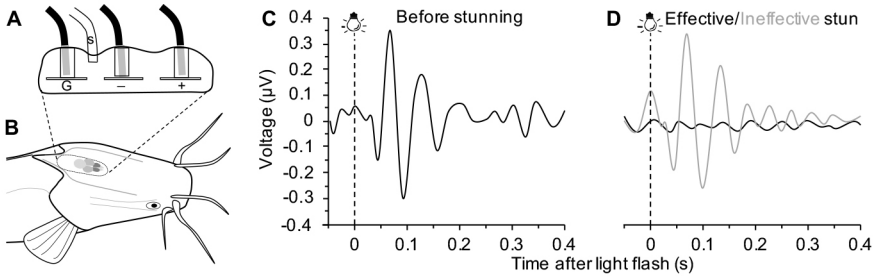

Figure 1: A schematic of the non-invasive EEG recording device (A) and its placement on an African sharptooth catfish (B). Representative examples of visually evoked responses (VERs) before stunning (C) and after an effective (black trace) or ineffective (grey trace) stun (D). Figure adapted from Brijs *et al.* 2020.

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Following the pre-stunning period, catfish were subjected to either ice chilling (*i.e.*, immersion in an ice slurry for 30 min), electrical stunning (*i.e.*, 2 s, 5 s or 10 s exposure to an electrical current of 1.69±0.09 A dm^{-2}), electrical stunning and exsanguination (*i.e.*, 10 s exposure to an electrical current of 1.69±0.09 A dm^{-2} followed by a throat cut), percussive stunning (*i.e.*, a sharp cranial blow by a fish priest), or isoeugenol immersion (*i.e.*, immersion in 10, 20, 30, 60 or 100 mg L^{-1} isoeugenol). EEG signals were then continuously recorded in response to light flashes to evaluate if, and how long, it took for VERs to disappear and then reappear (Fig. 1D). Behavioural indicators (*i.e.*, ventilatory/body movements and aversive behaviour) were also recorded throughout the stunning procedures.

Results and discussion

Based on the abolition of VERs:

Ice chilling induced insensibility between 2.6 and 7.6 min, during which catfish exhibited aversive behaviours (*e.g.*, thrashing around and trying to escape vigorously). Once VERs were lost, they remained absent so long as catfish remained immersed in the ice slurry.

Electrical stunning induced insensibility immediately but not irreversibly. Depending on the duration of the stun, catfish regained VERs within 0.5 to 4.9 min after the completion of the electrical insult.

Electrical stunning and exsanguination induced insensibility immediately and irreversibly if fish were immersed in an ice slurry following the throat cut. However, if placed in warm water following the throat cut, fish regained sensibility prior to death.

Percussive stunning induced insensibility immediately and irreversibly when administered correctly. However, 36% of catfish regained sensibility, which is likely explained by the difficulty associated with administering an accurate manual percussive stun of sufficient force on a live and struggling catfish.

Immersion in isoeugenol at doses exceeding that recommended for euthanasia in salmonids did not induce insensibility in catfish, which indicates that this substance may not be suitable for stunning this species. However, the potential for using isoeugenol as a pre-stunning sedative for improving handleability and reducing handling stress of this species warrants further investigation.

Conclusion and future directions

This study clearly demonstrates that when singularly administered, none of the abovementioned stunning procedures could reliably induce insensibility immediately and/or irreversibly without compromising fish welfare. However, these shortcomings can be resolved by using a combination of methods, as a correctly administered electrical or percussive stun can immediately induce a state of insensibility, which can be maintained until death if fish are exsanguinated and immersed in an ice slurry directly after the stun.

To further improve the welfare of fish during slaughter, a promising future direction is to develop a portable, user-friendly, and cost-effective diagnostic tool based on our laboratory-based neurophysiological technique. This tool can be used by various stakeholders, including stunning equipment manufacturers, fish farmers, and regulators, to verify, test, and optimize stunning methods before commercial use. By implementing these measures, we can significantly enhance the welfare of fish during slaughter and ultimately achieve more sustainable and ethical practices in aquaculture.

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[INCOMPLETE] USE OF RED ALGAE (*Palmaria palmata*) SUPPLEMENTATION IN START FEEDING OF ATLANTIC SALMON (*Salmo salar*): EFFECTS ON MICROBIOTA AND INTESTINAL HEALTH AND DEVELOPMENT

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Introduction
Aquaculture production is in continuous growth both for macroalgae species and fed fish species, like Atlantic salmon (*Salmo salar*) (FAO, 2020). The growth of fed fish species calls for novel feed resources to enable this growth, and in this regard, algae have been recognized as having promising potential relating to their nutritional value, sustainability in production, as well as their biologically active components (Naylor, 2009; Wan et al., 2019; Albreksten et al.,2022). However, the expected effects will depend on the species being fed, and the algae species in question.

The red seaweed species *Palmaria palmata* has been suggested as a feed additive candidate, mainly due to its high protein content and bioactive components that could have positive impacts on fish health (Florence, 1999; Holdt & Kraan, 2011;Grote et al., 2019). Wan et al. (2016) used different inclusions of *P. palmata* in feed for Atlantic salmon during the last feeding phase before slaughter, finding no difference in growth between algae-fed and control groups. This indicates the possibility of also using algae as a feed ingredient for carnivorous fish. However, the effects of such supplements in early feeding stages is yet to be determined. The fish are sensitive to environmental impact in the early phase (Lowe, 2021), suggesting that potential positive or negative effects from different factors like feed can be expected to be magnified. This study characterizes the effects of using a red algae (*Palmaria palmata*) as feed supplementation during the start feeding period of Atlantic salmon (*Salmo salar*) and discuss this potential use of this algae biomass.

Materials and methods
A 4-month start feeding trial was conducted by feeding tanks of Atlantic salmon fry up till an average weight of 10 grams. The algae used in the trial was sourced locally from the Trondheimsfjord shore (Storsteinan) during September. It was rinced, freeze dried and ground into a powder for feed production.

Triplicate groups were fed with control and algae supplemented (5% dried *P. palmata*) diet from start feeding until an average weight of 5 grams, then a standard feed without supplementation until the end weight of 10 grams. This enabled a comparison of which effects relate to the algae diet, and whether these would be sustained after a diet change. Sampling of tissue from liver and intestines were performed at average individual fish weights of 0.4, 1, 5 and 10 grams, and samples were used to assess growth, intestinal development and health, and microbiological flora.

Results and discussion
Preliminary results indicate no negative effects on growth from the algae addition to the diet, with a higher average end weight for the algae fed group (10.4±3.1 gram±SD) but no significant difference from the control group (10.2±2.9 gram±SD). There was a non-significant higher survival rate in the *Palmaria*-fed group. These results give a preliminary indication that the red algae supplement does not have a harmful effect on the growth and survival of salmon fry.

Most samples, including tissue for histological and microbiological analysis, are still being processed. Results from this will be included in the amended abstract.
Further discussions and results will be added to the amended abstract.

Conclusion
The study's initial conclusion is that the use of red algae as a feed supplement can give equal growth as commercial feeds used today. Further conclusions will be included with the amended abstract.

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BACTERIAL DYNAMICS IN A COMMERCIAL INTEGRATED ABALONE-*ULVA* FARM: FROM HATCHERY TO GROW-OUT

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Introduction

Buffeljags Abalone is a commercial abalone farm in South Africa that practices integrated multi-trophic aquaculture (IMTA) by growing the green seaweed *Ulva lacinulata* in D-shaped paddle-raceways receiving effluent water from adjacent *Haliotis midae* raceways. This practice allows for bioremediation of farm effluent, partial recirculation of water and the cultivated seaweed is often used as supplementary feed. Seaweeds cultivated in IMTA have also been reported to have a modulatory effect on the microbiome.

Microorganisms play vital roles in aquaculture systems, including DOM and POM decomposition, fermentation, nitrification, nutrient cycling and protection against pathogenic microorganisms. Host associated bacteria can synthesise essential amino acids, enhance digestion efficiency by supplying enzymes, produce essential micronutrients and metabolites (e.g., SCFAs), and enhance growth, health and development/morphology of the host. The microbiome of an IMTA and the associated species, is likely influenced by feeds and environmental factors but also by changing conditions during grow-out. Therefore, the aim of this study was to characterise the bacterial microbiome of hatchery-produced juvenile abalone (3 - 10 mm shell length (SL)) and the sources of bacterial introductions (feeds and seawater), and to compare this with the microbiome of adult abalone (± 70 mm SL), and their rearing environment, cultivated grown in an integrated abalone-*Ulva* IMTA system.

Materials and Methods

Hatchery-representative samples were collected from three tanks (L x W x D: 0.68 m x 0.50 m x 0.12 m) in the Buffeljags Abalone commercial hatchery, each stocked with 25 000 juvenile abalone. The juvenile abalone were fed a mixed diet of wild diatoms, formulated feed, *Ulva lacinulata* and *Gracilaria*. Samples (n = 36) for next-generation sequencing (NGS) included abalone intestines, abalone faeces, each feed, and bacterial cells in incoming seawater (500 mL) collected on 0.22 µm filters. Samples were collected in triplicate on the day that animals were moved from settlement plates to the rearing tanks and then once per month for two months. Grow-out representative samples (n = 60) were collected from three separate abalone-*Ulva* IMTA systems at Buffeljags over the course of a year. Samples included the abalone effluent water entering the *Ulva* paddle raceway, bioremediated water returning to abalone raceways after being mixed with 50% fresh seawater, and *Ulva* grown in the raceways. Simultaneously, the *Ulva* raceway that supplies the abalone hatchery with seawater was also sampled. *Ulva* in this system is not grown in abalone effluent and served as a non-IMTA control. Intestinal samples (n = 30) from adult abalone fed diets supplemented with or without IMTA grown *Ulva* (or components of *Ulva*) were also included in this study to compare to that of juvenile abalone.

A 16S rDNA fragment was amplified to characterise bacterial communities and raw sequence data was processed using QIIME2. Bacterial 16S reads were mapped against the SILVA 16S rRNA reference database for taxonomic identification of amplicon sequence variants (ASVs). MicrobiomeAnalyst was used to assess within- and between sample bacterial diversity, as well as to quantify and visualise taxonomic abundance, perform differential abundance analyses and to identify putative functional capabilities of the taxa. Data from the respective sample sets (hatchery, grow-out and adult abalone) were treated in the same way to allow for comparisons between the datasets.

Results & Discussion

Juvenile abalone digestive tract bacteria were dominated by the genera *Formosa* (36%), *Psychrilyobacter* (11%), *Vibrio* (11%) and *Mycoplasma* (5%), all of which are known colonisers of adult abalone digestive systems and had high abundances in adult abalone digestive systems in the current study. Over time, abalone digestive tracts and their associated microbiome became more specialised, with a lower overall diversity in adult abalone guts when compared to juveniles.

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Faeces collected from juvenile abalone largely reflected their gut bacterial communities with the exception of *Tenacibaculum*, which increased in abundance from 3% in the intestines to 17% in the faeces. This could be the result of nutrient availability causing bacterial proliferation or as a result of the abalone digestive systems being a niche environment capable of selecting for/against specific bacteria. Similar observations were made for the genera *Rubidimonas* and *Lewinella*, which were present across all environmental and feed samples, but absent from juvenile and adult abalone gut samples. These results are further supported by the lower bacterial diversity observed for juvenile abalone digestive tracts (Chao1; ANOVA F-value 22.23, $P < 0.05$) and adult abalone digestive tracts when compared to water and feed samples.

The gut bacterial communities of juvenile abalone were introduced by the incoming seawater, as well as the respective feeds, as a high abundance of *Vibrio* (19%), *Psychrilyobacter* (18%), *Tenacibaculum* (8%), *Formosa* (5%) and *Psychromonas* (4%) were observed in water and digestive tract samples. The *Ulva*-, *Gracilaria*- and diatom-associated microbiome also contributed to enteric bacteria of juvenile abalone, with the bacterial microbiome of *Gracilaria* and the diatoms showing a greater extent of similarity to that of juvenile abalone gut and faecal samples. Conversely, *Ulva* samples showed a distinct microbial profile, with a high abundance of *Granulosicoccus* (15%), Rhodobacteraceae (11%) and Saprospiraceae (9%), as well as other bacteria that are known for their involvement in *Ulva* morphogenesis and development. These results are similar to those obtained for samples from the grow-out IMTA system, where *Ulva* was also colonised by bacteria contributing to development of *Ulva* and nutrient cycling. In the abalone-*Ulva* IMTA, *Ulva* modulates its surface microbiome and that of the abalone effluent, reducing the abundance of certain genera, including known opportunistic pathogens, without causing a collapse in bacterial diversity of the bioremediated seawater, acting as a positive indicator for system health.

Conclusion

Incoming seawater and diet shapes the bacterial microbiome of the juvenile abalone gut, whereas inclusion (integration) of *Ulva* positively modulates the microbiome and contributes towards the functioning of IMTA on a commercial abalone farm. The complex interactions between microbial diversity, animal health and productivity has been observed in various aquaculture systems. This study contributes towards the understanding of the bacterial dynamics, their sources of introduction and their roles at different abalone production stages in an integrated abalone-*Ulva* system.

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CULTURING DELICACIES: POTENTIAL TO INTEGRATE THE ECONOMICALLY IMPORTANT GASTROPOD *Babylonia areolata* INTO POND CULTURES OF *Caulerpa lentillifera* (ULVOPHYCEAE, CAULERPACEAE)

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Introduction

More than 50% of the marine and coastal aquaculture products cultivated worldwide are macroalgae, with brown and red species dominating the sector and green algae contributing only to a small fraction (FAO 2022; Moreira et al. 2022) particularly in the last four decades, aquaculture is for the first time set to contribute half of the fish consumed by the human population worldwide. This reflects not only the vitality of the aquaculture sector but also global economic growth and continuing developments in fish processing and trade. Until a year or so ago, the production trends in aquaculture and capture fisheries were continuing without any drastic modification to those already in place at the start of this decade. The capture fisheries sector was regularly producing between 90 and 95 million tonnes per year, and aquaculture production was growing rapidly, albeit at a gradually declining rate. However, the substantial increases in energy and food prices, which started in 2007 and have continued into 2008, as well as the threat of climate change, mean that the conditions for capture fisheries and aquaculture are changing. That said, the combined effects of rising prices and climate change are complex, and they affect a very large number of fisheries and aquaculture operations in a mosaic of natural, social and economic contexts. Hence, it is too early to have a clear understanding of the cumulative impact worldwide on fisheries and aquaculture. Nonetheless, it is clear that there will be both winners and losers among fishers, aquaculturists and those employed in ancillary industries. On the one hand, rising prices for staple foods will also cause an increase in the price of many fish and fish products, and this will stimulate all in the sector to produce more. However, those who capture or culture fish, or other aquatic animals, using energy-intensive forms of production may find recent cost increases prohibitive. They may well face difficulties in continuing in their occupation, at least in the immediate future. On the other hand, low-intensity aquaculture and most small-scale and artisanal fisheries will attempt to expand production. This will increase the importance of improved governance in both aquaculture and low-energy-consuming fisheries (some near-shore fisheries, passive fishing gear, etc.. Among the green algae of economic interest is *Caulerpa lentillifera*, commonly known as “sea grapes” or “green caviar”. This edible seaweed is popular in the Indo-Pacific region for its unique texture and its high nutritional value (de Gaillande et al. 2017; Syakilla et al. 2022). In Viet Nam, the cultivation of *C. lentillifera* takes place mainly in earthen tidal ponds, where the algae are either planted directly into the sediment (sowing method) or grown on perforated plastic trays (tray method), depending on the characteristics of the pond’s substrate (Rabia 2016) www.m-hikari.com http://dx. Abstract Commercial cultivation of Caulerpa lentillifera is now gaining recognition because of the increasing demand in the domestic and international market. Studies on the different culture methods for large scale production of the species in the country are scarce. The present study evaluated the effects of two cultivation methods namely sowing and tray on the growth and biomass production of C. lentillifera cultured in brackishwater pond. For the tray method, propagules were clipped in two 0.75 m x 0.75 m tray and were hung in bamboo frame whereas for the sowing method, propagules were planted directly in the pond substrate with an interval of one meter. The weight gain using the sowing method was significantly higher and could be translated to an average of 1 kg every month of new or harvestable biomass. Specific growth rate of C. lentillifera grown in the substrate was at 3.85% day-1 during the first month and at 2.92% day-1 during the second month and was significantly higher compared to that of stocks grown in trays. High organic load of the soil (substrate. Also of economic interest in the region and used for human consumption is the spotted babylon snail *Babylonia areolata*. Co-cultivation of extractive species like seaweeds and fed species has been shown to effectively reduce negative impacts of aquaculture such as eutrophic wastewater (Chopin et al. 2001)especially where activities are highly geographically concentrated or located in suboptimal sites whose assimilative capacity is poorly understood and, consequently, prone to being exceeded. One of the main environmental issues is the direct discharge of significant nutrient

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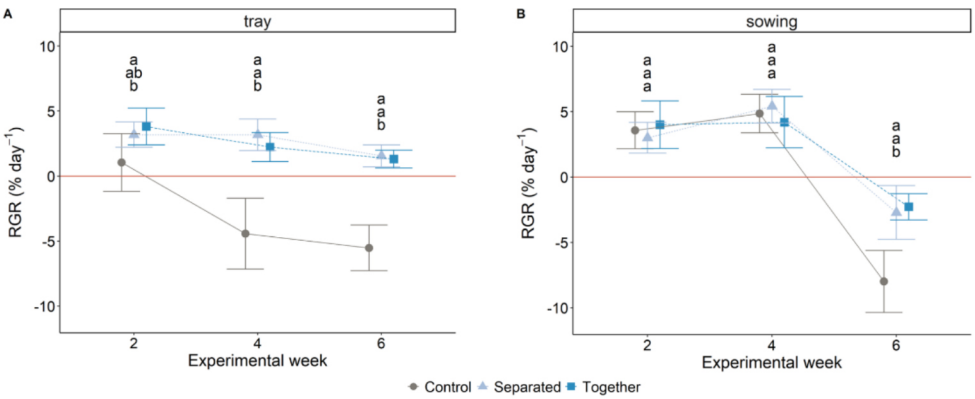

Figure 1: Relative growth rate (RGR) of *Caulerpa lentillifera* in tray (A) and sowing cultures (B) over an experimental run of 6 weeks. Treatments: Control: Only *C. lentillifera*; Separated: *C. lentillifera* and *Babylonia areolata* spatially separated; Together: *C. lentillifera* and *B. areolata* together. The red line separates positive and negative growth rates. Significant differences between the treatments per day are shown by different letters (One-way ANOVA with post-hoc test, $p<0.05$). Data are mean values ± standard deviation, n=6.

loads into coastal waters from open-water systems and with the effluents from land-based systems. In its search for best management practices, the aquaculture industry should develop innovative and responsible practices that optimize its efficiency and create diversification, while ensuring the remediation of the consequences of its activities to maintain the health of coastal waters. To avoid pronounced shifts in coastal processes, conversion, not dilution, is a common-sense solution, used for centuries in Asian countries. By integrating fed aquaculture (finfish, shrimp. The objective of this study was to assess whether the integration of *Babylonia areolata* into existing sea grape ponds could potentially increase the economic profit of sea grape farmers without reducing the yield of *Caulerpa lentillifera*. The factors of (1) sea grape culture method (tray vs. sowing), (2) co-culture approach (spatially separated vs. common bottom space), and (3) *B. areolata* stocking density were investigated.

Material and Methods

The study consists of two experiments that were conducted at the Institute of Oceanography (IO) in Nha Trang, Khánh Hòa Province, Viet Nam: an outdoor terrace (six weeks) and an indoor laboratory experiment (four weeks). The outdoor experiment investigated how the culture method of *C. lentillifera* (in the following referred to as *Caulerpa*) and the co-culture approach with *B. areolata* (in the following referred to as *Babylonia*) affect the growth and physiology of *Caulerpa.* Three different treatments were assigned to nine 400L tanks (n=3): Only *Caulerpa*; *Caulerpa* and *Babylonia* together; *Caulerpa* and *Babylonia* spatially separated. Each tank contained *Caulerpa* in two different culture methods, on perforated plastic trays and directly in the sediment, respectively. The second experiment focused on different *Babylonia* stocking densities. Four different snail densities were set up in 10L aquaria: Control, low, medium, and high with 0, 2, 4, and 8 snails, respectively (n=5). The response parameters weight of both organisms, harvestable biomass, photosynthetic efficiency (maximum quantum yield of photosystem II, F_v/F_m), antioxidant activity (AOA, ABTS assay, following Re et al. 1999), and total phenolic content (TPC, Folin-Ciocalteu assay, following Gillespie and Ainsworth 2007) of the algae as well as NO_x and PO_4 in the water were measured. AOA and TPC will not be considered further in the following (article in progress).

Results and Discussion

Overall, the presence of *Babylonia* had a positive effect on algal growth and physiology. The tray method resulted in higher weight gain and harvestable biomass of sea grapes compared to the sowing method over the course of the experiment, but only when co-cultured with *Babylonia* (Fig. 1A, B). Algae in the monocultures exhibited lowest growth rates and even decreased in weight. In the monocultures, the sowing method resulted in better growth of the sea grapes in the first weeks due to the availability of nutrients in the soil (Rabia 2016)www.m-hikari.com http://dx. Abstract Commercial cultivation of Caulerpa lentillifera is now gaining recognition because of the increasing demand in the domestic and international market. Studies on the different culture methods for large scale production of the species in the country are scarce. The present study evaluated the effects of two cultivation methods namely sowing and tray on the growth and biomass production of C. lentillifera cultured in brackishwater pond. For the tray method, propagules were clipped in two 0.75 m x 0.75 m tray and were hung in bamboo frame whereas for the sowing method, propagules were planted directly in the pond substrate with an interval of one meter. The weight gain using the sowing method was significantly higher and could be translated

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to an average of 1 kg every month of new or harvestable biomass. Specific growth rate of C. lentillifera grown in the substrate was at 3.85% day-1 during the first month and at 2.92% day-1 during the second month and was significantly higher compared to that of stocks grown in trays. High organic load of the soil (substrate. The poor growth of *Caulerpa* in the monocultures in the tray cultures was probably due to nutrient deficiency since NO_x and PO_4 concentrations in the monocultures (0.06±0.08 µmol L^{-1} and 0.48±0.13 µmol L^{-1}, respectively) were significantly lower than in the snail treatments (NO_x: 7.7-33.68 µmol L^{-1}; PO_4: 0.9-2.53 µmol L^{-1}). Limitations of nutrients are known to inhibit metabolic activities of seaweeds, negatively affecting photosynthesis and growth (Roleda and Hurd 2019)nitrogen and phosphorus are the main elements required by seaweeds for photosynthesis and growth. This review focusses mainly on nitrogen, but the roles of carbon and phosphorus, which may interactively affect seaweed physiological processes, are also explored. Fundamental concepts such as limiting nutrients, sources, and ratios, mechanisms of nutrient uptake, nutrient assimilation and storage, patterns of uptake and preferences for different nitrogen sources are discussed. The roles of abiotic (water motion, light, temperature, salinity and desiccation, which is why nutrient deficiency would also explain the decreased F_v/F_m values in the monocultures. *Babylonia* growth and survival were not affected by spatial separation from *Caulerpa*. *Babylonia* growth was consistent in both treatments and the snails nearly doubled in weight over the course of this study. Survival of the snails was 100% in both treatments. The indoor laboratory experiment showed that *Caulerpa* weight gain was positively correlated with snail density. The high increase in algal biomass in the high density-snail treatment indicates that snails can be implemented at high quantities without negative implications for the seaweed. The study showed the high potential of integrating *Babylonia* into existing sea grape ponds. Co-culture of these two organisms could increase the economic profit of sea grape farmers without reducing the yield of *Caulerpa*.

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SOUNDSCAPES AND OFFSHORE FISH FARMS: PRELIMINARY RESULTS FROM SAUDI RED SEA

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Introduction

The influence of sound and noise on living organisms is widely recognized and well described. Most of the past research effort was dedicated to addressing the impacts of noise on humans and terrestrial life, mainly for practical reasons. Today's evidence is pushing scientists to extends the impacts over several groups of marine life. Most of the existing literature about underwater noise is currently dedicated to marine mammals because of their well-known use of sounds for every aspect of their life cycle. Deadly effect of loud SONAR impulses on several marine mammal species has been clearly demonstrated. Currently there is an increased interest in the relevance of underwater sound in other marine animal groups both for ecological reasons (e.g. reef larval migrations following acoustic paths) and economic reasons (e.g. productivity of aquaculture farms carrying fish exposed to noise).

In this work we present results of a preliminary study, connecting these two aspects, thanks to the ongoing long-term investigation conducted on an offshore aquaculture farm in the Saudi Red Sea.

Area, materials and methods

The area lies in the Red Sea, in front of the Saudi Arabian coast, from 24.00°N to 18.50°N. In this large region, four locations were equipped with autonomous acoustic recorders. Two of these devices were in a fish farm t areas (Tharawat 1 and 17) while two were deployed in "pristine" sites (Yanbu, Al Lith,). Tharawat 1 was located at the centre of the fish farm, while Tharawat 17 was 1.3 km apart.

Within all stations, sea bottom is mostly sandy and ranges from -20m to -40m; coral reef spots and small islands are scattered around.

Two rounds of sampling were made in 2022 and 2023, obtaining about 600 hours (2022) and 1400 hours (2023) of wideband acoustic recordings in total. The analysis relied on skilled scientists displaying spectrograms (organized in chunks from 10 minutes to 24 hours) and through direct listening and in-deep spectrographic analysis when interesting slots were detected.

Results

Here we present a snapshot of results based on the analysis of the 2022 round of recordings (Yanbu, Tharawat 1 and 17, Al Lith).

Data were analyzed from the point of view of the differences between stations and depicting different soundscapes as shown in Fig. 1.

A 24h third octave spectrum (Fig. 2) and a SPL (Sound Pressure Level) in third octave (Fig. 3) for each station (as the one presented below, Tharawat 1 "farm" site) were also considered.

Discussion and perspectives

This screening shows a general uniformity of soundscapes among the areas, with the exception of Tharawat 1 where the soundscape is dominated by man-made sounds related to the fish-farming activity (recording station was in close proximity with the farm).

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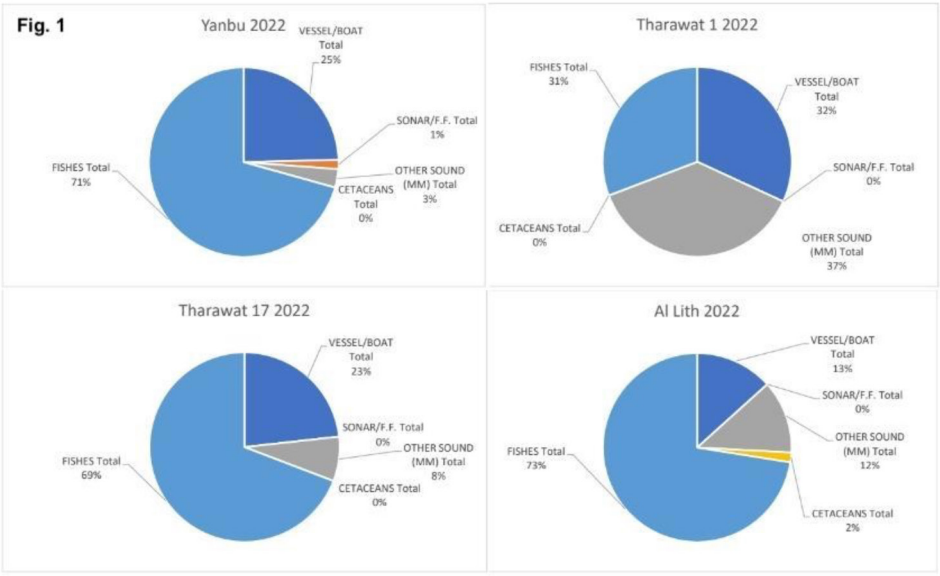

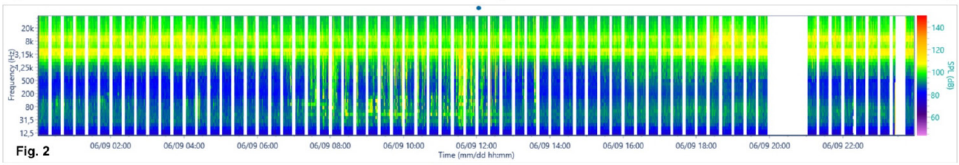

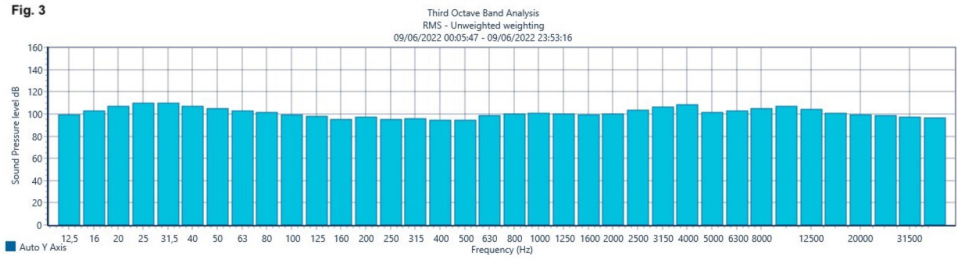

In general, all stations seem not to be affected by intense man-made noise. RMS (Root Mean Square) cumulated for 24h showed Tharawat 1 as the station with the highest levels of acoustic energy, followed by Tharawat 17. The other two stations have lower acoustic levels that differ just by a few dB. Most of the energy is concentrated in the lower frequencies (below 20 kHz), as shown in figures 2 and 3, with some differences in the distribution of energy over frequencies between "farm" and "pristine" sites.

Current further analysis is targeted on describing fish farms related noise. Results will be used to address potential interaction between plants noise *vs.* farmed species as well as the natural environment. Vast area soundscape characterization will provide an additional decision parameter for future fish farm siting. This point is of major importance, given the expected offshore aquaculture development in the Saudi Arabian Red Sea.

Coupling offshore aquaculture farms with the use of Passive Acoustic Monitoring is a promising approach to increase knowledge about wild and anthropized environments, the understanding of the soundscapes related to aquaculture plants, the potential productivity of the farms themselves and the possible impacts of anthropogenic sounds on the marine environment.

IMPACT OF THE SUSTAINABLE BIOFLOC REARING SYSTEM ON THE COMPOSITION, FUNCTION, AND STABILITY OF THE INTESTINE BACTERIAL MICROBIOTA IN THE RIVER PRAWN *Cryphiops caementarius*

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Introduction

Biofloc technology (BFT) has emerged as a sustainable aquaculture strategy because it does not require water exchange and supports heterotrophic bacterial growth, which increases nitrogen recovery by converting it into biomass that serves as a nutritional supplement. Juvenile river prawn *Cryphiops caementarius*, an endemic species of the arid regions of Chile and Peru, shows higher survival and growth in BTF than in the traditional clear water (CW) system. Thus, we aimed to compare the impact of these culture systems on the dynamics, stability, and functional potential of bacterial communities in the rearing environment and intestines of *C. caementarius*.

Materials and methods

The bacterial microbiota was characterized for the culture water (BF and CW) and in the intestines of juvenile prawns every 4-6 months for a total culture period of 14 months, using partial 16S rRNA gene sequencing. Additionally, after 8 months of rearing, prawns were subjected to an immune challenge using microbe-associated molecular patterns (MAMPs) to assess the stability of the intestinal microbiota. Taxonomic assignment was performed using the Genome Taxonomy Database GTDB Release 08-RS214 and the functional prediction was computed using FAPROTAX. The relative abundance of ASVs in each sample and community analysis (observed composition, alpha-diversity, and beta/diversity indexes) were calculated from subsampled datasets with the phyloseq package. The differential abundance of ASVs in each condition and of the functional potential was evaluated using the LEfSe method (linear discriminant analysis with size effect).

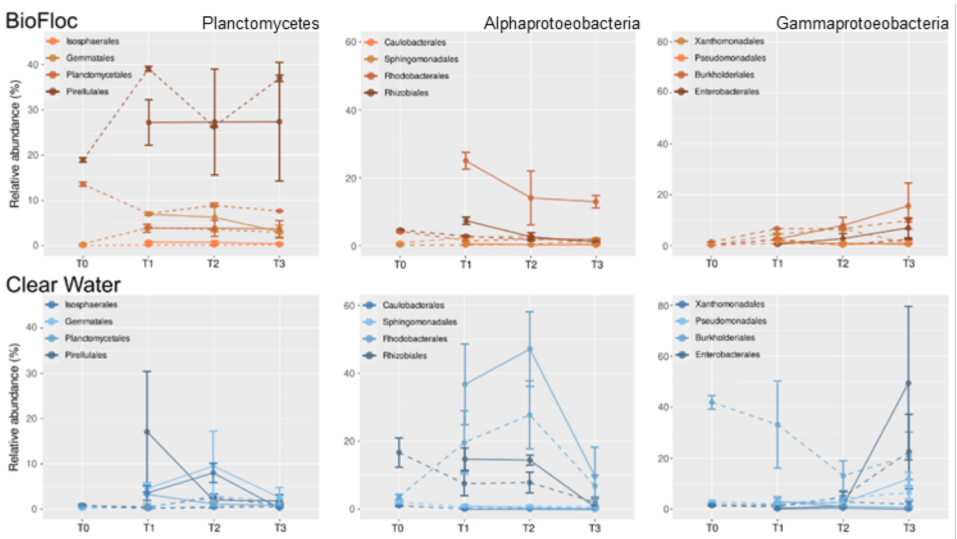

Figure 1. Relative abundances of the main bacterial groups present in the water (dashed line) and intestine (continuous line) microbiota in juvenile river prawns *Cryphiops caementarius* cultivated in Biofloc Technology (BTF) and Clear Water (CW) systems for 14 months. Samples of water and intestines were analyzed each 4-6 months (T1-T2), and an additional sample of water (T0) was analyzed before the incorporation of the prawns in the systems.

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Results

Bacterial diversity was greater and more stable in the environment and Biofloc-cultivated prawns compared to the CW system. Planctomycetes (~40%) dominated and remained stable over the 14-month both in the environment and the intestines of prawns grown in BFT. Alphaproteobacteria (mainly Rhodobacterales) were initially abundant (30 to 15%) but gradually transitioned to Gammaproteobacteria (10 to 30%) (mainly Bhurkolderiales) (Fig. 1). In contrast, Alphaproteobacteria (mainly Rhodobacterales) initially prevailed (60 to 10%) in the environment and intestines in CW but were replaced by Gammaproteobacteria (10 to 60%) (mainly Enterobacteriales) towards the end of the culture period. The intestines and water microbiota in the CW system show enhanced genomic potential for beneficial processes like aerobic denitrification and degradation of toxic substances. However, undesired side effects such as the proliferation of antimicrobial resistance genes and potential emergence of pathogens were observed. In contrast, the stable community in BFT is metabolically versatile, with genetic potential for fermentation, aerobic nitrification, and breakdown of complex organic compounds. Prawn intestines were significantly over-colonized by Gammaproteobacteria, particularly from the Aeromonadales, a well-known pathogen of aquatic animals, in both BFT and CW-cultivated prawns after 48 h immune challenge with MAMPs.

Our results emphasize the microbial stability and higher diversity by BFT. Added to a bacterial composition that could foster improved exploitation of nutritional resources, together they could promote the productive performance of *C. caementarius* by BFT technology.

THE BIOFLOC TECHNOLOGY SUPPORTS HIGH STOCKING DENSITY FOR JUVENILE RIVER PRAWN *Chryphiops caementarius* FARMING IN ARID ZONES

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Introduction

Biofloc technology (BFT) involves the cultivation of dense microbial communities within the water column, which serve as a natural food source for reared organisms. These microbial communities also help to maintain water quality by consuming excess nutrients and organic matter, reducing the need for water exchanges. This is particularly beneficial in areas with limited water resources like northern Chile, where the endemic river prawn *Cryphiops caementarius* is being cultivated. Increasing stocking density to an intensive or super-intensive level will help to support productive sustainability of this prawn. It has been shown that by using BFT, shrimp farmers can effectively increase the carrying capacity of their culture systems, allowing for higher stocking densities without compromising water quality. Therefore, in this study we evaluated whether BTF allows increasing the culture density of *C. caementarius*, comparing the physiological performance and productive parameters between individuals cultivated at different densities in the Biofloc or the traditional clear water (CW) systems.

Materials and methods

Juvenile prawns (0.44±0.07 g; 7.3±0.04 mm) were cultivated for 9 months in BFT and CW systems at initial stocking densities of 100, 200, and 400 individuals per m^2. At months 4 and 9, the components of the energy balance (energy intake allocated into the standard metabolic rate - SMR, and into energy losses by ammonia excretion and feces) and derived physiological index (scope for growth - SFG), and the status of chronic physiological stress state were determined under the different culture conditions. Physiological stress was measured through the transcriptional expression levels of the antioxidant enzyme superoxide dismutase (*SOD*) and the stress protein *HSP70*. In addition, the productive indices for each of the densities were determined via the growth and survival rates.

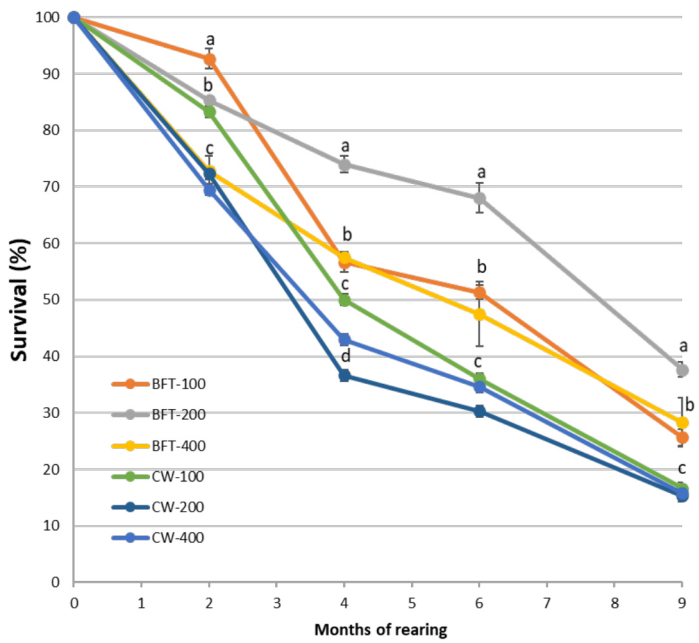

Figure 1. Survival rates of juvenile river prawns *Cryphiops caementarius* cultivated in Biofloc (BFT) and Clear Water (CW) systems for 9 months, at three densities (100, 200, and 400 individuals per m^2). Survival was measured each 2-3 months samples.

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Results

Juvenile prawns from BFT cultivated at 400 ind./m^2 showed higher SFG after 4 months of cultivation than CW-juveniles, which was associated with a higher energy intake and lower SMR in the formers. Energy balance and SFG were similar among juveniles cultivated in different densities and systems after 9 months, but superior in BFT considering that at this time the real densities in this system were two-fold the densities of CW due to higher mortality observed in the latter (Fig. 1). Chronic physiological stress was higher under CW than in BFT, as revealed by higher *HSP70* transcriptional levels, which additionally increased directly with the stocking density. Survival was significantly higher in the BFT than in the CW-juveniles in each of the stocking densities assessed, with the highest survival observed in juveniles maintained at 200 ind./m^2 in BFT. However, growth was inversely associated with the stocking density and independently of the rearing system. However, given that at the end of the rearing period, the real density in BFT was approx. two-fold the CW density, it is suggested that BFT supported growth at higher densities than the CW system.

Overall, the results revealed BFT allows for higher stocking (to an intensive level) density than the traditional CW system. BFT would support an efficient energy balance and lower physiological stress, which could be the basis of the observed enhanced productive performance in terms of growth and survival of juvenile *C. caementarius*. Thus, this technology emerges as an effective tool for river prawn farmers looking to increase their stocking densities and improve the efficiency of their production systems in arid zones.

DIFERENT LEVELS OF FISH MEAL IN DIET TO *Mugil cephalus* REARED IN BIOFLOC SYSTEM: EFFECT ON GROWTH PERFORMANCE AND NUTRITIVE EFFICIENCY

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Introduction

Biofloc technology (BFT) is an example of sustainable aquaculture due to its zero or minimal water exchange and its potentiality to reduce the input of artificial feed (fish meal) thanks to the establishing a nutritionally rich microbial biomass that can be used as natural food by the reared animals (Wasielesky et al. 2006). Mugilidae (especially *Mugil cephalus*) are excellent candidates for sustainable Mediterranean aquaculture due to its herbivorous/omnivorous feeding behavior. However, few information is currently available on the culture of this species in bioflocs combined with a reduction of fishmeal in the diet. Therefore, the objective of the present study was to evaluate the growth and nutritional efficiency of *M. cephalus* reared in bioflocs and fed diets containing reduced levels of fishmeal.

Material and Methods

For the experiment, 600 animals with a mean weight of 80 grams obtained from the Institute of Agrifood Research and Technology (IRTA, Tarragona), were transported to the facilities of Universitat Politècnica de València. The animals were randomly distributed in 9 tanks of 3.3 m^3. Three isolipidic feeds (10%; Table 1) were tested in tanks in triplicate with decreasing levels of fishmeal inclusion (15, 5 and 0% corresponding to treatments HP15, HP5 and HP0, respectively). The ratio between essential amino acids (AAE) and non-essential amino acids (AAnE) was similar for the three feeds, being at 0.73 and 0.88. The animals were fed until apparent satiety three times per day (9:00, 13:00 and 17:00h). The water quality parameters were monitored during the whole experimental period, which had a duration of 27 days. Results were analyzed compared using one-way ANOVA and to significant differences between treatments were verified by applying the Newman-Keuls test (significance level of 5%). Additionally, at the end of the experiment, the effect of the diet on the intestinal microbiota was evaluated through the analysis of 16S ribosomal DNA.

Results and discussion

The mullets adapted quickly to BFT, and a total substitution of fish meal meant in lower survival whit significant differences (Table 2). Additionally, HP0 showed the highest feed conversion ratio (FCR) and the lowest protein efficiency ratio (PER) and specific growth rate (SGR), but without significant differences. Nevertheless, HP5 did not reported differences with HP15 neither growth nor survival.

In relation to microbiote composition, the phylum with the highest representation was Proteobacteria (43.76 ± 6.74%), followed by Firmicutes (18.78 ± 5.38) and Spirochaetota (16.19 ± 5.47%). HPO reported an increase of Firmicutes (31.14 ± 10.71 vs 1.23 ± 0.19 %) and decrease of Planctomycetota (5.38 ± 2.62 vs 16.04 ± 0.83 %) presence respect to the initial point. No differences were observed independently of the diet respect diversity alpha (Shannon-index).

Therefore, low fish meal inclusion levels are possible in *M. cephalus* under BFT without affecting zootechnical performance and intestinal health. Similar results were observed by Nhi et. al (2018) in *Oreochromis niloticus*, also an herbivore/omnivore species, being able to reduce fish meal inclusion into the diets under BFT.

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Table 1. Formulation and proximate composition of each experimental diets.

Ingredients (g kg^{-1})	HP15	HP5	HP0
Fish meal	150	50	0
Wheat flour	352	289.5	260.5
Pig hemoglobin	40	50	55
Hydrolyzed wheat protein	30	50	60
Concentrated soy protein	50	70	80
Potato protein	55	75	85
Pulpa remolacha	35	45	50
Algae Lithonutri	50	50	50
Wheat gluten	20	70	95
Brewer's yeast	100	110	115
Fish oil	42	52.5	57.5
Soybean oil	23	18	15

Table 2. Growth performance (mean ± standard deviation) and nutritive efficiency indices of *Mugil cephalus* juvenile fed with three fish meal levels: 0 %, 5 % and 15 %.

	HP0	HP5	HP15
Initial weight (g)	82.68 ± 0.05	82.68 ± 0.05	82.68 ± 0.05
Final weight (g)	96.73 ± 3.55	100.13 ± 4.90	100.00 ± 4.97
FCR	0.59 ± 0.31	0.32 ± 0.09	0.31 ± 0.10
SGR (% day-^{1})	0.62 ± 0.15	0.76 ± 0.19	0.75 ± 0.19
FI (g 100 g fish^{-1} day^{-1})	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.00
PER	7.39b ± 3.19	10.36 ± 3.77	12.19 ± 4.88
Survival (%)	65.83 ± 8.67**a**	84.17 ± 3.97**b**	81.25 ± 3.61**b**

FI: Feed Intake. Different letters indicate significant difference between groups.

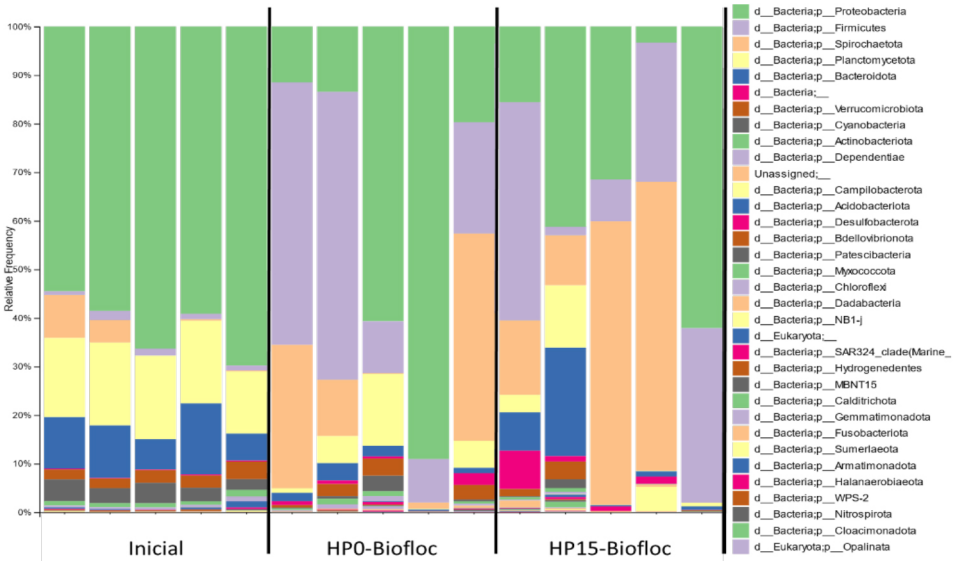

Figure 1. Phylum distribution of posterior intestinal microbiote in HP0, HP5 and PH15 diets (%).

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Acknowledgments

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RECIRCULATION AQUACULTURE SYSTEM VS BIOFLOC TECHNOLOGY: EFFECT OF REARING CONDITIONS ON INTESTINAL TRANSCRIPTION OF *Mugil cephalus*

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Introduction

The biofloc technology (BFT) and the clear water recirculation system (RAS) are two examples of closed aquaculture production systems that work with water recycling. In the case of the RAS system, the main difference to the biofloc system is its high structure and complexity of equipment for the filtration and treatment of the water, which is then returned to the rearing tanks (Badiola et al., 2018). On the other hand, the BFT mainly needs strong aeration to keep the bioflocs that are generated in the system active and in suspension, since there is zero or minimal water exchange in the system. Thus, there is the establishing a nutritionally rich microbial biomass that can be used as natural food by the reared animals (Wasielesky et al., 2006). Transcriptome analysis provides relevant information about gene expression, which can be influenced by the cultivation system. Thus, the objective of the present study was characterized and compare for the first time the intestinal transcriptome of *Mugil cephalus,* an excellent candidate for sustainable Mediterranean aquaculture due to its herbivorous/omnivorous feeding behavior, rearing in different systems, RAS and BFT.

Material and Methods

For the experiment, the animals were obtained from the Institute of Agrifood Research and Technology (IRTA, Tarragona) and transported to the facilities of Universitat Politècnica de València. The juveniles of *M. cephalus* were randomly distributed in 3 tanks of 3.3 m^3 to the BFT experiment (± 82.68 g) and in 3 tanks of 0.4 m^3 to RAS experiment (± 8.15 g). One isolipidic feeds (10%) were tested with 15% levels of fishmeal inclusion. The animals were fed until apparent satiety three times per day (9:00, 13:00 and 17:00h). The water quality parameters were monitored during the whole experimental period, which had a duration of 27 days. At the end of the experiment, the effect of the rearing conditions on intestinal trasncriptome was analyzed using omic approach (FISABIO, Spain). The reads were mapped to the Mugil cephalus reference genome (GCF_022458985.1) using the bowtie2 software, and read counts were generated using htseq-count. For the analysis of differential expression, the DESEQ2 tool was employed. Subsequently, we focused on transcripts with a padj value of less than 0.05 and an absolute log2FoldChange greater than 1. Enrichment analysis of GO and KEGG terms was conducted using the Fisher Exact Test.

Results and discussion

A total of 7651 differential expressed transcripts (DETs) were obtained when we compared the intestinal gene expression between fish reared under RAS o BFT. From them, 3022 and 4649 genes were down and up-regulated, respectively. The representation of DETs shows a clear differentiated distribution of the samples corresponding to BFT and RAS experimental group (Figure 1).

After GO enrichment analysis, results showed that 18 biological processes (BP), 6 cellular components (CC) and 5 molecular functions (MF), mainly related to transcriptional regulation, were significantly affected by the rearing conditions. If we focused on the GO enrichment based on the genes that were up-regulated in BFT, we obtained 121 biological processes (BP), 8 cellular components (CC) and 19 molecular functions (MF), mainly related to immune system, metabolism/cellular transport and cellular proliferation (Figure 2). Besides, KEGG analyses revealed 24 disturbed pathways, related to cellular transport and metabolism, meanwhile in the fish reared under BFT the enrichment KEGGs were more focused on lipid metabolism. Therefore, a differential intestine expression was reported based on rearing conditions.

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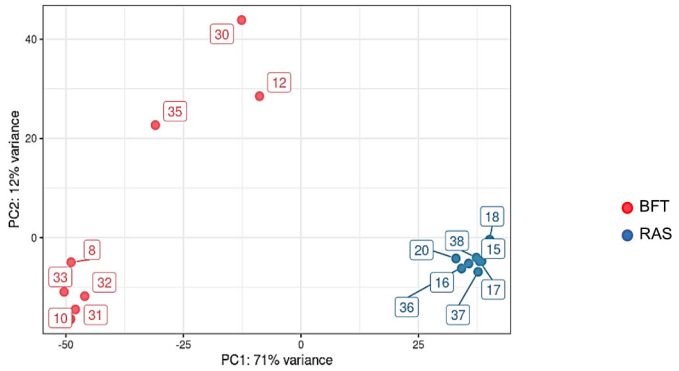

Figure 1. Principal componente analyses based on DETs of RAS and BFT samples

Figure 2. Interactive graph of enriched biological processes based on up-regulated DETs in BFT.

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Acknowledgments

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AN OVERVIEW UPDATED TO 2022 OF STURGEON AND CAVIAR PRODUCTIONS

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Sturgeon populations decline have dramatically increased throughout their range for the last 50 years due to anthropogenic impacts: overfishing, barriers to migrations and pollution have adversely impacted sturgeon species and populations worldwide. At about the same time sturgeon farming took hold in many countries in the range where sturgeons were native, and also in countries outside this range. The motivating cause of this farming, in addition to an initial interest in new species both for the market and for sport fishing, was the production of caviar from the nineties onwards. This product of great quality and of great economic value has stimulated many farmers to produce this good in their facilities, given the drastic reduction of natural resources and the maintenance of the product's value to the final consumer. Thus, since the 2000s there has been a very rapid increase in the production of both meat and caviar which in 2022 probably exceeded 800 tons, with China as the first producer and exporter in the world. For over 30 years, meat and caviar production data have been collected by the WSCS, not only to provide an information service to farmers, traders and researchers, but also to have an overview of where and which species are farmed in the world, in foresight of the use of these banks of potential breeders for the production of suitable animals for wildlife recovery and repopulation projects. In fact, natural stocks are drastically rarefied, and the rules and regulations of almost all states prohibit fishing of these species, and resorting to farmed populations is often the only option left. The present work therefore aims to provide an overview of sturgeon and caviar production updated to 2022, despite all the difficulties in the collection of complete and reliable data by all farmers and/or all countries.

THE RISE AND FALL OF THE ZOOPLANKTON IN THE ARCTIC. UNRAVELLING THE CONTRIBUTION OF LIPIDS TO COPEPOD DIAPAUSE WITHIN THE ARCTIC

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Introduction

Copepods such as *Calanus hyperboreus* and Calanus finmarchicus, are a rich source of long chain omega 3 fatty acids, such as EPA and DHA, and are an integral part of the north Atlantic and Artic ecosystems. However, these fatty acids exist in the wax-ester form rather than as triacylglycerols, possibly owing to their role as a modulator of copepod buoyancy, allowing them to maintain a neutral buoyancy and hibernate below the thermocline during winter, in a process known as diapause. Changes in climate are predicted to have implications for both biodiversity, resulting in changes in the phytoplankton community, as well as physical ocean characteristics, which may impact the life cycle of Arctic copepods. The interplay between copepod diet, intact lipid composition, buoyancy and diapause is not well understood, with the substantial role of copepods to sequester carbon to the seafloor an integral part of the biological pump. Therefore, it is crucial to understand how intact lipids respond to dietary inputs, and whether different copepod species vary with regards to their wax-ester profiles. This additional knowledge will help improve our modelling of this vital trophic link and potential aquaculture resource, and the limits to which it can be exploited. To this end, copepods from several species, locations and depths were profiled, as were several diets, with regards to their intact wax-ester composition to ascertain initial trends relating to these parameters.

Material and Methods

Copepods were collected during the Nansen legacy cruise (Nansen and Amundsen basins) and the Meriam cruise (2022, West Greenland) from various locations and depths, with several species identified. Calanus finmarchicus (females) was also grown under controlled conditions from eggs to stages C4-C5, using two salinities and 3 diets (*Thalassiosira weissflogii*, *Rhodopirellula baltica* and *Heterocapsa*). All copepods were weighed into polypropylene bead beating tubes, with 10 μl of antioxidant solution added (0.2 mg/ml BHT and EDTA in 50:50 v/v methanol/water) followed by 350 μl of 90 % v/v methanol. Samples were then bead homogenised for 30 seconds, followed by the addition of 1 ml of MTBE. Following vortexing, samples were left at room temperature, followed by the addition of 250 μl of water. Samples were again vortexed, centrifuged, and the upper MTBE layer removed and placed into a new Eppendorf tube. A second MTBE extraction was carried out, which was pooled with the first, with the extracts dried under nitrogen. Samples were then resuspended in approximately 0.25-1 ml of 2:1 v/v MTBE/MeOH, with the volume dictated by the sample weight. A 1:80 dilution was then made, with 1.5 μl injected on to the SFC-MS/MS system for intact wax-ester analysis. An Acquity UPC2 connected to a Xevo G2-XS QTOF (Waters, Milford, MA) was used for the analysis, with data processed using Progenesis v3.0 (Waters). Further data processing was conducted in Excel, with hierarchical clustering conducted using the online Morpheus tool.

Results

Copepods showed a clear separation based upon species, as well as location for the Nansen legacy cruise. The two locations (Nansen and Amundsen basins) showed distinct copepod wax-ester profiles, with group 1 (Nansen basin) being more unsaturated, containing a greater proportion of wax-esters with 3-7 double bonds. Group 2 (Amundsen basin) however was found to be more saturated, enriched in wax-esters containing 1-2 double bonds, though found to be on average shorter, 36-38 carbons in length, compared to group 1, which were on average longer, 40-44 carbons in length. Regarding species, *Pseudocalanus* species were found to contain much shorter chain wax-esters, ranging from 30-38 carbons long, with *C. glacialis* and *C. finmarchicus* generally being less distinguishable from one another. However, these species were distinguished from *C. hyperboreus* in that they contained elevated levels of 36 carbon wax-esters whereas *C. hyperboreus* contained elevated levels of 42 and 44 carbon wax-esters. When comparing *C. hyperboreus* species across regions (West Greenland and the Nansen and Amundsen basins), copepods from different regions could be differentiated by the wax-ester composition, however, no overall defining trend emerged to describe the separation, as intra-species separation was also found within these regions. With regards to *C. finmarchicus* under different dietary and salinity regimes, diet had the greatest impact on the copepod lipids, with the intact wax-esters capable of clustering samples based on diet primarily, though separation based on salinity was also observable.

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Discussion and conclusion

Overall, it was found that the intact wax-esters were able to discriminate Antarctic copepods based on species, as well as their trawl location. *C. hyperboreus* was generally found to be more distinct from the two other resident copepod species, *C. finmarchicu*s and *C. glacialis*, though *C. hyperboreus* showed wax-ester discrimination based on location. Whilst there was not a strong location signal, i.e., separation of *C. hyperboreus* populations between West Greenland and the northern basins, mainly due to intra-location groupings, this difference in wax-ester composition between populations may be due to age, sex and diet, as well as abiotic factors such as depth of isolation and sea temperature. It was found that diet does in fact play a large role in the copepod's lipid storage composition, with climatic change postulated to change not only abiotic factors, such as sea temperature and salinity, owing to increased freshwater inputs from melting sea ice, but also species distribution, including algal food sources. These are all postulated to have an impact on the life cycle of copepods, including the spring feeding periods and winter diapause. Changes in copepod lipid compositions are likely to have an impact on multiple processes, such as copepod buoyancy, carbon sequestration and lipid profile for both human and aquaculture utilisation. Monitoring the impact of these alterations will be key in managing these marine resources, as they provide both an ecosystem service and a feedstock for long chain polyunsaturated fatty acids.

Acknowledgement

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HAPLOTYPE-RESOLVED TELOMERE-TO-TELOMERE GENOME ASSEMBLY AND ANNOTATION OF THE AFRICAN CATFISH (*Clarias gariepinus*)

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Introducrtion

Air breathing catfishes (*clariids*) are a group of stenohaline freshwater fish that can withstand various environmental conditions and farming practices. Therefore, considerable efforts are being made to increase and optimize industrial production of the African catfish (*Clarias gariepinus*).

However, there is tremendous lack of genomic resources indispensable to study genetic traits and variations, that are of high relevance in domestication and adaptation of *clariids* to aquaculture environments. In particular, there is a need of a high quality assembled and annotated draft genome as reference basis for addressing questions in catfish research in general, and specifically, to understand and enhance aquaculture production and performance traits of the catfish.

Here, we sequenced the genome of the African catfish *C. gariepinus*, one of the most commonly farmed *clariids*, and generated a gapless telomere-to-telomere (T2T) de novo chromosome-level assembly with high-resolution haplotypes, by integrating long-range sequencing (Hi-C) with PacBio single-molecule (HiFi), Oxford Nanopore, and Illumina sequencing data.

Results

The diploid genome assembly yielded 58 contigs with a total length of 969.72 Mb and a contig N50 of 33.71 Mb. We reported 25,655 predicted protein-coding genes and 49.94% repetitive elements in the *C. gariepinus* genome. Interspersed repeat are the most abundant class of repetitive elements (46%). Retroelements and DNA transposons accounted for only 12 and 6 percent of the repeatome, respectively. Approximately 99% of the assembled genome is spanned by the 28 chromosomes of the primary assembly, without gaps. The distribution of genes and repeats across the chromosomes followed the typical distribution in vertebrate genomes, with higher gene densities in GC-rich regions and lower gene densities in repeat-rich distal and pericentromeric regions.

We performed various assessments to support the high quality and completeness of our African catfish genome assembly. The BUSCO completeness was 97.5% with only 2% of the genes missing, showing that the gene space spanned by our genome assembly is nearly complete. Furthermore, approximately 92% of the *C. gariepinus* transcripts could map on our assemblies (>90% coverage and >90% identity), indicating their high functional completeness. We also mapped genomic reads to our assemblies to assess structural accuracy and found that more than 96.69% of raw PE reads were concordantly aligned. The alignment rate of ONT, HiFi, and Hi-C reads to the primary assembly was 99.91%, 99.95%, and 100%, respectively.

Furthermore, we annotated 6,403 full-length ribosomal RNA, 154 microRNA, and 13,536 transfer RNA throughout the African catfish genome. Remarkably, 96% (6150/6406) of the predicted 5S rRNA genes were all found in a single cluster on a 2-Mbp region on both chromosome 4 (n = 2455) and chromosome 13 (n = 3725). Similarly, 84% (21/25) of the predicted 18S rRNA genes were clustered within the first 500 kbp upstream in the terminal telomeric region of chromosome 27 (Figure 1).

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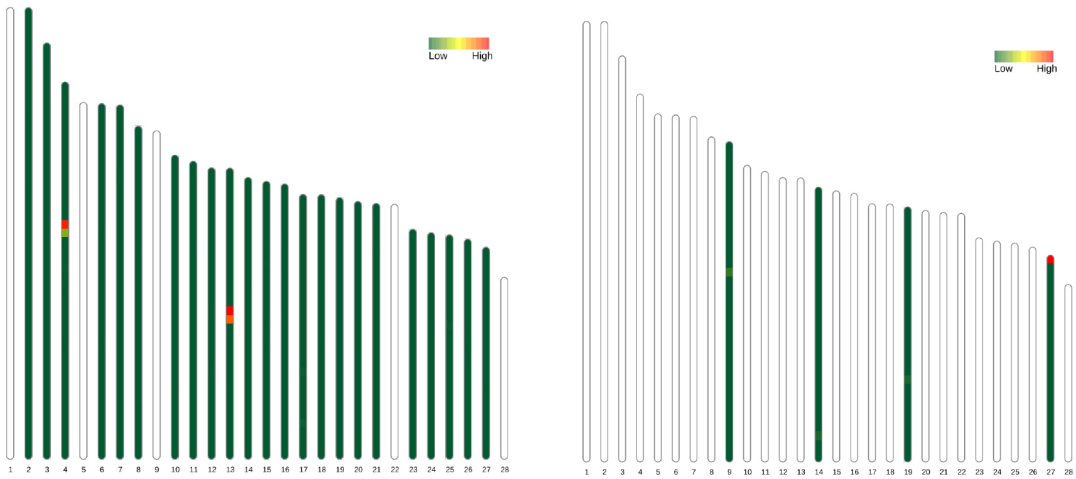

Figure 1: Chromosomal loci of 5S (left) and 18S (right) rRNA genes, clusters are red coloured, green chromosomes show remaining loci of 5S and 18S RNA

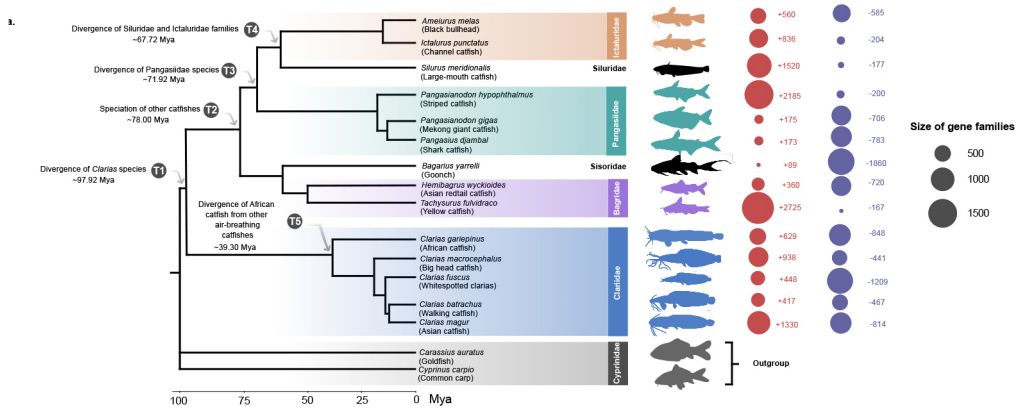

Figure 2: Phylogenetic divergence between the catfish species

The comparative phylogenomic analyses performed with OrthoFinder assigned 336,681 (94%) of 390,198 genes to 27,587 orthogroups shared among catfishes and two outgroup species (common carp and goldfish). 16,281 genes in *C. gariepinus* were found to be orthologous between the 14 catfish species, with 378 of them being single-copy orthologs. According to our estimated phylogenetic tree using protein sequences of all homologous single-copy genes, air breathing catfishes (Claridae clade) split as a monophyletic group around 98 Mya, which is roughly comparable to the divergence time between rodents and humans (96 Mya) (Figure 2).

Conclusion

Our genome assembly provides the first comprehensive gene annotation and haplotype information, such as the male-specific haplotype, enabling us to identify critical genes and molecular mechanisms underlying amphibious traits and terrestrial adaptation of air breathing catfishes. We found that several gene families involved in ion transport, osmoregulation, oxidative stress response, and muscle metabolism were expanded or positively selected in *clariids*, suggesting a potential role in their transition to air breathing capabilities.

The reported findings expand our understanding of the genomic mechanisms underpinning the resilience and adaptive mechanisms of *C. gariepinus* to adverse ecological conditions. They will serve as a valuable resource for future studies in elucidating these unique biological traits in related teleost's and leverage these insights for aquaculture improvement.

DETERMINATION OF NON-MYOFIBRILLAR AMINO ACIDS IN CAUDAL AND DORSAL MUSCLE FROM RAINBOW TROUT

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Introduction

Amino acids perform different metabolic and physiological functions in animal organisms and, in fish, they are also associated with specific dynamic action with an important role in satellite cell activation and muscle growth. Fish muscles are extremely plastic and adaptable, being organized in compartmentalized structures containing different types of fibers (white, pink and red ones). Thus, the objective was to evaluate the profile of non-myofibrillar amino acids in the dorsal and caudal muscles of rainbow trout.

Material and methods

Five trout from the same batch and age (14 months after fertilization), 261.66±40.76 g and 23.00±1.06 cm were caught after 48 hours of fasting. The fish were stunned (benzocaine), slaughtered by the rupture of the cervical spine. Muscle tissue samples were removed from the middle portion of the body, below the dorsal fin (white fibers) and from the peduncle (red fibers more abundant) and they are frozen. About 200 mg of dorsal muscle sample was crushed in 0.6mL of Milliq water added with 0.2mL of 5% mercaptoethanol and 0.1mL of 5% perchloric acid, kept in the refrigerator for 30 minutes. For caudal muscle, 200mg of tissue was triturated with 0,2mL of water, 0,2mL of 5% mercaptoethanol, and 0.12mL of 8% perchloric acid. After 30 minutes in refrigerator, the solution was centrifuged at 10,000 rpm for 10 minutes at -10 °C. The pH of the supernatant was corrected to pH=4 with 0.1mol/l ammonium hydroxide for dorsal muscle. For the caudal muscle we used 60% ethanol for precipitation of compounds rather than pH correction. The samples were kept in a refrigerator for 12 hours, after which they were centrifuged again at 10,000 rpm for 10 minutes at -10 °C and filtered through 0.45um millipore. And it injected 20uL into the HPLC Yl 9300 using a Luna C18 column, acetonitrile solvent (ranging from 4-50%) and acetate buffer pH=4.7 (ranging from 50-96%) at a temperature of 35 °C. Amino acid readings were performed using the Clarity 8.8 software.

Results

The aminoacids detected from dorsal and caudal muscle are presented in Figure1 A and B, respectively. Dorsal muscle was characterized by high detection of Cys and Lys (666.46±191.20 and 1710.90±413.29 μM). Val and Leu were detected only in caudal muscle (583.08±64.47 and 82.08±6.21 μM, respectively) and it was observed higher concentration for Arg and Phe (375.43±34.83 and 5596.63±623.43 μM, repectively) when compared with dorsal muscle (33.04±4.79 and 576.30±115.80 μM, respectively). Pro and Ile were more abundant in dorsal muscle too (377.11±42.33 and 291.33±63.48 μM). Traces of Pro and Ile were detected in the caudal muscle and of Trp and Gly in both tissues.

The utilization or metabolism of AAs in fish is complex and compartmentalized and participate in biological oxidation, gluconeogenesis and lipogenesis in a cell- and tissue-specific manner (Li et al, 2021). Among the wide variety of functions, Arg and Phe are associated with energy storage and metabolism and regulation of basal energy metabolism, respectively (Wu et al, 2013). Val is associated with glucose metabolism action (Ahmad et al, 2021), indicating the important rule of caudal muscle in this process if we considerer that fish were 48 hours fasting. Cys is associated with anti-oxidative process, Lys with long-chain fatty acid transport (Wu et al, 2013) and this find indicates that diferent role of dorsal and caudal muscle in the metabolism maintenance.

Non-myofibrillar amino acids profile was diferent in muscle dorsal and caudal in a 48h fasting, reflecting the different metabolic and physiological roles of these tissues.

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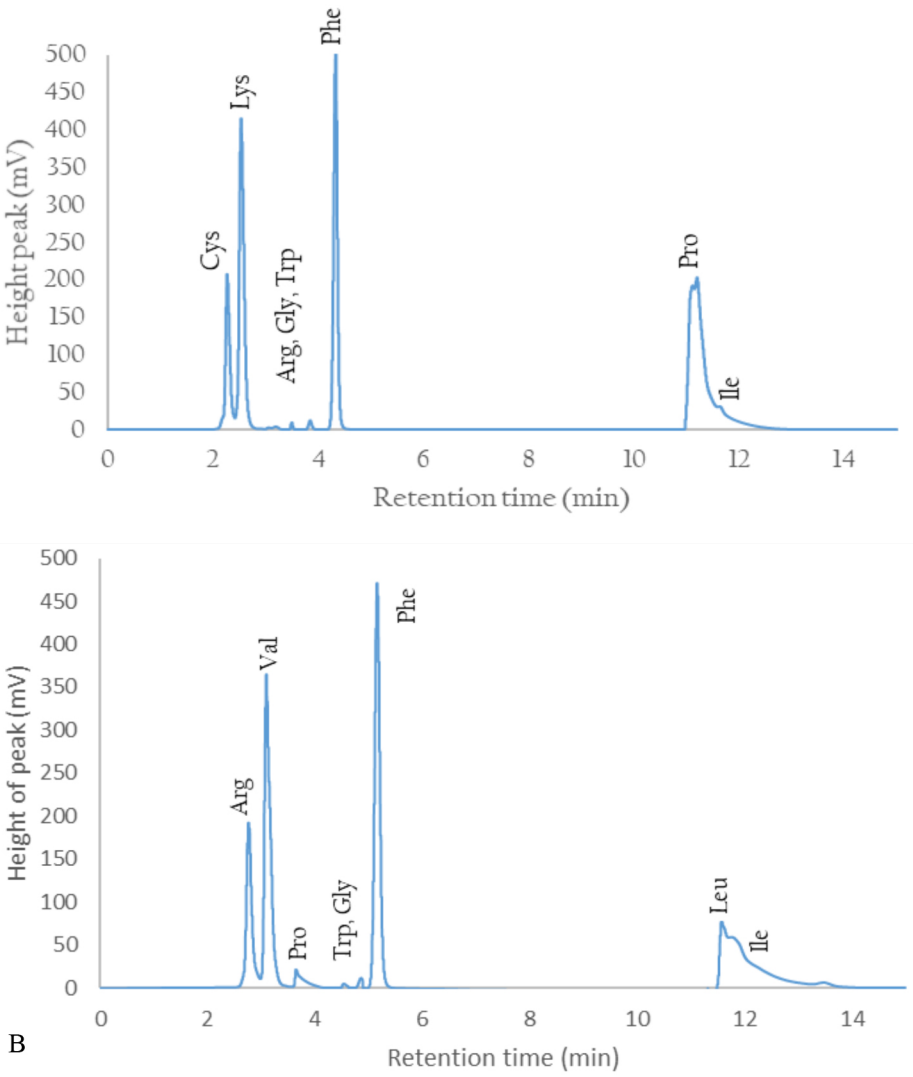

Figure 1. HPLC non-myofibrillar amino acid chromatogram extracted from rainbow trout dorsal (A) and caudal (B) muscle.

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EXPONENTIAL GROWTH MODEL OF RAINBOW TROUT STRAINS

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Introduction

In Brazil, the majority of trout farms are small-scale due to limited availability of cold water. Value addition and product diversification have been employed as alternatives to enhance the profitability of this activity. In this context, the establishment of different skin color strain has garnered attention, both in research as potential phenotypic markers, and commercially from fish farms involved in recreational fishing. Thus, the objective was to characterize the growth of trout strain, differentiated by their color pattern.

Material and Methods

Trout of four different colors (wild-type, blue, yellow-albino and white-albino) were obtained through the crossbreeding of white trout with wild-type trout. Obtaining eggs and semen involved the abdominal compression extrusion of sexually mature trout aged 2 to 3 years. Five white-albino males were suitable for reproduction and were paired with wild-type females. The semen from each male was mixed with a pool of eggs from females (N=80, average weight 1,327.67±79.35 g). The eggs were incubated under the same conditions, and the fry were cultivated in circular tanks of 2 m^3 (in triplicate) in an open system with a constant water flow until reaching a commercial weight of 350 g. The minimum water temperature during the period was 13.55±1.89, and the maximum was 15.64±1.56. Dissolved oxygen was at 6.75±0.52 mg/l.

The fish were fed ad libitum with extruded feed specific to each phase, three times a day. As the fish grew, 30 trout from each tank were randomly sampled and individually weighed at 1, 60, 90, 150, 210, and 270 days of cultivation after a 24-hour fasting period.

All weight-to-age data were fitted to an exponential model represented by $y = Ae^{Kx}$. In this model, y represents the observed weight of each fish; A represents the initial weight estimate; e is the base of the natural logarithm; K is the specific growth rate; and x is the age of each fish. 95% confidence intervals were used to compare the curve parameters for each strain. Equations and R^2 adjusted statistics were provided. The estimates were obtained using weighted least squares due to the lack of homoscedastic variance between days 1 and 270, and considering autoregressive errors (Draper and Smith, 1998; Santos et al., 2013).

Results

The screening was carried out by separating the color patterns, excluding undersized or oversized fingerlings, resulting in 2031 white-albino fingerlings, 1969 yellow-albino, 1971 blue, and 2306 wild-type ones. This quantity of the four colorations aligns with the color inheritance pattern from the crossbreeding of heterozygous white-albino trout with wild-type colored trout (25% wild-type aabb; 25% white-albino AaBb; 25% yellow-albino aaBb; and 25% blue Aabb) (Hattori et al., 2020). The fingerlings started the experiment with a similar average weight of 3.07±0.14 g ($P>0.05$).

The estimates of initial weight "A" showed differences between trout strains ($P<0.05$). The estimate for the wild-type strain was higher than that for the white-albino strain (4.4983 and 3.4449 g, respectively), and they did not differ significantly from the blue and yellow-albino strains (3.6621 and 3.8656 g, respectively). The estimates of "K" indicated differences between strains ($P<0.05$). The white-albino strain exhibited the lowest growth rate (0.00159 g/day) when compared to the yellow-albino and blue strains (0.0174 and 0.0173 g/day). The growth rate of wild-type trout was 0.0163 g/day. The estimated final weight (at 270 days of cultivation) for the wild-type, blue, yellow-albino, and white-albino strain were 366.76 g, 391.13 g, 424.16 g, and 252.12 g, respectively.

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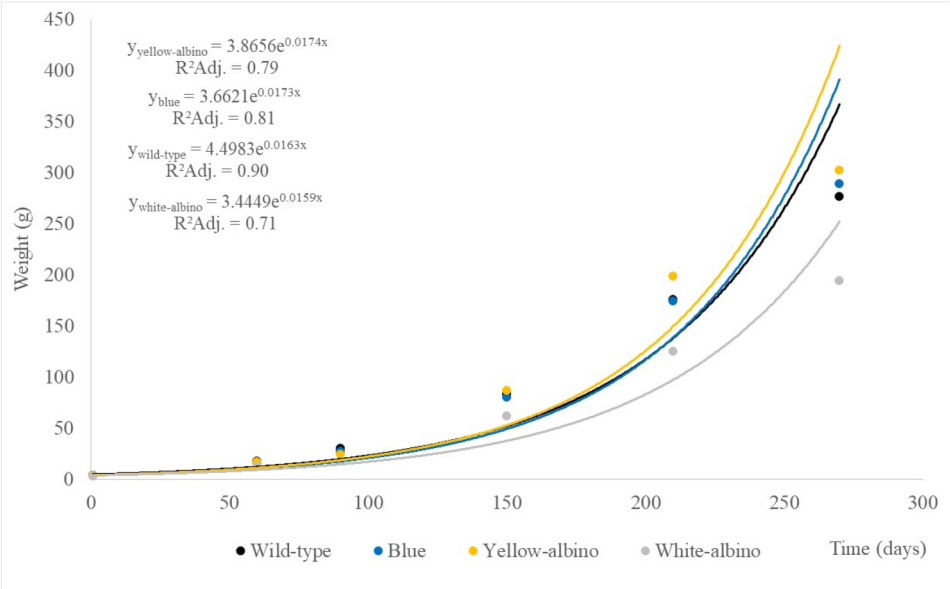

Figure 1. Exponential growth model of rainbow trout strains. The confidence limits for parameters A and K were respectively: 3.9116 - 5.0623 and 0.0156 - 0.0170 for wild-type strain, 3.321 - 4.0079 and 0.0167 - 0.0179 for blue, 3.5007 - 4.235 and 0.0168 - 0.0180 for yellow-albino and 3.1686 - 3.7269 and 0.0154 - 0.0165 for white-albino trout.

In the last few decades, the vast majority of fish farmers have been cultivating genetically improved lineages of certain species in their production systems (Gjedrem and Rye, 2018). Some traits that directly affect production costs are growth rate, fillet yield, and feed conversion. However, more recently, other traits such as body composition, nutrient content, well-being, and environmentally friendly production systems have gained prominence in consumer preferences. In this context, the establishment of lineages with different skin colors has garnered attention, especially among fish farms engaged in recreational fishing. Thus, the cultivation of lineages with lower performance but possessing consumer-attractive characteristics like coloration can translate into a competitive advantage, allowing for higher prices to be achieved.

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IRREVERSIBLE SPERM MOTILITY LOSS IN RAINBOW TROUT DUE TO POST-MORTEM LACTIC ACID ACCUMULATION AND PROLONGED LOWERED PH EXPOSURE

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Introduction

Post-mortem semen collection is a common approach in fisheries for breeding wild fish species prone to high post-capture mortality. Additionally, breeders are interested in utilizing milt from fish that have died during the spawning season. Following death of an organism, anaerobic metabolism leads to lactic acid build-up, causing *post-mortem* pH reduction. This lactic acid accumulation extends beyond blood and muscles. Previous studies have shown that lactate concentration under normal circumstances in epididymal semen collected from rats and dogs ranges from 0.6 to 0.9 mM, but this concentration surges nearly tenfold in samples taken two hours *post-mortem* [1].

Therefore, this study aimed to investigate: (1) the possibility of revitalising rainbow trout sperm with the use of short-term storage of undiluted and diluted semen samples collected *posthumously*; (2) the relationship between elevated lactate concentration, pH decrease, and the revitalisation process of sperm collected *post-mortem* from rainbow trout (*Oncorhynchus mykiss*) males.

Materials and methods

Semen samples were collected *post-mortem* from the sperm duct during mid-spawning from 3-year-old male rainbow trout with an average body weight of 0.44 kg, using a catheter (n=18). Sperm samples were collected at 2-hour intervals over 16 hours (n=6). The collected sperm were divided: one undiluted (50 µl) and the other diluted 1:10 (sperm:diluent) with artificial seminal plasma (ASP) composed of: 100 mM NaCl, 40 mM KCl, 3 mM $CaCl_2$, 1.5 mM $MgCl_2$, 20 mM Tris, and a pH of 8.2. Both samples were stored at +4°C in sealed 1.5 ml Eppendorf tubes on crushed ice. Immediate motility recording followed collection. Additional motility analyses occurred at 0.5, 1, 1.5, 2.5, 6, and 24 hours from the start of storage.

The significance of monitoring lactic acid and pH levels in seminal plasma was examined using an *in vivo* model. The concentrations of these parameters were measured in semen samples collected *posthumously* (n=18). Subsequently, semen samples from 6 males were diluted 1:10 with ASP at pH 8.5, 7.5, and 7.0. To study the effect of lactic acid concentration on sperm survival under *in vitro* conditions, we added 10, 20, and 50 mM of lactic acid to ASP solution with a pH of 7.5. Diluted sperm samples (50 µl) were stored in closed Eppendorf tubes at +4°C. Sperm motility was assessed immediately (time 0) and again after 24 hours. After 24 hours, some preserved samples were further diluted in a 1:4 ratio with ASP at pH 8.5, while the rest remained undiluted. Motility analyses were performed on all samples after another 24 hours. Motility analyses were carried out with the use of a computer-assisted sperm analysis system (CRISMAS, Image House Ltd., Denmark). For sperm motility activation 1µl of sperm was mixed with 100µl of a buffer containing 20 mM Tris, 154 mM NaCl, 1 mM $CaCl_2$, and 30 mM glycine supplemented with 0.5% bovine serum albumin (BSA, Sigma-Aldrich). L-lactate content in seminal plasma was measured using a colorimetric L-lactate Assay Kit (Abcam) at 450nm, following the provided instructions.

Results

We observed that nearly immotile rainbow trout sperm retrieved from the fish body regained motility following short-term storage when collected 2, 4, 6, and 8 hours after death. We also observed that sperm collected 2 hours after fish death regained their motility potential as soon as 1 hour of storage. Regained motility in samples diluted with ASP was higher than in undiluted samples (i.e. 94 and 71% respectively for samples collected 2 hours *posthumously*). However, samples collected 10 hours after the fish's demise showed considerably reduced sperm revitalisation compared to those obtained at earlier time points. Beyond this period, only samples diluted with ASP managed to recover around 20% motility.

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In collected *post-mortem* rainbow trout sperm samples, rising lactic acid levels were linked to lower seminal plasma pH. Within 24 hours, sperm motility in samples diluted with pH 7.0 ASP dropped significantly. However, samples diluted with pH 7.5 ASP showed no change in sperm motility during the same period. For samples diluted with lactic acid in pH 7.5 ASP, motility decreased from 80% to 30% within 24 hours. Meanwhile, samples diluted with pH 8.5 ASP showed higher motility than the initial level after 24 hours. Additional dilution with pH 8.5 ASP after 24 hours increased motility in all sperm samples. In undiluted samples, sperm motility gradually decreased, particularly in those diluted with pH 7.5 ASP compared to pH 8.5. Adding lactic acid at doses ranging from 10 to 50 mM led to a swift reduction in sperm motility within 24 hours of dilution. Subsequent additional dilution after 24 hours increased motility only in samples diluted with the lowest lactic acid dose (10 mM).

Discussion

While earlier studies indicated a decline in rainbow trout sperm quality during *post-mortem* storage [2], our research reveals the potential for a minimum 50% recovery in sperm motility within 8 hours after the fish's demise. Furthermore, our observations demonstrate that sperm revitalisation occurred more rapidly when samples obtained from deceased fish were diluted with ASP. Notably, dilution with ASP proved effective in restoring sperm motility, particularly for samples collected 10 hours after fish death.

Chiba et al. [3] demonstrated a significant increase in lactic acid concentration in fish muscle and blood after death. In our study, we made a novel observation that lactate accumulates in the sperm duct following fish death and contributes to the decline in sperm motility. Through *in vitro* experiments, we discovered that this effect could be reversed, but only when the lactic acid concentration does not exceed 10 mM. We suspect that a combination of pH and lactic acid is responsible for the irreversible immobilisation of rainbow trout sperm after the fish's death. However, it is evident that other factors, in addition to pH and lactic acid, play a pivotal role in fish sperm immobilisation or degradation after death, as the observed sperm decrease ratio in the dead fish body is much higher than in the *in vitro* experiments. Notably, the impact of lactic acid on sperm immobilisation has been previously confirmed in avians during sperm storage in tubules [4]. While the physiological implications might differ between fish and birds, the molecular mechanism of this action could be similar in both animal groups.

Acknowledgments

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OFFSHORE LOW-TROPHIC AQUACULTURE IN MULTI-USE SCENARIO REALISATION

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Introduction

Global food security, human health and well-being are at serious jeopardy, as marine resource production can no longer be sustained by ecosystems and natural fisheries production only. Broad evidence supports the potential of low trophic aquatic food to reduce food and nutrition insecurity (SDG 2) in a changing climate, while we hypothesize restorative impacts on natural ecosystem services by contributing to SDG 12, 13 and 14.

Expansion of low trophic aquaculture (LTA) for increasing seafood production are faced with opportunities in unexploited regions and environments and maximizing benefits of marine space by considering low impact multi-use (MU) of space such as combining offshore wind farm (OWF) areas and integrated multi-trophic aquaculture (IMTA).

Details of the project

In the following, the EU-funded OLAMUR (Offshore Low-trophic Aquaculture in Multi-Use Scenario Realisation) project will be presented. The main objective of OLAMUR is to bring together MU-LTA related key sectors, to demonstrate sustainable commercial solutions for both the North and the Baltic Sea. All data, information, products and standards for establishing, operating and evaluating will be monitored, simulated, stored and customized as an "OLAMUR digital MU-LTA farm service" (Fig. 1). This will provide a solid basis for MU-LTA upscaling. Through a transdisciplinary holistic approach, OLAMUR will ensure substantial contributions towards demonstrating and documenting the possibilities for low impact co-use of the marine space. Multi-disciplinary data will be collected and integrated from the demonstration sites via monitoring and modelling efforts. A databased service system will be developed for policymakers for knowledge-based decisions, and innovative governance/policy arrangements will be developed towards achieving a holistic, effective and sustainable solution for multiple uses. OLAMUR will focus on three pilots that will serve as testing and demonstration sites. Two of these pilot studies are located in the Baltic Sea (Denmark and Estonia) and one study is being conducted in the North Sea (Germany) (Fig. 2). Strategies are being developed for multi-use of OWF and seaweed as well as bivalve aquaculture, and the combination of existing fish farms and the multi-use with seaweed and mussels.

An important pathway towards impact in OLAMUR is the science-policy-industry-community interface. With that, OLAMUR ensures advancement in developing optimal and carbon-neutral use and enabling a quantum leap towards long-term sustainable, healthy and rich European marine spaces.

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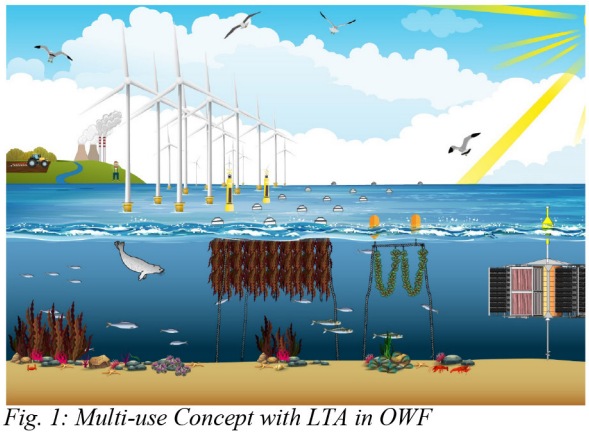

Fig. 1: Multi-use Concept with LTA in OWF

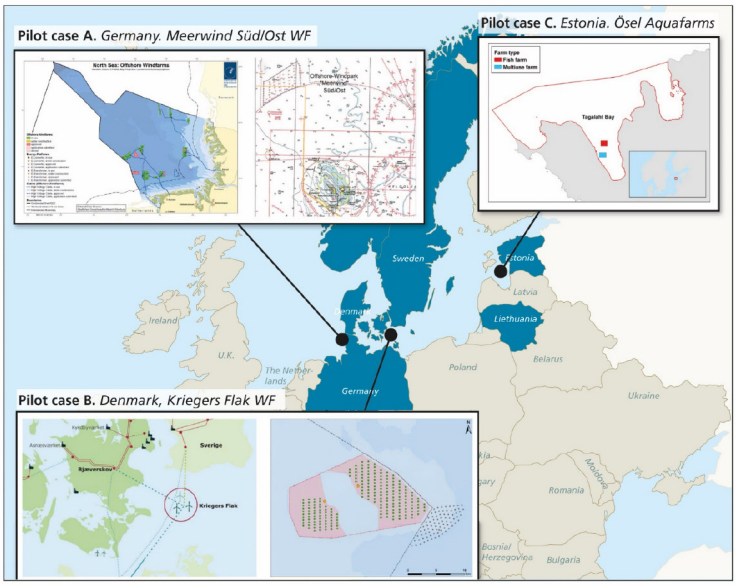

Fig. 2: Case Study Sites in OLAMUR.

Funding Details
The OLAMUR project has 25 partners from eight nations, which deal with the topics of technical realization in the wind farms, as well as site selection, LTA performance, environmental monitoring, and much more. The OLAMUR project is funded by the European Union, grant no. 101094065.

AQUACULTURE IN DISTANT AND EXPOSED ENVIRONMENTS: SCIENTIFICALLY DETERMINED INDICES AND APPLICATIONS FOR AQUACULTURE SITE CHARACTERIZATION IN A TRANSDISCIPLINARY CONTEXT

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Introduction

The terms "offshore" and "open ocean" have been used to describe aquaculture sites that are further from the coast and/or in higher energy environments. Neither term has been clearly defined in scientific literature or in a legal context and the terms are often used interchangeably. These and other related terms (e.g., "exposed", "high-energy"), refer to specific aspects of a site, usually the geographic distance from shore or infrastructure, or the level of exposure to an extended fetch leading to large waves and strong currents. The term "offshore aquaculture" has hitherto encompassed various perspectives, including technology, geographical location, legal jurisdiction, and more. To resolve the ambiguity in this term and understand its implications for current and future aquaculture, "offshore" should be resolved into two separate metrics: (1) Distance from shore and (2) exposure. Consequently, "offshore is defined by the distance from the shore while exposure can be applied as additional character or aspect of any site".

Details of the project

The ICES Working Group for Open Ocean Aquaculture (WGOOA) therefore established a need to define the terminology to reduce ambiguity for characterising these types of aquaculture sites or more precisely, to: 1) promote a common understanding and avoiding misuse for different classifications; 2) enable regulators to identify and designate the characteristics of a marine site; 3) allow farmers to be able to assess or quantitatively compare sites for development; 4) equip developers and producers to identify operational parameters in which the equipment and vessels will need to be operating; and 5) provide insurers and investors with better means to assess risk and premiums.

The United Nations Convention on the Law of the Sea (UNCLOS) delineates zones in which States are largely free to regulate aquaculture and other exploitation activities (e.g. inland and territorial waters, exclusive economic zones). Within this framework, different coastal states have developed policies and laws that specifically govern aquaculture. Neither UNCLOS nor national aquaculture laws, however, provide a precise definition of the term "offshore". Such vague geographical concepts alone cannot aid in identifying, assessing, and geographically pinpointing suitable aquaculture sites.

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The metrics of distance from shore and exposure are seen as a range rather than a specific threshold, allowing for a continuum. Distance from shore is readily quantified as distance from the baseline. To rigorously quantify the exposure, six indices were generated, which covered various oceanic parameters (i.e., water depth, water currents, wave height and period). The influence and interaction of the oceanic parameters were all considered using the indices to determine site characteristics. Two indices were selected for utilization on the analysis of sites based on their ease of use and applicability.

Finally, we applied these indices to their use in aquaculture with different species, technologies and in O&M. We also considered the costs of expanding aquaculture from protected to more exposed sites. The influence of these definitions on socio-economic aspects is addressed. Negative public discourses on the expansion of nearshore aquaculture are one of the most prominent aspects driving public opinion against aquaculture. Expansion of offshore aquaculture out of sight from the coasts is a major advantage. Finally, we suggest necessary research areas to enable the expansion of aquaculture activities to “offshore” and “exposed” waters.

Funding Details
This research has been supported by the institutes of the scientists involved. The resulting 8 publications were compiled within the framework of the WGOOA (Working Group on Open Ocean Aquaculture) of the intergovernmental scientific organisation ICES (International Council for the Exploration of the Sea - Copenhagen/Denmark). Likewise, this project was supported by the OLAMUR project with 25 partners from eight nations dealing with the issues of technical implementation in the wind farms, as well as site selection, LTA performance, environmental monitoring and much more. The OLAMUR project is funded by the European Union under grant number 101094065.

SOCIETAL PERCEPTION OF AQUACULTURE: COMBINING SCOPING REVIEW AND MEDIA ANALYSIS

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Introduction

Aquaculture has been recognised as a potential food industry to contribute to multiple United Nations Sustainable Development Goals; its growth and development depend on understanding societal perceptions in a broader context. However, to date, no comprehensive reviews have been conducted to understand whether scientific publications and newspaper outlets portray societal perceptions of aquaculture – in terms of sustainability – in positive, negative, or neutral terms. To fill this gap, this study synthesises articles on societal perceptions of aquaculture to understand whether scientific and newspaper articles mention multiple sustainable dimensions before portraying aquaculture as positive, negative, or neutral.

Methods and materials

Web of Science, Scopus, and Google Scholar were consulted to search research articles published from 1 January 2015 to 15 January 2023 with search terms: *aquaculture*, *farmed fish*, *aquafarming*, *mariculture*, *polyculture*, *perception*, *belief*, *attitude*, *image,* and *opinion.* Scientific articles following inclusion criteria were thematically clustered employing the visualisation of similarities (VOS) software. Further, 100 newspaper articles (n = 100) were selected from each of the following countries: UK, Denmark, France, Spain, Turkey and China; 79 were selected from Poland, while articles from Hungary (n = 29) and India (n = 70) were also selected for media content analysis.

Results

A scoping review identified 151 studies for inclusion in our five identified clusters of scientific publications: (1) social acceptability, (2) growth and development, (3) media coverage, (4) sustainable aquaculture, and (5) consumer perception. Further, with triangulation, the findings from this study suggest that scientific and newspaper articles mention one or more aspects of aquacultural sustainability in an abstract form to base their perception as positive, negative, or neutral. Key stakeholder groups include the fish farming industry (fish farmers, aquaculture associations or groups), civil society groups (environmental NGOs, activists, community groups, media), governmental officials, scientists, and business leaders (retailers/wholesalers, technology industry, other industries such as fisheries, tourism), and the public (indigenous groups, residents, and consumers). The stakeholder groups perceive aquaculture differently, both between them and depending on the circumstances and context; their perception ranged from positive to negative. Multiple factors influence their perceptions, including aquaculture's impact on multiple sustainability dimensions, knowledge, transparency, personal interests, types, and location of aquaculture practises, regulations, experience, food-related and dietary lifestyles, as well as sociodemographic characteristics such as age, gender, education, household size, occupation, and income.

Implications

We recommend that aquaculture practitioners focus on context-specific multifaceted strategies – prioritising transparency, communication, and accountability – and provide essential knowledge to ensure that societal perceptions of aquaculture are based on accurate, empirical information.

SUPPLEMENTATION OF THE PLANT *Solanum glaucophyllum* TO DIETS FOR ASIAN SEA BASS *Latas calcarifer* INCREASES GROWTH

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Introduction

Although the vitamin D metabolism in fish is similar to the one described in other vertebrates, there are some marked distinctions. While the conversion of vitamin D to 25-hydroxyvitamin D (25(OH)) occurs in the liver, the main sites for the second conversion step from 25(OH) to the active metabolite 1,25-dihydroxyvitamin D (1,25$(OH)_2D_3$) are both the kidneys and the liver (Lock et al., 2010). In addition, 1,25$(OH)_2D_3$ seems to be the main circulating vitamin D metabolite in fish and not 25(OH) as in other species (Lock et al., 2010). Last but not least, the role of vitamin D on the calcium metabolism in fish is unclear (Lock et al., 2010).

Recently, additional effects of vitamin D and its metabolites on aquatic species have been examined. Observed effects were changes in lipid metabolism and thus growth (Lin et al., 2022) as well as effects on the immune system (Cheng et al., 2020).

As the conversion of vitamin D to 1,25$(OH)_2D_3$ might be impaired by other factors such as age or dietary mycotoxins (Abe et al., 1982; Sauvé et al., 2023), directly supplementing 1,25$(OH)_2D_3$ to the diet could be a way to close this gap. *Solanum glaucophyllum* (SG, waxy-leaf nightshade) is one of only a few plants naturally containing a high amount of 1,25$(OH)_2D_3$ in glycosidic form (G-1,25$(OH)_2D_3$). The aim of this study was to test the effect of different dosages of SG added to standard feed for Asian sea bass (*Latas calcarifer)* on performance.

Material and Methods

A total of 120 male and female Asian sea bass were distributed with a density of 15 fish/m^3 in 24 300-liter tanks with a salinity of 15 ppt. Initial body weight was 44.5 ± 2.3 g. The fish received 3 times daily one of 6 experimental diets during 8 weeks: Control diet (CON), CON + 2.5 µg 1,25$(OH)_2D_3$ (T1), CON + 7.5 µg 1,25$(OH)_2D_3$ (T2), CON + 15.0 µg 1,25$(OH)_2D_3$ (T3), CON + 22.5 µg 1,25$(OH)_2D_3$ (T4), and CON + 30.0 µg 1,25$(OH)_2D_3$ (T5). The control diet was formulated according to standard recommendations and contained 39.5 % crude protein, 11,2 % crude lipids, 3.8 % crude fiber, 1.31 % Ca and 0.90 % total P. 1,25$(OH)_2D_3$ was added as G-1,25$(OH)_2D_3$ from SG (Panbonis®, Herbonis Animal Health GmbH, Switzerland). Performance (body weight (BW), daily weight gain (DWG), feed conversion ratio (FCR), and specific growth rate (SGR)) was measured from 0 to 4 and 4 to 8 weeks after the start of the experiment. Protein efficiency ratio (PER) was calculated for the whole experimental period.

Results

There was no mortality recorded in any of the tanks. All performance data were lowest in CON and highest in T2. Additionally, performance of T5 tended to be lower than performance of animals fed T2, T3 or T4. Effects were more pronounced at the end of the experiment than after 4 weeks. Final individual BW in CON was 87.2 ± 15.2 g and 146.0 ± 8.9 g in T2, respectively ($p < 0.05$). There were no differences among the treatments in SGR until week four with an overall mean of 1.68 ± 0.38 % per day. The value increased to 1.86 ± 0.38 % per day for the whole experimental period, showing a difference of 0.96 % per day between T2 and CON ($p < 0.05$). Similarly, the body weight SGR was lowest in CON and highest in T2 ($p < 0.05$). FCR for the whole experimental period was between 1.08 ± 0.04 (T2) and 1.78 ± 0.46 (CON), which was significantly lower than any other diet supplemented with SG. PER was 1.50 ± 0.38 for fish fed the CON diet and 2.36 ± 0.08 for fish supplemented with 7.5 µg G-1,25$(OH)_2D_3$ ($p < 0.05$).

Discussion

This trial showed that fish react in a dose dependent manner to the supplementation of SG. Interestingly, the dosages required to result in any changes are much higher than used in terrestrial animals. There, the standard dosage is 1.0 µg G-1,25(OH)2D3/kg feed. This might be related to differences in metabolism (ectotherms vs. endotherms) and digestive physiology. Further studies are planned to better understand the underlying mechanisms of the influence of 1,25(OH)2D3 on fish performance and to narrow down the optimal dosage for practical application.

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Conflict of Interest

The trial was funded by Herbonis Animal Health GmbH. Neither K. Bühler nor H. Bachmann were involved in data collection or data analysis.

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LOW INCUBATION TEMPERATURE MAY INFLUENCE SURVIVAL AND DEVELOPMENTAL PLASTICITY OF INNATE IMMUNITY IN ATLANTIC SALMON LARVAE EXPOSED TO *Yersinia ruckeri*

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Introduction

Norwegian aquaculture shares a large proportion (more than half) of global production, where Atlantic salmon (*Salmo salar*) is one of the most intensified farmed fish species. However, there is a significant number of fish mortality in the production line resulting in huge economic losses due to various infectious agents, selective breeding practice and high rearing temperature causing impaired immune functions. Moreover, higher temperature can be detrimental for fish health and can pose a threat for developing deformities during early life stages in Atlantic salmon. Continuous attempts have been undertaken to produce robust fish and rearing circumstances, particularly by adjusting water temperature during early life stages, which have been shown to be effective in regulating adult salmon phenotypes. In this study, we aim to assess if low rearing temperature during embryogenesis of Atlantic salmon can manipulate innate immunity which may improve disease resistance potential against potent pathogens in later life stages such as *Yersinia ruckeri* which is predominant in salmonids. *Y. ruckeri* causes enteric redmouth disease (ERM) and is more prevalent in rainbow trout in countries such as Scotland, Australia and Chile. However, the number of outbreaks in farmed Atlantic salmon has increased in Norway in recent years. *Y. ruckeri* is often a problem during the freshwater stage of production. Therefore, it is crucial to find sustainable options on how to improve disease resistance in Atlantic salmon. Manipulating embryonic rearing temperature may help producing robust phenotypes, as it is a strong regulator of immunity and changes occurring during early life stages may have a pervasive influence on the fish.

Materials and methods

Fertilized eggs of Atlantic salmon were reared under different temperature regimes of 4, 6 and 8°C until the eyed-egg stage (~320 day degrees), and thereafter reared under similar conditions as the 8°C group. Larvae (before start-feeding) were exposed for 2h to a *Y. ruckeri* bath challenge in a multi-well plate and followed for 72h. Bacterial localization was assessed by immunohistochemical (IHC) detection and followed up by histological examination in the gills and skin. Multi-gene expression analysis was performed on the BioMark HD platform to assess early immune competence (ImCom). Multiplex fluorescent *in situ* hybridization (FISH) was established, using RNAscope technology, to detect monocyte and lymphoid progenitor cells in whole larvae.

Results

Larvae from the 6°C group showed a better survival probability (up to 16.69%) than in the 8°C at 72h post challenge. IHC staining showed localization of the bacterial antigen mostly in the secondary lamellae of gills, and the epithelial and basal layers of skin. However, very mild histopathological changes such as sparse epithelial lifting of gills secondary lamellae and epithelial disruption of skin tissue were noticed after 24 and 72h. ImCom results showed that the larvae of the 4°C group had a significantly differential expression pattern of innate immune genes compared to the 8°C group. For example, *toll-like receptor 13 (tlr13)* involved in pathogen recognition and stress proteins such as *heat shock protein 70 (hsp1a1)* in the gills, whereas *gelsolin (gsn)*, *collagen 1 (col1a)* and *claudin 4 (cldn4),* which are important extra cellular matrix (ECM) and tight junction proteins for their regulatory roles during inflammatory and antimicrobial response, were differentially expressed in the skin after 24 and 72h of challenge. However, *gsn*, *hsp1a1* and *cldn4* were also upregulated in 8°C, but immune response was overall more pronounced in the gills than in the skin. Moreover, 6°C group had a relatively suppressed immune genes expression in both gills and skin compared to 8°C group. Other pro-inflammatory genes also showed higher tendencies in the low temperature groups. The FISH method demonstrated the detection of monocyte lineage cell population in the head kidney region and lymphoid progenitor cells, which were more abundant in the thymus tissue in all temperature group. However, 4°C group larvae showed a relatively low number of these long living immune cells which could relate to thermal sensitivity of progenitor cell differentiation during embryogenesis.

Conclusion

Altogether, our results depict that low rearing temperature during early life stage may have an influence on developmental plasticity of innate immunity and better survival in later life.

Acknowledgements

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INVESTIGATING SALMON BEHAVIOURAL CHANGES TO A STRESSOR FOR AUTOMATED FEEDING AND IMPROVED WELFARE IN AQUACULTURE FARMS

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Introduction

Aquaculture is expanding globally, valued at USD 281.5 billion in 2020, with Atlantic salmon dominating finfish production at 2.7 million tonnes annually (FAO, 2022). As the industry grows, more sophisticated technology is needed to monitor farms and ensure their sustainability. Using behaviour as a non-invasive form of monitoring, in combination with artificial intelligence and machine learning, can allow for higher control over farm management (Yang et al., 2020). The development of algorithms to analyse fish behaviour related to feeding may be used to fully automate the feeding process and reduce environmental and economical waste. The goal of this study is to identify changes to Atlantic salmon (*Salmo salar*) behaviour related to responses to stressors such as the well boat treatment for gill disease (freshwater) and sea lice (FLS).

Materials and Methods

For this study, 5 cameras were deployed at a Scottish Atlantic salmon farm consisting of 10 cages, each 100 m in circumference and ~15 meters in depth. The cameras were deployed in one cage in the following orientation: 3 down the centre (4 m, 9 m, 14 m), 2 at 9 m on the inner and outer areas of the cage, respectively. An algorithm was created by Observe Technologies to process video footage from these cameras and transform it into behavioural data useful for farmers (*e.g.,* activity, speed, schooling, shoaling). For this project, daily internal validation occurs whereby experts compare the videos to the output from the algorithm. The analysis for this study used activity as a proxy for distribution, as an increase in activity corresponds to an increase in the number of fish observed in the videos. Activity was recorded for 5 days surrounding the mechanical treatment and statistical differences were determined with two-sided Kolmogorov-Smirnov tests. Additionally, latency to feed was documented as the time between when feeding was turned on to when activity increased at camera 2.

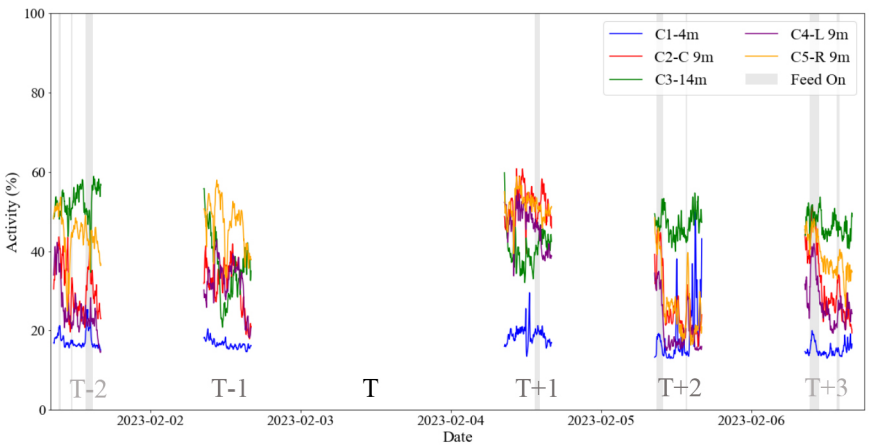

Figure 1. Activity (%) for each camera (blue: centre at 4 m; red: centre at 9 m; green: centre at 14 m; purple: left at 9 m, yellow: right at 9 m) during daylight hours for 5 days surrounding gill disease and sea lice treatment (T-2 to T+3). The grey bars indicate when feed was turned on.

(Continued on next page)

Results

The fish were distributed predominantly in two areas of the cage, at mid-depth towards the inner farm and at the bottom of the cage, which was observed 2 days prior to treatment (T-2; Fig. 1). The day prior to treatment (T-1), the fish were starved, and started to move towards the middle of the cage, indicated by the higher activity at cameras 2 and 4. On the day of treatment (T), the cameras were pulled from the water, so the day post-treatment (T+1) was used to analyse fish stress response. On T+1, there was significantly higher activity in cameras at mid-depth, cameras 2 (30.8% to 51.9%; D=0.96, $p<0.001$), 4 (25.2% to 46.3%; D=0.92, $p<0.001$), and 5 (43.9% to 51.3%; D=0.69, $p<0.001$), compared to on T-2. This coincided with a significant decrease in activity in the bottom of the cage (52.3% to 40.5%; D=0.90, $p<0.001$). Two days post-treatment (T+2), the fish moved back down to the bottom of the cage, however they appear to swim to the surface sporadically. Finally, three days post treatment (T+3), the fish resume the distribution observed at T-2. It should be noted that while the latency to feed was highest on T+1 (39.5 min), it was similar on days T-2, T+2, and T+3 (14.75, 12, 8.75 mins, respectively).

Discussion

Behaviour is a useful welfare indicator as it is the first change to occur after exposure to a stressor (Sadoul et al., 2014). The distribution of fish during this study period is largely governed by temperature. Temperatures ranged from 8 ºC at the surface to 9 ºC at the bottom of the cage. As salmon show a temperature preference between 16-18ºC, within the lower range of temperatures available they will congregate in the warmest depth, which was the bottom of the cage (Johansson et al., 2006). There appears to be a subsection of "shy" fish within the inner cage, which prefer to stay away from the bottom of the cage and the potential for predation. After treatment, the fish stress response was to move towards the centre of the cage, likely due to their innate defence behaviour mechanism of shoaling away from areas of higher potential for predation (Sadoul et al., 2014). On day T+2, while there were sporadic increases in activity at the surface, the latency to feed was not significantly different from days T-2 and T+3, indicating that changes to feeding behaviour only occurs 1-day post-treatment. Understanding these timelines can provide insight to the farmers on how to structure their feeding post-treatment to ultimately reduce feed waste.

T-2 T-1 T T+1 T+2 T+3

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DIETARY EFFECT OF ALTERNATIVE PROTEIN AQUAFEED ON JUVENILE AND SUBADULT EUROPEAN SEABASS GUT MICROBIOME

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Introduction

Recently, the gut microbiome has been at the focus of nutritional aquaculture research because it has been shown to regulate nutrient uptake (Semova et al., 2012) and changes in fish metabolism (Ni et al., 2014)immunity and health maintenance. The present study focused on factors affecting the gut microbiome of grass carp (Ctenopharyngodon idella. Such functional flexibility of the gut microbiota likely increases the adaptability of fish digestion, and studies of the gut microbiome are becoming a necessity for aquaculture nutrition research. In this study, the effects of experimental diets on the gut microbiome of juvenile and subadult European seabass were investigated after a 120- and 147-day feeding trial, respectively.

Material and methods

In both experiments, diets were formulated to be iso-proteic (45%), iso-lipidic (20%), isoenergetic (20.3 MJ kg^{-1}) and to meet the dietary requirements of juvenile and subadult European seabass. We used plant protein-based diet (CV), two plant-based diets in which graded amounts of plant protein mixtures were replaced with partially defatted *Hermetia illucens* pupae meal alone (VH10) or in combination with poultry byproduct meal (VH10P30), a fish meal diet (CF), and a fish meal diet supplemented with *H. illucens* (FH10).

Bacterial DNA was extracted from the whole intestine content of 15 juvenile and 20 subadult experimentally fed European seabass (3 juvenile replicates and 4 subadult replicates per treatment) using the Invitrogen PureLink Microbiome DNA Purification Kit (Carlsbad, CA, USA) following the manufacturer's protocol. The commercial services of Microsynth AG (Balgach, Switzerland) were used for library preparation based on Nextera two-step PCR. Variable region V4 of the 16S rRNA gene was successfully amplified from 15 juvenile and 18 subadult DNA extracts using the MiSeq2000 Next Generation system (Illumina, San Diego, CA, USA). The USEARCH algorithm (Edgar, 2010) was applied to generate clusters (OTUs) and taxonomic assignment for the representative sequence of each OTU based on the RDP database (Cole et al., 2014). Downstream analysis was performed using the Phyloseq package (McMurdie and Holmes, 2013) for R (version 4.2.2).

Results and discussion

The results suggest that the gut microbiota of juvenile European seabass is more stable than that of subadult European seabass using the same experimental diet formulations, regardless of the diet tested. Although the majority of phyla detected were the same in juveniles and subadults (Actinobacteria, Bacteroidetes, Cyanobacteria/Chloroplast, Firmicutes, and Proteobacteria), the observed richness was lower in juveniles and there were no statistically significant differences between treatments for alpha and beta diversity. Interestingly, the overall growth performance of juvenile fish was worse on plant-based diets than on fish-based diets, whereas the opposite was observed in subadult fish. There is a possibility that changes in the gut microbiota of subadult fish increased their digestive adaptability and contributed to better performance on alternative plant-based diets containing poultry by-products and/or insect meal, while this effect was absent in juvenile European seabass. These results are important for the development of sustainable aquafeeds and the aquaculture industry.

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Acknowledgments
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EFFECTS OF SINGLE-CELL PROTEINS FROM *Paecilomyces variotii* ON GROWTH PERFORMANCE, PLASMA BIOCHEMISTRY, AND GUT HEALTH OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

The increasing global need to find alternative and sustainable protein sources has promoted research in the field of non-conventional feed ingredients, such as single-cell proteins (SCPs). SCPs production is based on the fermentation of microorganisms such as yeast, bacteria, microalgae, and filamentous fungi. They have a high protein content with a suitable amino acid profile[1]. Specifically, single-cell protein products obtained from fungi contain 30 to 50% of proteins, other nutrients including different vitamins mainly from vitamin B-complex, and a relatively high amount of nucleic acid. To date, various substrates have been utilized to cultivate microorganisms for SCPs production, such as agricultural wastes, fruit processing wastes, methane, and animal wastes[2]. This study was undertaken to assess the effects of different inclusion levels of SCPs on growth, feed utilization, plasma biochemistry, and gut health of gilthead seabream reared under normal conditions and after a crowding stress period. The product utilized in this trial is a dried, inactive *Paecilomyces variotii* produced from sustainable industrial wood residual feedstocks to partly or fully replace traditional protein sources in fish feed.

Materials and methods

One trial with gilthead sea bream was conducted in a closed recirculation aquaculture system (RAS). Fish (initial weight: 120.3 ± 0.3 g), were fed over 104 days with four experimental diets containing different inclusion levels of SCPs meal (0% CTRL, 5% SCP5, 7.5% SCP7.5, and 10% SCP10) in substitution of fish meal (FM). After the end of the trial, fish were subjected to crowding stress for two hours (density 80 kg/m^3). At the end of the growth trial, distal intestine content was collected for gut microbiota analysis and growth and feed efficiency parameters (specific growth rate, SGR, feed intake, FI, feed conversion rate, FCR), were assessed. Furthermore, blood and a portion of the distal intestine were collected at the end of the growth trial (T0), after the 2 hours (T2) and after 24 hours (T24) from the crowding stress in order to get information on the physiological status of the fish and to investigate on the expression of genes correlated to several gut functions, including immune regulation (interleukin 1β, IL-1β, interleukin 6, IL-6, interleukin 8, IL-8, interleukin 10, IL-10, tumour necrosis factorα, TNF-α, T-cell receptorβ, TCRβ, histocompatibility complex I, MHC I, Proliferating cell nuclear antigen, PCNA, Lysozyme, Lyz), stress response (glutathione reductase, GR, glucocorticoid receptor, GCR, Heat shock protein 70, Hsp70), nutrient absorption (peptide transporter 1, Pept 1, sodium-glucose transporter, SLC5A1, fatty acid binding protein 2, FABP2, Chemerin Chemokine-Like Receptor 1, cmklr1), and epithelial function (aquaporin8, Aquap8, Claudin15, Cldn15, Aminopeptidase N, AminopN). Data were analyzed by one-way, and a two-way ANOVA followed by Tukey's multiple comparison test, using GraphPad Prism, version 9.1.1.

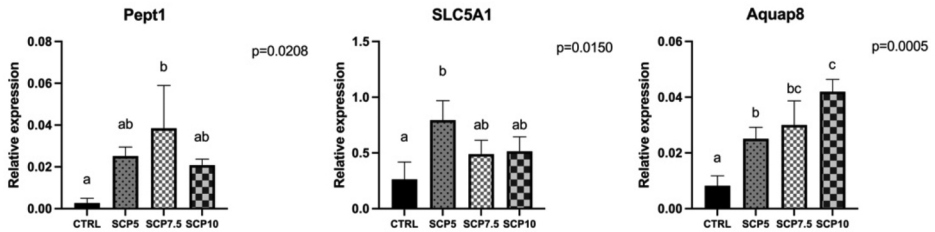

Figure 1. Relative expression of genes Pept1, SLC5A1, and Aquap8 at T0. Different superscript letters a, b and c denote significant differences ($p < 0.05$) among diets.

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Results

At T0, no significant differences ($p > 0.05$) due to the different treatments were observed in final body weight, FI, SGR, and FCR. Regarding gene expression analysis, genes related to immune regulation showed no differences among diets ($p > 0.05$). Regarding genes involved in nutrient absorption, Pept 1, showed higher values in SCP7.5 with respect to the control diet ($p < 0.05$), while SLC5A1 showed higher expression in SCP5 as compared to the control diet. Even if not significant ($p > 0.05$), the FABP2 showed a similar trend. Among genes with epithelial function, Aquap8 showed a dose-response effect ($p < 0.05$). In fact, its expression increased with the increasing inclusion of SCPs (Fig.1). Considering T2 and T24, time effect, diet effect, and a significant interaction between diet and time were observed in the expression of several genes including interleukin 8, heat shock protein 70, glutathione reductase, glucocorticoid receptor, and FABP2.

Discussion and Conclusion

The results show that experimental diets containing different levels of SCPs inclusion from *Paecilomyces variotii* can replace 5%, 7.5% and 10% of FM without compromising the growth and feed utilization. Regarding the immune genes, there is no difference in immune response among treatments at T0, a result that matches growth performances. SCPs inclusion affected genes related to nutrient transport and water absorption indicating a probable higher capacity for nutrient and water uptake. Finally, both diet and time clearly affected gene expression.

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EFFECT OF PARTIAL SUBSTITUTION OF FISH MEAL BY RICH PROTEIN SPENT GRAIN ON MEAT QUALITY, MICROBIOTA AND CHEMICAL COMPOSITION OF WHITE SHRIMP (*Litopenaeus vannamei*) FARMED IN BIOFLOC

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Introduction

Fishmeal and fish oil are essential elements of diet of many species in aquaculture due to their excellent balance of nutrients however, the increase in its demand has generated shortages and increased prices (FAO, 2020). Recent research has focused on the development of replacement strategies for fishmeal and fish oil by alternative sources such as those of vegetable origin, among which those derived from cereals, legumes or vegetable oils stand out; however, other research areas that has emerged today is the search for industrial by-products that can be incorporated in diets for aquaculture species. Currently, the brewing industry generates large amounts of by-products such as spent grains and others (Mussatto et al., 2006). Spent rains from the brewing industry contain approximately 18 to 35.4% protein (Fărcaş et al., 2017), which does not make it the ideal candidate to replace fishmeal in a high percentage; however, have begun to be investigated with favorable results (Estévez et al., 2021). Besides, recently have been reported some methods to increase the protein of spent grain (He et al., 2019).

Although vegetable ingredients may present some deficiencies derived from anti-nutritional factors, one alternative to face these deficiencies are the biofloc cultures, since the bioflocs developed in these cultures can supplement part of the deficit of amino acids and essential fatty acids in diets with little or no inclusion of fishmeal. Roy et al. (2009) replaced fishmeal with alternative sources of animal and vegetable origin in shrimp diets and Bauer et al. (2012) verified that the complete replacement of fishmeal with alternative sources of vegetable origin (soybean meal) and biofloc meal does not affect the growth, FCA, or survival of L. vannamei, concluding that natural productivity acted as a source nutrient supplement. These studies suggest that bioflocs could play an important role in the diet when fishmeal and fish oil are substituted (Ekasari et al., 2010). That is why the objective of this work is to investigate the feasibility of using protein-rich spent grain from the brewery to partially replace fishmeal and its effect on growth parameters, chemical composition, meat quality and microbiota of Pacific white shrimp (*Litopenaeus vannamei*).

Materials and Methods

The spent grain from the brewery was processed according to the method published by He et al (2019). Subsequently, 4 experimental diets (37% protein and 10% lipid) were prepared. The Control Diet (DC) was formulated with fishmeal, soybean meal and poultry meal as protein sources, while in the experimental diets fishmeal was substituted by beer bagasse by 7.5% (D1-7.5), 15% (D2-15%) and 22.5% (D3-22.5). Subsequently, a batch of 2000 larvae (PL-7) of Litopenaeus vannamei shrimp was transported from the company Productos pesqueros del Evora, Sinaloa, Mexico to the aquatic organisms laboratory of the Veterinary Sciences Research Institute-UABC, where they were received in a tank. of 1000 liters that is part of a recirculation system with seawater and were fed with a commercial diet until they reached the approximate weight of 0.5 grams. At that time, 300 juveniles (0.498 ± 0.01) were randomly stocked in 12 tanks of 100 liters each with biofloc culture (the density was 250 org x m3 at the time of stocking).The crop is developed with zero water changes and will last 45 days. The photoperiod is maintained in a scheme of 10 hours of darkness and 14 hours of light (6:00 a.m. and 8:00 p.m.).Dissolved oxygen (mg L-1) and temperature are monitored daily at 08:00 a.m. and 5:00 p.m. (YSI model 550A) and salinity is measured once a day (YSI30). Nitrogenous compounds (ammonium, nitrites and nitrates), alkalinity and dissolved solids are determined every four days using commercial reagents and Imhoff funnels, respectively.At the end of the experiment, samples of the biofloc and the digestive tract of the organisms will be taken for the identification of the bacterial flora. Likewise, biofloc and shrimp samples will be taken to determine their proximal composition, and shrimp to evaluate the performance and quality of the meat. Data for growth, proximal composition, and meat quality will be analyzed by linear regression using sigma stat 3.5 software.

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Results

Table 2. Formulation and chemical composition of experimental diets with different percentages of substitution of fishmeal for brewers' spent grain

INGREDIENTES	DC	D1-7.5	D2-	D3-
Brewers' spent grain	0	7.5	15	22.5
Soja (NRC)	25	25	20	17.5
Poultry meal PC	5	5	10	10
Fish meal	20	12.5	5	0
Beef tallow (NRC)	3.88	3.4	3.4	3.4
White wheat flour	12	12	12	12
Corn gluten (NRC)	4	4	4	4
Stay c (NRC)	0.2	0.2	0.2	0.2
ROVIMIX (Vitamins	2.5	2.5	2.5	2.5
Cholesterol (NRC)	0.5	0.5	0.5	0.5
DHA Natur	3	3	3	3
Sodium benzoate	0.1	0.1	0.1	0.1
Gelatin	6.9	6.9	6.9	6.9
Phospholípidos	1	1	1	1
Raw cornstarch	0.78	4.55	4.55	4.55
Cornstarch	15.13	11.84	11.84	11.84
BHT	0.01	0.01	0.01	0.01
TOTAL	**100**	**100**	**100**	**100**
Chemical analysis				
Protein	37.5081	37.0191	37.3031	37.0756
Lipid	10.3759	9.94015	10.6014	10.83665
Ash	10.77225	9.3699	8.70015	7.7154
Moisture	3.8	3.8	3.8	3.8
Gross energy (kJ/g)	16.45	16.59	16.84	17.05
P/E (mg/kJ)	22.81	22.32	22.15	21.74

Conclusions

Through this project, valuable knowledge will be generated about the use of industrial by-products that are currently waste matter from the brewing industry in various parts of the world and that with the correct treatment can be used for inclusion in the diet of aquatic organisms; which, complemented with the nutritional contribution of the biofloc, could generate significant savings in food costs without affecting the health of the organisms or the quality of the meat.

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EXPLORING THE EFFECTS OF FUNCTIONAL DIETS BASED ON TAURINE TO INCREASE RESILIENCE OF EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

In recent decades, there have been ongoing efforts to replace marine proteins in fish diets with alternative and environmentally sustainable sources. The most used alternative proteins in aquaculture nowadays derive either from plants or from by-products from the land animal production sector, and these are usually deficient in taurine when compared to protein marine sources. Taurine is considered to be a conditionally essential amino acid for marine fish species and has been shown to have an important role in a wide variety of biological functions, such as control of endocrine tissues physiology, cellular osmoregulation, bile acid metabolism and immune response modulation, among others.

Aquaculture fish are usually submitted to several stress factors throughout their production cycle. Stressors in fish farming can arise from both anthropogenic factors, such as grading, vaccination, and fishing procedures, as well as natural factors, like sudden increases in water temperature. Changes in water temperature have been demonstrated to significantly affect the immune system of fish.

The aim of this study was to evaluate functional diets based on alternative proteins and supplemented with taurine, as a tool to increase resilience in European seabass (*Dicentrachus labrax*) submitted to a thermal stress.

Materials and Methods

Four isonitrogenous and isolipidic diets (51% crude protein and 17% crude fat) were formulated with practical ingredients: a Marine, including 25% of marine ingredients (fishmeal and squid meal); a PAP, including 24% of animal by-products (poultry meal and poultry blood meal) and only 5% of fishmeal to increase pellet palatability; and two other diets based on the PAP formulation but containing two different levels of taurine supplementation, 0.5% (TAU0.5) and 1% (TAU1). The experiment was conducted at the Centre of Marine Sciences of Algarve (CCMAR, Faro, Portugal). Groups of 27 European seabass juveniles (12.9 ± 0.90 g) were distributed into 12 flat-bottom 100 L tanks under natural photoperiod conditions (Spring-Summer) at a density of 3.5 kg/m^3. These tanks were part of a recirculating aquaculture system (RAS), and fish were reared following standard procedures with a controlled water temperature (18.0 ± 0.3 ºC) and oxygen saturation (96.2 ± 1.9 %). Fish were hand-fed three times a day until apparent satiety for 33 days. At the end of this period, 10 fish per tank were anaesthetized, weighed and measured. Blood samples were collected from the caudal vein. Plasma, liver and intestine samples were collected and snap-frozen in liquid nitrogen for further analysis of physiological indicators, antioxidant status and immune parameters. All samples were collected after a 24 h period of fasting. After this sampling, the water temperature in the tanks was increased 5 ºC, and fish were kept under the same dietary regime for 7 days. At the end of this period, a similar sampling procedure as described before was performed.

Results and Discussion

Preliminary results indicate that after 33 days, no significant differences were observed in the final biomass, weight gain, daily growth index, feed conversion ratio and protein efficiency ratio among the treatments.

No significant differences were observed in voluntary feed intake, indicating that the replacement of fishmeal with alternative ingredients did not influence the feeding behavior of fish, nor taurine acted as a feeding stimulant.

No significant differences were found in the hepatosomatic index and condition factor among treatments. Fish survival was high (>99.5%) and not significantly affected by the dietary treatments.

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Concerning the post-stress sampling, significant differences were found in fish size. Specifically, fish fed with the PAP diet ended the trial with a significantly greater total length compared to those fed with TAU0.5. However, no significant differences were observed in the condition factor nor in the hepatosomatic index. Fish survival during this period was high (>99.6%) and no differences were found among treatments.

Plasma, liver, and intestine samples are currently being analyzed. These findings will shed light on whether taurine supplementation enhances the fish's readiness to respond to a thermal stress, providing valuable insights into its potential effects.

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SEABREAM WELFARE DURING SLAUGHTER: BEHAVIOUR AND HEART RATE ASSESSMENT

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Introduction

Seabream (*Sparus aurata*) is usually slaughtered by asphyxia on ice or ice-slurry, a method that does not immediately stun the fish and cause notable suffering. With the goal of finding a slaughter method that do not compromise the welfare of seabream, in this study we evaluated the welfare of adult seabream during the slaughter process using different stunning methods followed by one of two killing methods.

Methods

We stunned the fish with either electronarcosis as an alternative method for the industry, an anaesthetic (2-phenoxyethanol) as a control, or not applying any stunning as the current practice. Following stunning, we started a killing phase of either ice-slurry or *ikejime*, a Japanese technique consisting of impairing the brain of the fish with a sharp tool followed by exsanguination. We evaluated the welfare of the fish by using behavioural indicators such as the latency to lose opercular movement, eye-roll reflex, equilibrium, and swimming activity. We also implanted heart-rate bio-loggers that measured the heart rate activity and internal temperature of the fish as an additional method to evaluate fish welfare.

Results

We found that electronarcosis stuns the fish immediately and 2-phenoxyethanol renders the fish unconscious within 100 s. No fish stunned with electronarcosis gained consciousness while they were in the ice-slurry, while only one fish stunned with anaesthesia gained consciousness after 18 min in ice-slurry. Ice-slurry did not stun the fish and the fish showed signs of distress within 55 s of being placed in ice-slurry and only losing consciousness within 15 min on average. Slaughtering with ice-slurry can take up to 40 min and it varies depending on the stunning method used and the individuals (Fig.1). *Ikejime* caused an immediate death, however performing the technique correctly required practice.

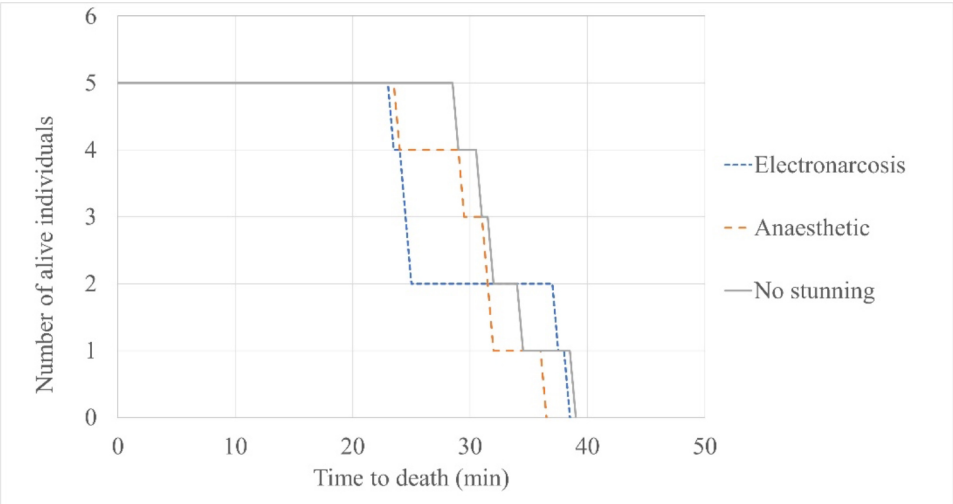

Figure 1. Time to death during ice-slurry exposure of each individual subjected to either electrical stunning (dotted blue line), 2-phenoxiethanol (dashed orange line), or no stunning (grey line).

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The bio-loggers showed that the internal temperature of the fish lowers at a very slow rate when exposed to ice-slurry, taking 24 minutes to go from 28 °C to 10 °C. The heart rate decreases over time, getting below 50 bpm within 2 min of exposure when no stunning method was applied beforehand, and increasing again after 23 min of exposure, showing some arrhythmias. Exposure to 2-phenoxyethanol reduced the heart rate from 90 to 70 bpm, and exposure to ice-slurry lowered it below 50 bpm within the first minute of exposure and maintained it low until the fish died. Electronarcosis lowered the heart rate from 83 to 65 bpm, and lowered it below 50 bpm within 1 min, also maintaining it low until the fish died.

Conclusions

Our results show that both anaesthesia with 2-phenoxyethanol and electronarcosis followed by ice-slurry are appropriate slaughtering method for seabream, and the slaughtering process can be even faster using the *ikejime* technique. Slaughtering only with ice-slurry induces suffering for 28 to 38 minutes and therefore should be avoided.

EFFECTS OF INTRAMUSCULAR INJECTION OF CARRAGEENIN, LIPOPOLYSACCHARIDE, AND POLYINOSINIC: POLYCYTIDYLIC ACID TO GILTHEAD SEABREAM (*Sparus aurata*) SPECIMENS ON SERUM AND SKIN MUCUS HUMORAL IMMUNITY

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Introduction

Inflammation is a well-characterized process in mammals, but scarcely studied in fish (Esteban, 2012). In this regard, our studies focused on the development of a model of skin inflammation with carrageenin to study and reproduce inflammation in gilthead seabream (*Sparus aurata*) (Campos-Sánchez *et al*, 2021a). Carrageenin is a mucopolysaccharide derived from the cell wall of red algae (Rhodophyceae family), used for decades as a model of acute inflammation in rodents (Winter *et al,* 1962). However, other inflammation triggers frequently used in mammals such as the bacterial lipopolysaccharide (LPS) or the viral mimic Polyinosinic:polycytidylic acid (poly I:C) have not been studied in depth in this context (Zhao *et al*, 2023). For this main reason, the present study aims to evaluate and compare the modulation of the humoral immune response in the serum and skin mucus of gilthead seabream after an intramuscular injection of either carrageenin, LPS, or Poly I:C.

Material and methods

In this study, 72 juveniles (59.94 ± 1.05 g, 15.26 ± 0.10 cm) of gilthead seabream (*Sparus aurata*) originated from broodstock of the aquaculture Research Station of Olhão (Portuguese Institute for the Ocean and Atmosphere) and maintained in the same facilities, were injected intramuscularly with 50 μL of phosphate buffered saline (PBS, as control), carrageenin (1%, Sigma), LPS (1%, Sigma), or Poly I:C (1%, Sigma) solutions. At 3, 6, and 24 hours post-injection, blood samples and skin mucus were collected by following the methods described by Guardiola *et al* (2014). The following parameters were analysed: peroxidase activity, esterase activity, lysozyme activity, bactericidal activity against *Photobacterium damselae* and total immunoglobulin levels in serum and skin mucus. Haemolytic complement activity was also studied in the serum (Guardiola *et al*, 2016). The results were expressed as mean ± standard error of the mean (SEM) and data were analysed by two-way ANOVA (followed by Tukey tests) to determine differences between experimental groups and each group in relation to time, respectively. The level of significance used was $p < 0.05$ for all statistical tests.

Results and discussion

Haemolytic complement activity increased in the serum of fish from the carrageenin group at 3 h post-injection, compared to fish from the control (injected with PBS) and LPS group. In addition, this activity also increased at 24 h in fish injected with carrageenin and LPS compared to fish injected with Poly I:C. The rest of the activities studied in the serum (peroxidase, esterase, lysozyme, bactericidal, and total immunoglobulin levels) were not altered by none of the inflammatory triggers at any experimental time. In skin mucus, peroxidase and esterase activities increased in fish injected with carrageenin and studied at 3h compared to fish in the control and LPS groups. Furthermore, peroxidase activity increased at 24h in the Poly I:C group compared to the carrageenin group, while esterase activity values increased at 24 h in the Poly I:C group compared to the control group. On the other hand, lysozyme and bactericidal activities increased at 3h post-injection in all three fish groups compared to the control group. However, at 6h post-injection, lysozyme activity only increased in the carrageenin-injected fish group compared to control fish. In addition, bactericidal activity decreased in the Poly I:C fish group compared to the LPS group. Thus, all three inflammatory triggers used in this study appeared to stimulate the release

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of proinflammatory molecules into the cutaneous mucosa from cells neighbouring the injection site, exercising a very localised action (Campos-Sánchez *et al*, 2021b). Furthermore, while carrageenin and LPS seemed to activate the innate immune response of gilthead seabream, which is a shorter and non-specific response with granulocytes and macrophages involved, Poly I:C could activate the adaptative immune system with a later, but more specialised response developed mainly by lymphocytes.

Conclusion

The present results suggest that carrageenin, LPS, and Poly I:C are able to trigger inflammation in fish and may modulate the (innate or adaptive) immune response in gilthead seabream. These data not only could be used in further studies to elucidate inflammatory mechanisms, but also in the search for anti-inflammatory drugs of great relevance to the aquaculture sector.

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A NUTRACEUTICAL COMPOUND ADDED TO A PLANT PROTEIN-BASED DIET PREVENTS HISTOLOGICAL/METABOLIC ALTERATIONS AND IMPROVES RESISTANCE TO STARVING CHALLENGE IN JUVENILE GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

In recent years, the use of micro- and macroalgae as functional ingredients has been contemplated to improve the growth and general condition of cultured fish. The "health diets", which include ingredients with functional properties on the organism, are a very promising option. In this context, nutraceuticals develop a fundamental role, providing numerous benefits for the health and physiological status of animals, including the prevention or treatment of diseases. LB-LIVERprotect is a compound formulated from microalgae that improves fish hepatic performance, contributes to cell structure maintenance, acts as a lipotropic agent that prevents the development of fatty liver and has a multibiotic effect. On the other hand, although starvation is a common situation in aquaculture, which can induce some damage to the metabolic status and welfare of cultured animals, it is still necessary to deepen the study on its negative effects and the way to prevent them. The present work aimed to evaluate the potential benefits on the metabolism concomitantly with hepatic and gut well-being of supplemented plant-based diets enriched with the nutraceutical LB-LIVERprotect for the gilthead seabream (*Sparus aurata*) in the face of a starving challenge.

Materials and methods

Three diets were formulated: i) control diet (C+) with a nutritional composition based on fish meal (FM) and fish oil (FO); ii) vegetalized diet with a high substitution (75%) of FM/FO by plant proteins and oils (C-); and iii) the C- diet supplemented with 1% of the LB-LIVERprotect compound (LP). Juveniles gilthead seabream (*S. aurata*) of 27-28 g initial mean weight were distributed in nine tanks (3 tanks per experimental diet) and fed *ad libitum* for 90 days. Subsequently, two tanks per treatment were starved for 14 days (St) and the rest of the animals were kept on continuous feeding (Cf) with the same diets. Sampling was carried out in which biometric parameters were measured and biological samples were collected. Somatic and zootechnical indices were calculated and histomorphological and histochemical analysis for the intestine and liver was carried out. Additionally, some metabolic parameters were also analyzed.

Results and discussion

C- experimental group (both fed and starved) showed the development of subepithelial spaces consequence of the separation of mucosa and submucosa layers in the anterior intestine. The ratio of the mucosa surface and width of the mucosa/submucosa layers did not present differences between treatments and/or experimental diets, but a significant increase of goblet cells was observed in starved fish from all experimental groups. In addition, starved fish presented alterations in the *tunica muscularis* of the intestine. This may be due to variations in the length and diameter of the intestine during the feeding/starving process (Liu *et al*., 2018). At the hepatic level, adipocytes number surround the exocrine intrahepatic pancreas increased in C- group (both fed and starved), and a slight decrease was observed in the LP group (Fig. 1A, B, C). This agrees with the enhancement of hepatic triglyceride content and MSI index observed in LP group (Fig. 1D), suggesting that a metabolic orchestration could be taking place for the protection of the liver in specimens receiving this diet. In this way, the excess lipids generated by the plant-based diet and influenced by the nutraceutical compound may be stored fundamentally as perivisceral fat but not as hepatic fat. Starved fish from C+ and C- groups, showed significant atrophy of the hepatocytes, while in LP-starved group this atrophy was less evident. These results are related to those obtained for the HSI index which decreases in all starved groups, although this decrease was mitigated in LP group (Fig. 1E). So the diminishing of hepatocyte volume (cellular atrophy) contributes to the reduction of liver weight and thus, the

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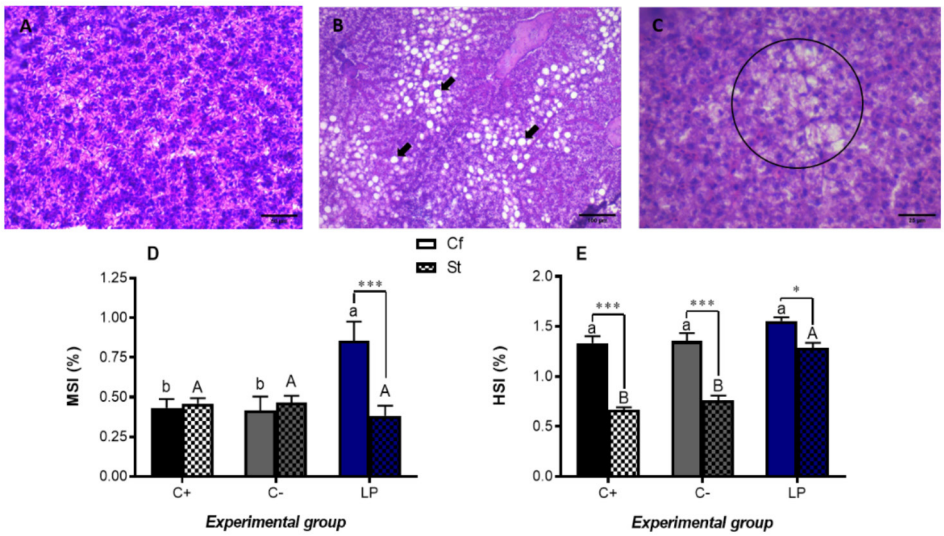

Fig. 1. A. The hepatic histological architecture from fish fed with C+ diet (H&E); B. Liver from fish fed with C- diet. Note the high number of unilocular adipocytes (black arrows) (H&E); C. Liver of fish starved from LP experimental group. Focus of multilocular adipocytes (circle) (H&E); D. Mesenteric index (MSI); E. Hepatosomatic index (HSI) in *Sparus aurata*. Results are expressed as mean ± SEM for each experimental group.

HSI value. The decrease in the volume of the hepatocytes can be also due to the depletion of glycogen stores observed in starved groups (Karatas *et al*., 2021).

Our results indicated that administration of the nutraceutical compound has positive effects on the histology and metabolism of *S. aurata*, providing the fish with metabolic protection to prevent significant hepatic damage during the starving period.

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Acknowledgements

The authors wish to thank the support of the Experimental Feeds Service of the University of Almería. This work was funded by the European Union under the 2014-2020 ERDF Operational Programme and by the Regional Government of Andalusia (FEDER-UCA18-107182), and co-financed by the spin-off from the University of Almería *LifeBioencapsulation S.L.* A. Caderno has been supported by a Ph.D. fellowship from the Regional Government of Andalusia 2021 (PREDOC_02015).

NUTRACEUTICAL COMPOUND WITH IMMUNOSTIMULATORY EFFECTS INFLUENCED METABOLISM AS WELL AS HEPATIC AND INTESTINAL STRUCTURE IN GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES

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Introduction

Diseases and infections caused by pathogenic agents in fish, as well as stress generated due to alterations in the homeostatic state of the specimens, are two of the critical points facing aquaculture. In recent years, the search for healthier and more environmentally sustainable alternatives for the feeding of farmed fish has increased. The concept of "functional feed" is an emerging paradigm in the aquaculture industry. It involves developing nutritionally balanced diets supplemented with food additives to improve health and disease resistance. In this context, microalgae are good candidates as a dietary supplement and ingredient because they possess immunostimulant properties, increase stress tolerance, and have an excellent nutritional profile. Likewise, nutraceutical compounds have acquired enormous potential in the aquaculture industry and are currently one of the main strategies to boost production in the sector. The present work aimed to evaluate, at metabolic and histological levels, the possible positive effects of the LB-IMMUNOboost nutraceutical compound in gilthead seabream (*Sparus aurata*) juveniles previously fed with a plant-based diet alone or supplemented with this compound and submitted to an inflammatory response induced by administration of IFA (Incomplete Freund's Adjuvant) oleaginous solution.

Materials and methods

Three diets were formulated: i) control diet (C+) with a nutritional composition based on fish meal (FM) and fish oil (FO); ii) vegetalized diet with a high substitution (75 %) of FM/FO by plant proteins and oils (C-); and iii) C- diet supplemented with 1 % of the LB-IMMUNOboost compound (IB). Juvenile gilthead seabream (*S. aurata*) of 27-28 g initial mean weight were distributed in nine tanks (3 tanks per experimental diet) and fed *ad libitum* for 90 days. Subsequently, eight animals per experimental diet were injected intraperitoneally with two treatments: i) Saline (control injection) and ii) IFA (100 µL of IFA solution/100 g fresh weight, vaccine adjuvant that induces intestinal inflammation). At the end of experimental time and 72 hours post-injection, specimens were sampled for biometric parameters and biological samples. Histomorphological and histochemical analysis for the anterior intestine and liver was carried out. Plasma and hepatic parameters related to metabolism were also analyzed.

Results and discussion

At the metabolic level, mobilization of some metabolites seems to be taking place after IFA injection. In these animals, hepatic glycogen concentrations decreased, constituting a common strategy in some fish species to maintain circulating carbohydrate levels after an acute stress event. However, this carbohydrate mobilization was only significant in animals fed with C- diet since, in both C+ and IB groups, plasma and hepatic glucose levels remained constant between both treatments (Saline and IFA). Differences in plasmatic lactate values were only observed in the C+ group, increasing significantly in IFA-treated animals. TAG concentrations followed a similar pattern in plasma and liver, being higher (both, Saline and IFA) in animals fed with the two plant protein-based diets (C- and IB) concerning the control diet, so, in this case, it may be an effect of the diet itself and not of the treatment.

Fish of the Saline-C+ experimental group showed normal histological architecture in the intestine and liver. Mucosa/Submucosa surface Ratio (MSR), a measure of the absorption surface, was lower in all experimental groups injected with IFA (Fig. 1A). However, groups fed with IB diet enhanced this MSR. A significant increase in the thickness of mucosa/submucosa layers was observed in individuals injected with IFA and fed with C- diet. However, in fish fed with IB diet, this effect was not observed (Fig. 1B). Goblet cells increased in individuals treated with IFA in all experimental groups, especially in group C- where this enhancement was most significant (Fig. 1C). Hepatocyte area diminished in C- and IB groups (Fig. 1D); although the nutraceutical compound seems to have a partial protective impact on this parameter after IFA injection.

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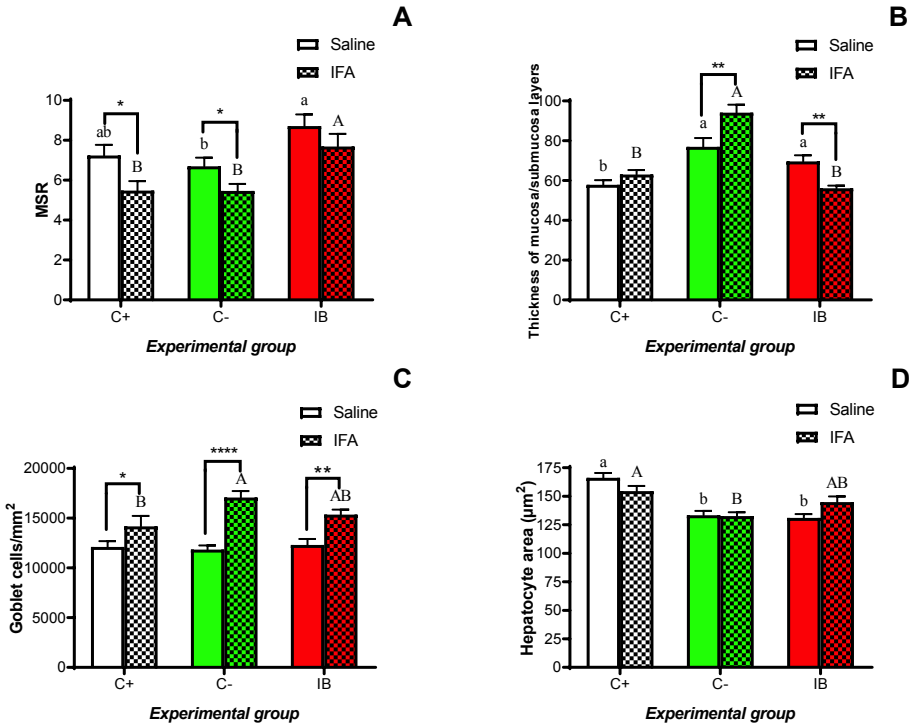

Fig. 1. A. Mucosa/Submucosa surface Ratio (MSR); B. Thickness of mucosa/submucosa layers; C. Goblet cells/mm^2; D. Hepatocyte area (μm^2) in *Sparus aurata*. Results are expressed as mean ± SEM for each experimental group.

Our results indicated that administration of the LB-IMMUNOboost nutraceutical compound has some positive effects on the metabolism and histology of *S. aurata*, providing the fish with metabolic and histological protection in the face of possible infection/inflammation challenges that can commonly occur in aquaculture practice.

Acknowledgements

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REPLACING FISH MEAL WITH FERMENTED PRODUCTS- A DIET TRIAL WITH POST-SMOLT ATLANTIC SALMON

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Introduction

Fish meal is mainly derived from wild caught fish where landings have reached a maximum capacity and cannot expand further without detrimental effects on fish stocks. In contrast, the aquaculture industry is the fastest-growing animal food-producing sector worldwide [1,2]. Therefore, great efforts have been made to find sustainable, healthy and low-cost solutions for replacing fishmeal for carnivorous fish like Atlantic salmon [2]. Salmonids that are fed diets in which fish meal is completely replaced with plant-based proteins show reduced growth, lower FCR, altered gut microbiota, metabolic and intestinal dysfunctions [3,4]. Biodegradation processes such as fermentation have proven efficient in improving the nutritional quality of several plant-protein based diets [5]. Nevertheless, knowledge on this topic is still limited and the inclusion of fermented products is less than 0,5% in common Atlantic salmon feeds [6]. The aim of this study was to assess the efficacy of substituting fish meal for fermented products (rape seed, soy and seaweed) and map how these diets affect fish performance, welfare and gut- microbiota in Atlantic salmon post-smolts.

Materials and methods

This 12-week diet-trial was performed at the NIVA research station, Solbergstrand. PIT tagged Atlantic salmon (*Salmo salar*) were stocked in 9 tanks with full strength sea water in flow-through. Fish were allowed >14 days for acclimation before the trial started. At the start of the experiment, the mean fish weight was 250 ± 48 g. Two diets, EP199 (fermented rapeseed and soy, 15% inclusion), EP299 (fermented seaweed and soy 30% inclusion), were evaluated against a control diet (CTRL) (Table 1). Diets were produced by SPAROS R&D and all diets were tested in triplicate tanks. Fish were fed *ad libitum* with automatic feeders according to the daily feeding regime: 10:00, 12:00, 14:00, 00:00, 02:00 and 04:00. Water quality was monitored daily. Feed waste was continually collected in order to calculate feed conversion ratio (FCR) and feed intake. All fish were weighed at the start, middle and end of the experiment to assess condition factor and specific growth rate (SGR). 9 fish from each diet were sampled at the start, after 6 weeks and at the end of the trial (12 weeks). From these samples microbial composition and diversity in the gastro-intestinal tract was mapped using 16S rRNA amplicon sequencing of the V4-V5 region. Sequence reads were analyzed using DADA2 [7] and community composition and ordination analysis identified the most important drivers of observed diversity Blood plasma samples were analyzed for the immune health biomarkers (Lysozyme, protease, superoxide dismutase and peroxidase activity) using commercial kits. To assess if diet affected the fish's ability to respond and recover after a stress event a standardized stress test was performed at the end of the trial according to [8].

Results and discussion

Preliminary results from this 12-week diet trial revealed that post-smolts fed with the EP199 diet had a higher condition factor compared to fish fed with CTRL and EP299 diets after both 6 and 12 weeks, and a better SGR and FCR compared EP299 fed fish at the end of the trial (Fig 1.). In contrast, fish fed EP299 besides having a lower condition factor also had a lower FCR and SGR compared to fish fed CTRL and EP199 diets after 12 weeks. Preliminary results from sequencing of the microbial community in the gut show no difference in microbial diversity (Richness and Shannon-diversity) between fish fed different diets, more data on the effects of diet on microbial composition will be presented. Post-smolts fed with EP199 also had increased levels of lysosome, peroxidase and protease activity in the plasma compared to fish fed a CTRL diet. Furthermore, EP199 fed fish had a higher stress responsiveness, measured as a larger increase in plasma levels of cortisol after a stress test. Overall, these results suggest that fish fed a diet with reduced fishmeal and a 15% inclusion of fermented rapeseed and soy perform as good or better than fish fed a CTRL diet. Furthermore, results suggest that the EP199 fed fish may have a better primed immune system and responsiveness to stress. Future studies are needed to assess and optimize inclusion of fermented seaweed products for Atlantic salmon feed.

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Table 1: Experimental diets and inclusion percentage of fermented products and fish meal.

Diet	Fermented products	Inclusion (%)	fish meal (LT70) (%)	Fish protein hydrolysate (%)
CTRL			36	4
EP199	Rapeseed and soy	15	18	4
EP299	Seaweed and soy	30	18	4

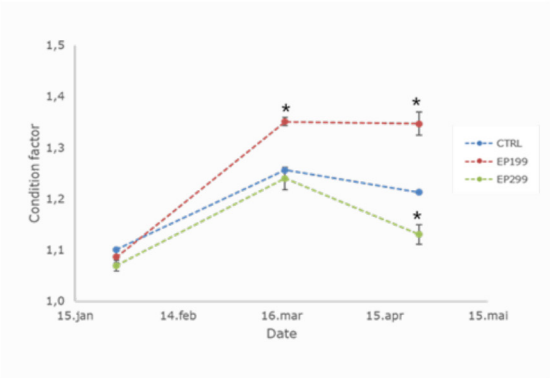

Fig.1: Fulton`s condition factor of Atlantic salmon post-smolts fed either EP199 (fermented rapeseed and soy, 15% inclusion), EP299 (fermented seaweed and soy 30% inclusion) or a control (CTRL) diet.

Acknowledgements

We would like to thank the staff and students at Solbergstrand for help carrying out this experiment. This study was funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 818431 (SIMBA).

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EFFECT OF REPLACING FISHMEAL WITH OTHER ORGANIC PROTEIN SOURCES ON THE GROWTH OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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In the present study, 5 diets were formulated, to fed *L. vannamei*, with organic raw materials (vegetal mixture, viscera Iberian pig, rainbow trout sub products, organic insect and a control diet with 30% fish meal), as an alternative to conventional fishmeal, studying its effects on growth and shrimp survival. No significant differences were found between the different treatments in terms of growth and neither in terms of survival. In summary, this study demonstrated that it is possible replace fishmeal with ecological alternative ingredients in shrimp diets without impairing its growth.

Introduction

Organic aquaculture consists in a aquatic production that is carried out respecting the certification codes that guarantee a production system that respects the environment and animal welfare in general and the sustainability of fisheries in particular (Regulation CE834/2007). In accordance with this context, the objective of this study is to replace the use of fishmeal in the feed of *L. vannamei* with completely organic ingredients for ecological and sustainable aquaculture. This was carried out by evaluating the different sources of protein in the replacement of fishmeal and studying their effects on growth.

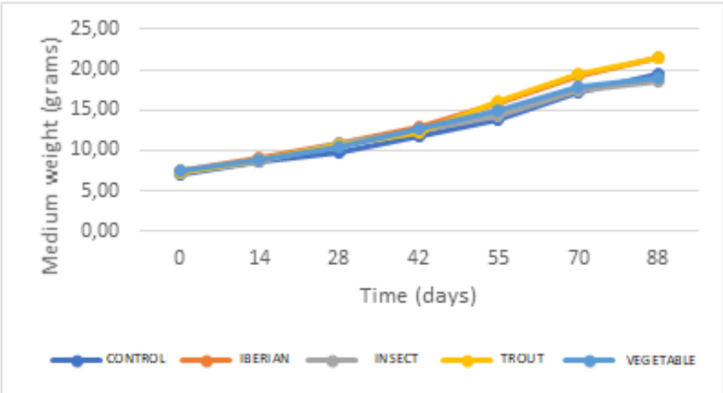

Figure 1. Average weight evolution of the shrimps fed with the experimental feeds during 88 days.

Table 2. Growth and nutritional parameters of shrimp fed with the different experimental feeds.

Diets	CONTROL	IBERIAN	INSECT	TROUT	VEGETABLE
Initial Weight (g)	1,16	1,12	1,13	1,15	1,13
ΔWeight (g/100g)	1589,6	1792,8	1546	1778,2	1563,7
Survival (%)	71,6	81,5	75,5	67,4	61,9
FR (g/100 g pez día)	4,44	4,63	5,15	4,34	4,76

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Materials & Methods

5 experimental diets were formulated and manufactured with different raw materials of organic origin, substituting fishmeal. These ecological sources were; a mixture of vegetable flours, organic trout by-product flour, Iberian pig offal meal and insect meal (VEGETABLE, TROUT, IBERIAN, INSECT). A control feed whose main protein source was fishmeal (CONTROL with 30% fishmeal) was also used. The experiment took place within the facilities of the aquaculture laboratory of the Polytechnic University of Valencia, 15 shrimp were placed in each of the 30 experimental tanks within a RAS system. The duration of the experiment was 88 days.

Results & discussion

Figure 1 shows the growth of *Litopenaeus vannamei* fed with the different experimental diets. As shown in Table 1, no significant differences were found between the treatments in terms of relative weight gain or survival. The Iberian and trout diets were those that reached a higher final weight, without significant differences (21.2 and 21.6 grams, respectively). While the Iberian and Insect diets obtained the highest values in terms of survival level, again without significant differences with the rest.

Conclusions

Feeding white shrimp *Litopenaeus vannamei* organic protein ingredients can eliminate the use of fishmeal without adverse effects on growth and survival. The organic trout evisceration by-product meal, as well as the Iberian pig viscera meal, are especially promising in terms of growth and given their animal origin. However, they do not present significant differences between them.

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SUSTAINABILITY ASSESSMENT OF NOVEL FISH FEED INGREDIENTS: A FRAMEWORK PROPOSAL WITH AN APPLICATION TO *Calanus finmarchicus* AND NORWEGIAN SALMON AQUACULTURE

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Introduction

The aquaculture industry faces a significant challenge in obtaining affordable and sustainable feed raw materials. As a result, new potential ingredients have been researched in recent decades as replacements for traditional aquafeeds, which are already overexploited. To ensure long-term environmental sustainability, any potential replacements for the feed ingredients must have their environmental performance assessed, which is why many studies in the literature have emerged assessing the environmental impacts of alternative feed ingredients for the aquaculture sector using tools such as a life cycle assessment. However, the environmental dimension is not the only criterion to consider when looking for sustainable feed ingredients. The social and economic dimensions of sustainability are also relevant, as demonstrated by Elkington (1998) in the Triple-bottom-line approach, and are frequently overlooked in the case of fish feed ingredients. The use of alternative feed ingredients may have an economic impact on producers and other stakeholders of the industry because raw material prices vary, and any changes may affect the entire production supply chain. In addition, depending on the source of the raw material, the use of alternative ingredients may affect local employment positively or negatively, while consumers may change their purchasing decisions based on their willingness to pay for more sustainable fish feed ingredients.

Future challenges for alternative feed ingredients include developing oceans in a way that is economically and environmentally sustainable while reducing reliance on the human food chain for seafood production. The dependence on edible fish (such as anchoveta) and land-based feed ingredients (such as soy) for aquaculture feed will be reduced by using species unused for human consumption. There are many species in the ocean, especially in the lower trophic levels, which are either not harvested or are only marginally utilised. One of these species is Calanus (*Calanus finmarchicus*), a lipid-rich zooplankton that is present in large amounts in the North Atlantic Ocean. Calanus is mainly used for high-value n-3 fatty acid products for human consumption in Norway, while the protein fractions are used as attractants in starter/shrimp feed and as taste enhancers in pet food. Although the production volume is currently quite small, it has the potential to provide new and substantial quantities of marine raw materials to support the sustainable expansion of Norwegian aquaculture. With an estimated biomass of 290 million tonnes, *Calanus finmarchicus* is a resource with high opportunities for its harvest and use.

Following the previous, the objectives of this investigation are twofold: (1) Develop a framework for the sustainability assessment of fish feed ingredients encompassing environmental, economic and social impacts, and (2) apply the analysed framework in the sustainability assessment of Calanus-based feed production for Norwegian salmon aquaculture.

Materials and methods

The framework will consider methodologies such as life cycle assessment, cost-benefit analysis, and semi-structured interviews and questionnaires, to address the environmental, economic and social impacts of alternative feed ingredients. Life cycle assessment is a technique for assessing the environmental aspects associated with a product over its life cycle. Meanwhile, cost-benefit analysis and semi-structured interviews serve as a base for developing economic and social indicators.

Furthermore, because a comprehensive approach with environmental, economic, and social aspects is difficult due to the many different single results obtained and the use of both qualitative and quantitative information, the Multi-Criteria Decision Analysis (MCDA) method is used to address this issue. MCDA is well-known for its ease of use, transparency, and robustness in eliciting stakeholder preferences, as well as for determining the relevance and importance of each criterion used in sustainability assessments (Deshpande et al., 2020).

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Results and discussion

The framework developed consists of the following summarized steps:

1. Mapping the life cycle stages of the production of feed's raw materials: to ensure a comprehensive assessment, the life cycle stages of the alternative raw material from harvest to the finished product are mapped. Additionally, the effect of incorporating the ingredient into a commercial diet could be examined. The Cradle-to-Gate system boundary can be used in conjunction with systems engineering methods to evaluate the environmental, economic, and social effects of the production method.
2. Data gathering and stakeholder involvement: Based on the stages of the life cycle, material, energy, and monetary flows are mapped with the participation of pertinent industrial stakeholders. As research techniques, questionnaires and semi-structured interviews are used to gather both qualitative and quantitative data relevant to the assessment.
3. Data processing and sustainability assessment: Processed data is fed into the framework for evaluating sustainability. The most pertinent environmental, social, and economic evaluation criteria are determined. For the chosen feed alternative, the life cycle assessment, cost-benefit analysis, and multi-criteria decision analysis research methods are used to evaluate the environmental, economic, and social aspects of sustainability.
4. Uncertainty analysis: Preliminary results are processed to estimate and reduce the uncertainty in the assessment to ensure a reliable analysis. In particular, to better understand the effects of emphasising one dimension (social, economic, and environmental) over another, a sensitivity analysis of the weights assigned to each of them is carried out.

In terms of the application of the framework to *Calanus finmarchicus* production, preliminary results indicate that harvesting and freezing on board presents the highest environmental impacts within the supply chain. Moreover, economic impacts are highly influenced by the production method and price of the feed material. Furthermore, it is expected that consumers are willing to pay more for salmon products in which local-based sources of feed such as Calanus are used. Also, there is a positive effect on local employment driven by a future scenario in which outsourced feed ingredients are partially replaced with local feed ingredients such as Calanus. More concrete and updated results will be presented at the conference.

Funding

This investigation is part of an industry-academic collaborative research project (CalaFeed - Enhancing the potential of Calanus as raw material for sustainable aquaculture feed ingredients) funded by the Norwegian Research Council (NRC). This investigation is part of WP4, which aims to perform a comparative sustainability assessment encompassing environmental footprints, economic feasibility and social impacts of Calanus-based feed production compared to traditional feed ingredients.

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HOW LARVAL FEEDING STRATEGIES FROM MEDITERRANEAN AQUACULTURE CAN BE APPLIED TO TROPICAL SPECIES? THE CASE STORY OF BARRAMUNDI

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Introduction

Asian Seabass or Barramundi, *Lates calcarifer,* is one of the 15 species of finfish most produced in the world through aquaculture systems. In the last 20 years, the production of this species showed an increase of 80%, from 18.1 thousand tons in 2000 to 105.8 thousand tons in 2020 (FAO, The State of world fisheries and Aquaculture 2022).

Barramundi aquaculture industries are well-established in Thailand, Malaysia, Singapore, Indonesia, Hong Kong, Taiwan, the Philippines and Australia.

The feeding regime for larval rearing of Barramundi is similar to other marine species; the green water technique is used in the initial stage of larval development, together with rotifers, followed by co-feeding of Artemia and dry diets (Aquaculture of Asian Seabass or Barramundi, Global Aquaculture Advocate, 2020).

The present study, conducted on a research scale between BRINE research centre (Indonesia) and SFA marine aquaculture centre (Singapore), investigated the application of Natura pRo and ExL in warm water culture conditions in the backyard and advanced production systems.

The effect of rotifer replacement was also investigated by introducing dry feed from the first days of exogenous feeding. Moreover, the importance of live feed enrichment was shown by comparing different feeding regimes.

Materials and methods

Asian Sea bass experiments

Experiment 1: Backyard system (Indonesia)

Hatched Asian Seabass larvae, originating from the same pool of eggs, were stocked at the density of around 15 larvae. l^{-1} in 4,000l larval rearing tanks. No pure oxygen and no continuous water renewal were used. The water temperature during the larval rearing was 28±1°C.

The effect of dry algae, live feed enrichment and early dry feed introduction was compared to the standard protocol (using fresh algae and not enriched live feed) from 0 to 25dph.

In both treatments, Control (Tr1) and RS (Tr2), algae were used from day 2 to 18ph. Enriched rotifers were fed from day 2 to 12ph in the Experimental treatment (reduced by 50% compared with the Control) and rotifers cultured by fresh algae were fed until 19dph in the Control. In Tr2 AF *Artemia* was fed from 10 to 13 dph, and enriched *Artemia* was fed from 13dph to 23dph. While in the Control group, non-enriched *Artemia* was provided from 13 to 23 dph. Different dry diets were used in the two groups: in the Control, Otohime was used from day 8 onwards and in the RS (Rotifer Substitution) group Natura was used from day 3 onwards.

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The trial finished at 25 dph with the first grading.

A natural photoperiod was used (12L/12D), and the treatments were done in triplicate.
During the larval rearing period, from 5dph, biometrics were carried out to compare growth rates, every 5 days. A salinity stress test was done to determine the stress resistance before the grading. At 25dph, growth, survival rate, final fish number per tank, fish size distribution and feed consumption were determined.

Experiment 2: Advanced system (Singapore)
Hatched Asian Sea bass larvae, originating from the same pool of eggs, were stocked at the density of around 30 larvae.l^{-1} in 4,000l larval rearing tanks under controlled conditions: pure oxygen and continuous water renewal were used. The water temperature during the larval rearing was 30±0.1°C.

The effect of the rotifer substitution and early dry feed introduction was compared to the standard protocol from 0 to 21dph.

In both treatments, Control (Tr1) and RS (Tr2), fresh algae (*Nanochloropsis)* were used from day 1 to 14ph. Rotifers were enriched in both treatments and were fed from day 2 to 14 dph (Tr2 was fed with 50% less of rotifers compared with Tr1). *Artemia* AF was fed from day 10 to 14dph, and enriched *Artemia* was fed from 15dph to 21dph. Different dry diets were used in the two groups: in the Control, Otohime was used from day 14 onwards and in the RS group, Natura was used from day 2 onwards.

The trial finished at 21dph with the first grading.

A photoperiod of 12L/12 D was used, and the treatments were done in duplicate.
During the larval rearing period, from 2dph, biometrics were carried out to compare growth rates, every 5-7 days.

At 21dph, deformity, survival rate, final fish number per tank and feed consumption were determined.

Results
The Asian Seabass larvae reared in a regime of reduced enriched rotifers and early use of dry diets showed similar growth, better performance and survival if compared with a standard feeding protocol. The adaptation of the protocols resulted in 42% rotifers substitution in Singapore and 69% in Indonesia.

Conclusions
This study shows that Asian Sea bass larvae can be reared under a feeding regime with reduced offers of rotifers, obtaining a good survival rate and good fry quality when enrichment for the live feed and balanced diet is used from the first days of exogenous feeding. The importance of live feed enrichment, well known in European aquaculture, is a central theme that must be transmitted in the Asian aquaculture hatcheries.

A high-quality diet is fundamental to obtain optimal growth and quality in the larval rearing of Asian Seabass, showing that Natura pRo and ExL, developed for the European finfish species, demonstrated a high versability in tropical conditions.

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METAGENOMICS TO CHARACTERIZE THE MICROBIOTA OF MANILA CLAMS (*Ruditapes phylippinarum*) FARMED IN THE VENICE LAGOON

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Introduction

The North Adriatic lagoons host the important aquaculture activity represented by the rearing of Manila clams *Ruditapes philippinarum*. The filter-feeding habit of these animals enhances the association of several marine bacteria within their edible tissues and among them the *genus Vibrio* is one of the most recurrent. Several studies demonstrated the increase in spread and distribution of *Vibrio* and other pathogens as an effect of global warming (Froelich *et al*., 2020; Amato *et al*., 2022). In the aquaculture industry *Vibrio* infections are frequently the cause of great losses in stock and profits (Lafferty et al., 2015), but the identity of the pathogen is hard to determinate and the dynamics of interaction within the animal microbiota could modulate the pathogenic effect. In the last few years, the improvement of the sequencing methods based on NGS allowed the detailed characterization of the microbial community dynamic and evolution. In this study, we apply shotgun metagenomics from clam homogenate and from colonies from Marine Agar (MA) and Thiosulfate Citrate Bile Salts Sucrose (TCBS) plates (enriched samples) to define and characterize the composition of the clam microbiota at species and strain level.

Material and methods

Manila clams of commercial size were collected in the Venice lagoon from the farming site of Chioggia and from Porto Marghera a polluted site in which the collection of clams is forbidden. For each site, the batches of clams were analyzed before and after the depuration treatment. A total of 12 batches were collected, 8 in summer (2018-2019) and 4 in winter (2019). After collection, depurated and non-depurated batches (25 gr) of clams were homogenized by adding 225 ml of Alcaline Peptone Water. The homogenate samples (HO) were diluted tenfold. Subsequently, 100 µl of each serial dilution was plated on MA medium (Marine Agar) and TCBS medium. MA medium was incubated at 22°C for 24 h and TCBS medium was incubated at 22 and 37°C for 24 h. The bacterial DNA was extracted from the homogenate and from pellet of bacterial cell scraped from -1 dilution plates of MA or TCBS incubated at 22°C and 37°C (enriched samples) for a total of 32 samples (8 homogenates, 8 enriched_MA, 8 enriched_TCBS22 and 8 enriched_TCBS37). The preparation of shotgun metagenomics libraries and the analysis of the resulting data were performed as described by Zampieri *et al.*, 2020.

Results

The metagenomics analysis (after filtering the reads lower than 0.001%) reports a total of 145 bacterial species from HO samples including 21 different species of *Vibrio* (14.5 % of the total species). Regarding the enriched_medium samples that represent the cultivable part of the microbiota, 65,47, and 58 species were sequenced from the enriched MA, TBCS22 and TCBS37 respectively. As expected from enriched_TCBS samples most of the species belongs to the *genus Vibrio* (55% from TCBS22 and 91% from TCBS37). After *Vibrio* the most represented *genera* are *Pseudoalteromonas* with 17 species in TCBS22, 21 in MA and 19 in HO and *Shewanella* with 1 species in TCBS (22 and 37), 6 in MA and 7 in HO. Among the most represented species several pathogens were identified in the population of healthy clams: the human pathogenic *Vibrio* species (*parahaemolyticus, vulnificus, colerae* and *alginolyticus*), *Vibrio crassostreae, Vibrio splendidus, Vibrio harvey, Vibrio corallilitycus, Vibrio tubiashii*.

Discussion

The shotgun metagenomic approach allows the definition of the total community at strain level and evidences the presence of a very complex *Vibrio* community in clams. The human pathogenic *Vibrio* species were identified in the clam samples, in both depurated and non-depurated samples, demonstrating the inefficiency of the depuration treatment in the elimination of these bacteria. Several species reported as possible pathogens for marine organisms are part of the microbiota of healthy clams, both in winter and in summer season. This demonstrated the ubiquitarian distribution of these species. Adverse conditions and environmental stress make the animals susceptible to the action of this community of potential pathogens (Green et al 2018).These data confirm the difficulty in the identification of the pathogenic agents causing the mortality events of farmed marine organisms (Manchanayake et al 2023). The shotgun metagenomic approach could provide a

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complete list of the virulence genes (if previously identified as virulence genes and reported in the database) associated to the samples and could help to elucidate the pathogenic action-interaction among the different bacterial species. The metagenomics of the fraction of cultivable bacteria (enriched samples), has the negative effect of the loss of the part of the community not able to grow in the medium. However, it reduce the host contamination (reads from the host could be over 90%) allowing a better definition of the most recurrent living part of the microbiota.

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THE DIETARY ARACHIDONIC ACID CONTENT IMPACTS THE FATTY ACID METABOLISM, ENZYMATIC AND NON-ENZYMATIC METABOLITES OF LIPIDS AND THE RESPONSE TO ACUTE STRESSOR IN RAINBOW TROUT FRY

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Introduction

The importance of arachidonic acid (20:4n-6, ARA) in fish nutrition is receiving increasing attention. Indeed, this fatty acid (FA) plays a crucial role in growth, survival, stress resistance, and immunity in fish (Bell and Sargent, 2003). Freshwater fish are capable of synthesizing ARA from linoleic acid (LA, 18:2n-6), unlike marine species, which have a reduced ability. The endogenous capacity of freshwater fish to synthesize polyunsaturated fatty acids (PUFAs) and thus ARA differs between species. The ARA requirements of rainbow trout have not yet been determined. Therefore, the objective of the present study was to evaluate the consequences of variable dietary ARA intake on survival, growth but also PUFAs biosynthetic capacity and stress response in rainbow trout fry.

Materials & methods

For this purpose, rainbow trout fry were fed diets containing different proportions of ARA (*i.e.* 0.6, 1.1 or 2.5% of total FA) for 8 weeks and survival and growth were monitored during the whole duration of the trial. At the end of the experiment, fish were exposed to acute confinement (10 min at 10 kg/m3) to evaluate stress response. Whole fish were collected to investigate the effects of diets on (1) endogenous biosynthesis of PUFAs, (2) production of FA oxidation-derived compounds, and (3) stress response through serotonin (5-HT) and dopamine (DA) pathways by monitoring 5-HT/5-HIAA ratios (target/one metabolite of 5-HT) and L-DOPA/HVA ratios (direct precursor of DA/one metabolite of DA).

Results & discussion

The ARA diet level did not affect the growth, but the lowest level of ARA significantly increased mortality, although the percentage of mortality was very low (1.6% for the ARA0.6 diet compared to 0.7% for the other diets, p-value=0.01). This ARA0.6 diet also showed a significantly higher level of expression of fatty acid elongase 5 (elovl5). This enzyme converts, in particular, LA to ARA, suggesting that rainbow trout can synthesize ARA from LA when dietary supply of ARA is low.

Oxylipins are bioactive lipids generated by the oxidation of PUFAs. Those derived from ARA, such as prostaglandin D2 and E2, are considered to be pro-inflammatory and may, in excess, harmful to fish (Bell and Tocher, 2009)omega-3 (ω3 or n-3. When fingerlings were fed the diet containing the highest level of ARA (2.5% of total FA), the production of oxylipins derived from ARA significantly increased while that of oxylipins derived from other PUFAs, such as eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA), LA, and α-linolenic acid (ALA 18:3n-3) were not altered.

The serotoninergic and dopaminergic activities resulting from stress revealed a more effective response when the ARA content of the diet was 1.1% of the total FA. This was evidenced by higher 5-HT/5-HIAA and L-DOPA/HVA ratios with the ARA1.1 diet than with the other two diets. These results indicated that the ARA content of the diet can modulate the response of the fish to an acute confinement stress.

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Conclusion

In conclusion, a dietary intake of ARA corresponding to 1.1% of total FA seems to be the most appropriate to promote the robustness of rainbow trout fry. Indeed, with an ARA intake of only 0.6% of the total FA, survival decreases even though the capacity to synthesize ARA from its precursor LA increases. With an ARA level of 2.5% of the total FAs, the production of ARA-derived oxylipins rises, which could ultimately have negative effects on the health of the fish.

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OVERCOMING BOTTLENECKS IN SEAWEED CULTIVATION - NATURAL PROTOPLAST PRODUCTION IN *Ulva Lacinulata* AND ITS IMPLICATIONS FOR LARGE-SCALE CULTIVATION

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Introduction

The *Ulva* genus has proved to be a suitable candidate for large-scale cultivation and can serve multiple purposes in several industries (e.g., pharmacy, cosmetics, food, and feed) (Mantri et al. 2020; Leyva-Porras et al. 2021). However, to guarantee the success of its large-scale production, it is important to find solutions to the currently existing biological and technical limitations. Some *Ulva* species reproduce in predictable ways, and their reproduction can be induced by following established methodologies (see Mantri et al. 2020 for recent review). However, attempts at inducing the reproduction of the commonly cultivated species *Ulva lacinulata* (Kützing) have been unsuccessful, and large-scale cultivation of this species requires re-stocking from the wild or limiting the harvest to maintain a starting stock of biomass. While this species is often sought for cultivation because it grows well unattached and it does not sexually reproduce, which results in the loss of biomass, this species often degrades, which also contributes to a loss of biomass. The cause of this biomass degradation has until now not been understood. Here, we present evidence that tissue degradation in *U. lacinulata* occurs as a result of natural protoplast production and release. These protoplasts can then develop in several different directions. New blades can be formed, which either develop into adult thalli or undergo gametogenesis very early in their development, resulting in the release of gametes and the germination of new germlings. These results contribute to a new understanding of the life cycle of *U. lacinulata*, and provide the first evidence of natural protoplast production in this species. Because the induction of protoplasts in *Ulva* spp. is time-consuming and expensive, finding the trigger for this process will be an important step to accelerating the success of large-scale cultivation of this species.

Methodology

The biological material (previously molecularly identified as *Ulva lacinulata* by Cardoso et al. 2023) was collected in 2021 in a "green tide" area in Lagoa de Óbidos, Portugal (following the Nagoya Protocol) and then brought to the Alfred Wegener Institute, Germany where it has been cultivated in a closed system. Throughout its cultivation, it was kept in 5 L glass vessels at 15 °C (± 1 °C) and an irradiance of 70 µmol photons m−2 s−1 with a 16:8 h light:dark photoperiod (LD) in pasteurized artificial seawater (ASW) (30 PSU) supplemented with half-strength Provasoli in a concentration of 10 mL L^{-1}. The medium was replaced once per week and an aeration system guaranteed the continuous tumbling of the material inside of the vessels.

Upon observation of the onset of biomass degradation, fresh biomass (0.72 g) was collected. The biomass was distributed equally into four 1 L beakers. The experiment ran for four weeks under the previously mentioned cultivation conditions. Every week, the water in each beaker was filtered and centrifuged to collect the protoplasts. After filtering the water, the debris and small pieces of the original biomass were collected, weighed, and placed back into the beaker with newly added culture media. Calcofluor white (CFW) was used to observe the presence/absence of cell walls and disposable hemacytometers were used to quantify the protoplasts yields obtained in each beaker each week. A defined amount of the collected protoplasts were isolated in individual Petri dishes and their development was observed under the microscope. The number of protoplasts that germinated into new blades were counted after five weeks.

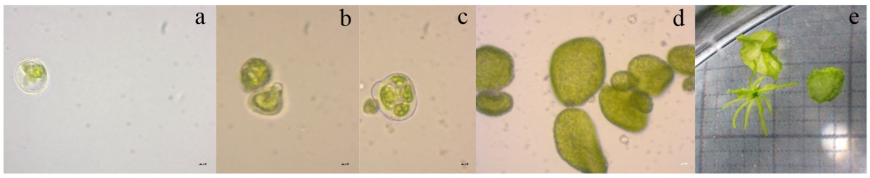

Fig. 1: Development of the regenerated protoplasts from *U. lacinulata* obtained during a "degradation" event. a: single protoplast with no visible cell wall; b: first cell division; c: second cell division; d: *Ulva* discs originated from cell division of protoplasts; e: discs and germlings originated from protoplasts. A, b and c at a 400 x amplification, d at an amplification of 100 x, e taken with a binocular (each square = 1 x 1 mm).

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Results and Discussion

Our observations under the fluorescent microscope confirmed the absence of cell walls in the cells dyed with CFW, thus confirming that these cells are naturally occurring protoplasts. The total protoplast yield obtained in our first experiment was 3.21 x 10^7 cells per gram of fresh biomass, which is comparable to those reported in studies where the protoplast formation of different *Ulva* species was enzymatically induced (Reddy et al. 2018). Approximately half of the protoplasts regenerated (40-60 %) and grew into discs or germlings in a similar fashion to what has been described in the literature for induced protoplasts of *Ulva* spp (Reddy et al. 2018; Fig. 1). Moreover, sexual reproduction was found to occur during gametogenesis in protoplasts, rather than in adult blades.

Additionally, by measuring the weight of the initial biomass each week, we observed that the original tissue did not completely degrade after protoplast formation and release, and a final total fresh weight of 5.79 g resulted in a 7 % daily relative growth rate. Thus, revealing that degradation does not necessarily result in a total loss of the culture.

Conclusion

Our observations of natural protoplast production in *U. lacinulata* close an important knowledge gap in understanding this species' reproductive cycle. This new knowledge can be beneficial when trying to understand the formation of "green tides" and the differences between "green tide" strains and non-"green-tide" strains. Additionally, the production of natural protoplasts can potentially be exploited to improve the efficiency of *Ulva* cultivation methods in the future and remove the bottlenecks existing today for safe and profitable large-scale production.

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CO-INFECTION WITH *Piscirickettsia salmonis* GENOGROUPS IN ATLANTIC SALMON: CHARACTERIZATION OF IMMUNE-RELATED BIOMARKERS IN HEAD KIDNEY, LIVER, SPLEEN AND SERUM

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Introduction

Intensive aquaculture production has led to an increased occurrence of infectious diseases. In addition, co-infections in fish are natural events that should be considered when studying the biology of the pathogens for control measures, since infection with multiple pathogens may increase the severity of disease in fish. The SRS (Salmon Rickettsial Syndrome) produces a systemic disease in Atlantic salmon that was responsible for the mortality of 54.2% (of total infectious diseases) during first semester 2022 in Chile, resulting in annual economic losses of USD $700 million for this industry.

The biological agent of SRS, *Piscirickettsia salmonis* (a facultative intracellular bacterium) has been found in all the others important salmon farming countries like Norway, Canada, Scotland, and Ireland, where the disease can be considered as a potential emergence disease (Long, A, *et al*. 2021). *P. salmonis* evades the salmon immune system by replicating within cytoplasmic vacuoles of macrophages, avoiding the respiratory burst in these cells. That is why is hard to control by the host innate and adaptive immune responses and it could also be one of the reasons why vaccines still do not provide efficient protection over time (Rozas-Serri, 2022). There are currently two genogroups (LF-89 and EM-90) identified for *P. salmonis* with different virulence levels that cohabit in Chile (Saavedra, J, *et al*. 2017). Thus, our study focuses how this cohabitation can be related to high fish mortality, as the within-host competitive interactions are linked to virulence, changing the development and persistence of diseases. This, also modulate the population dynamics of pathogens by cooperative or competitive interactions with different antigenic epitopes triggering a cross-reactive immune response in the host, altering its efficiency and the health and welfare of fish (Kotob *et al*. 2016).

Materials and Methods

In this study, we evaluated the initial effect of co-infection in Atlantic salmon with the two different genogroups LF-89 and EM-90 in an intraperitoneal challenge model to compare pathogenicity and host immune response. Fish (average weight of 60.4 g) were smoltified at VESO Vikan (Namsos, Norway) in brackish water (25‰ ± 2‰) at 15ºC and continuous 24 h light exposure (24:0). Then, fish were starved for 48 h prior to challenge and divided into groups of 80 fish each (in three identical tanks with a stocking density of 40 kg m^3). To perform the challenge, fish were sedated and intraperitoneally (i.p.) injected with 0.1 mL of a 1.0×10^7 cfu/fish (with one of the *P. salmonis* strain or in a ratio of 1:1 for co-infections). Fish were fed *ad libitum* and monitored daily. At day 0, 12 fish were sampled as negative control. Moreover, at 7, 14 and 21 days post challenge (dpc), 12 fish per tank were randomly selected for sampling of head kidney, liver and spleen for total RNA extraction and qPCR gene expression analyses of immune-related biomarkers. Serum samples were evaluated by ELISA after 14- and 21-dpc for total and specific IgM detection.

Results and discussion

The results revealed a variation in mortality between single- and co-infection. The LF-89 strain alone did not induce mortality, while the EM-90 strain produced 52.6% mortality at 22 dpc. However, during the co-infection challenge, mortality started earlier and reached 61.6% mortality after 22 dpc in a steeper curve. The gene expression of immune-related biomarkers at 14-dpc showed the up-regulation of *il-10* (anti-inflammatory cytokine) during the co-infection compared to EM-90 alone in spleen and in head-kidney. At the same time, *il-8* (chemokine) was significantly up-regulated in co-infection compared with EM-90 alone in spleen and liver, and *il-1b* in the liver. In EM-90 challenges this is already described (Rozas-Serri, 2022) but never with higher levels of expression in a co-infection study.

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ELISA analyses in serum showed an increase in the protein levels of total IgM (in EM-90 infections and in co-infections) at 14- and 21-dpc, being significative different to the control at day 0 and to LF-89 infection. Moreover, specific IgM against *P. salmonis* showed a peak production at 14-dpc for the three infected groups (EM-90, LF-89 and co-infection). Interestingly, serum from fish infected LF-89 had a higher cross-reaction using EM-90 as antigen than serum from fish infected EM-90 against LF-89.

Conclusions

Our study proposes that there is a synergistic interaction effect among the *P. salmonis* genogroups (LF-89 and EM-90) during the infection in Atlantic salmon that could modulate the host immune response, triggering higher mortality. This novel approach to SRS characterization as a multiple-genotype infection may offer better insights into the development and further control of the disease.

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IMPACT OF *Tenacibaculum maritimum* INFECTION ON JUVENILE GILTHEAD SEABREAM SUBJECTED TO DIFFERENT INFECTION METHODS AND CONCENTRATIONS

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Introduction

Intensification of the aquaculture industry can lead to several production-related issues that may cause poor fish welfare and impact human health and the environment. One of these problems is the emergence of disease outbreaks that affect fish production and consequently induce economic losses. A common disease found in aquaculture farms worldwide is Tenacibaculosis, which is caused by the bacteria *Tenacibaculum maritimum*. This bacterium affects a wide range of species, however further knowledge about its impact on some of these species is necessary. Therefore, the current study aims to explore the impact of different methods of infection and concentrations of this bacterium on the survival of juvenile gilthead seabream (*Sparus aurata*), one of the most produced species in the Mediterranean.

Material and Methods

Gilthead seabream juveniles, bred in the Experimental Aquaculture Station of IPMA, Olhão, Portugal, were selected from stock populations reared under standard conditions (20 m^3 fiberglass tank with a continuous flow of water at a 12:12 light-dark cycle). One-hundred and sixty-eight individuals with a mean weight of 19.5 ± 4 g were sorted and net chased for 1 min prior to the experiment. Five conditions were tested in triplicate in 40 L tanks, where eight fish per tank were injected subcutaneously with *T. maritimum* (CECT4276) at 1.6 x 10^6, 1.6 x 10^7 and 1.6 x 10^8 or subjected to bath infection with 1.6 x 10^4 or 1.6 x 10^5 bacterial concentrations for 24 h. In the control groups, fish were injected or bathed with PBS, and this experiment lasted 5 days. Morphological welfare indicators and survival rate analysis were performed to evaluate the impact of the different infection methods and bacterial concentrations on the fish.

Results and conclusion

By the end of the experiment, no mortality was recorded for seabream injected with *T. maritimum* at a concentration of 10^7 and a lower mortality rate of 4.2% and 8.3%, respectively, for infection with concentrations of 10^6 and 10^8. However, the mortality rate of the control group of injected fish was 4.2%. Seabream bathed with *T. maritimum* showed mortality rates of 41.7% and 83.3% in the groups infected with a concentration of 10^4 and 10^5, respectively. Fish in the bath groups exhibited clinical signs of infection such as hemorrhagic fins, tail rots, and skin lesions. Furthermore, no mortality was recorded in the control group. The presence of *T. maritimum* in the liver was confirmed by nested PCR. These results indicate that the bath challenge was more efficient than injection in recreating *T. maritimum* infection in juvenile seabream. However, the concentrations used in these methods were different and, consequently, could not be directly compared. Further studies to characterize *T. maritimum* infection in gilthead seabream are essential and are currently underway.

THERMAL PREFERENCE AS A WINDOW TO UNVEIL INFECTION SUSCEPTIBILITY IN SEABREAM UNDER DYNAMIC ENVIRONMENTAL CONDITIONS

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Introduction

Strategies for stress management, including diseases, are pivotal for ensuring the well-being and longevity of fish populations in aquaculture settings and natural ecosystems. These approaches are essential for safeguarding fish health by reducing the negative effects of stressful situations and enhancing their ability to withstand disease outbreaks. One such strategy involves providing fish with a thermal gradient where they can choose their preferred temperature, which positively influences their coping ability and survival when facing stress, as it is known that fish can exhibit behavioral fever. The current study aims to explore whether gilthead seabream uses temperature gradients to enhance its fitness and survival when facing stressful challenges, which may provide valuable insights into improving their well-being and resilience to support sustainable aquaculture practices.

Methods

Gilthead seabream juveniles bred in the Experimental Aquaculture Station of IPMA, Olhão, Portugal, were selected from the stock populations reared under standard conditions (20 m^3 fiberglass tank with a continuous flow of water at a 12:12 light-dark cycle). Six conditions were tested in triplicate, where unstressed, stressed, and infected fish with *Tenacibaculum maritimum* in groups of eight fish were subjected to either a constant temperature or a thermal gradient for 120h. The frequency distribution of the animals was recorded by video cameras in a custom-built multi-chamber tank under both constant temperature (21 °C) and a continuous thermal gradient profile (ranging from 18°C to 24°C), allowing the temperature preference of each group to be recorded over an 8h time period. Hematological, morphological welfare indicators and survival analysis were performed to evaluate the impact of thermal gradients on stress coping ability and fitness.

Results and conclusion

This experiment is ongoing, however some results can be described. The unstressed fish, exposed to a thermal gradient, preffered temperature was calculated as 21.06 ± 1.95 ºC, while for stressed fish was 23.65 ± 0.25 ºC. Additionally, the distribution within the first hour of acclimation was measured to assess differences in the acute stress response. Unstressed fish showed preference for 21.62 ± 0.90 ºC and stressed fish for 21.11 ± 0.35 ºC. Overall, these findings highlight the importance of considering thermal preferences and stress responses in fish when designing aquaculture or research environments. Understanding these thermal preferences could contribute to the development of more effective and fish-friendly aquaculture practices and management strategies.

A PROTOTYPE WITH SEDATIVE PROPERTIES BASED ON NATURAL COMPOUNDS TO UPGRADE WELFARE IN LIVE-FISH TRANSPORT: AMELIORATION OF STRESS RESPONSES IN SEABASS (*Dicentrarchus labrax*)

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Introduction

In a context of global change and increased food demand, the growth of aquaculture production must guarantee animal welfare. Handling or transport operations activate the stress responses, which may compromise the integrity and general welfare status of fish. To improve these processes, the use of anaesthetics has been proved useful to reduce the possible adverse effects (Sneddon et al., 2016).

MS-222 or benzocaine are synthetic chemicals commonly employed as anaesthetics in aquaculture. However, their use is controversial due to human safety issues and the physiological side-effects that induce in fish (Zahl et al., 2012)there are several situations in which fish are subjected to handling and confinement. Netting, weighing, sorting, vaccination, transport and, at the end, slaughter are frequent events under farming conditions. As research subjects, fish may also undergo surgical procedures that range from tagging, sampling and small incisions to invasive procedures. In these situations, treatment with anaesthetic agents may be necessary in order to ensure the welfare of the fish. The main objective of this paper is to review our knowledge of the effects of anaesthetic agents in farmed fish and their possible implications for welfare. As wide variations in response to anaesthesia have been observed both between and within species, special attention has been paid to the importance of secondary factors such as body weight, water temperature and acute stress. In this review, we have limited ourselves to the anaesthetic agents such as benzocaine, metacaine (MS-222. Essential oils (EOs) derived from plants have been studied for this purpose, but its use might be problematic because of its compositional variability and effectiveness (De Freitas Souza et al., 2019)stressful events initiate a hormone cascade along the hypothalamus-pituitary-interrenal and hypothalamus-sympathetic-chromaffin (HSC. Therefore, the use of synthetic nature-identical compounds (such as eugenol, menthol or thymol), the bioactive molecules naturally present in those EOs, is a promising approach to develop new sedative strategies to improve operational processes in aquaculture.

For this purpose, this study assesses the effectiveness of a prototype with sedative properties (TecV2, developed by TecnoVit-FARMFaes Ltd.) in seabass (*Dicentrarchus labrax*) for a live transport of 3 and 6 hours.

Materials and methods

Seabass juveniles (*D. labrax*) (n = 72, weight = 48.9 ± 18.4 g) were placed into 15 L-tanks and distributed in four different experimental groups in triplicate: i) 3 h transport without sedation (CTRL-3H); ii) 3 h transport with 10 ppm of TecV2 prototype; iii) 6 h transport without sedation; and iv) 6 h transport with 10 ppm of TecV2 prototype. Prototype concentration was selected according to previous tests.

Transport simulations took 3 and 6 h respectively. At the end of stressing trials, half of the animals from each tank were euthanized and sampled (n = 9). The remaining fish were transferred into clean water tanks to determine their status after 24 h of recovery. Additionally, 9 fish were euthanized and sampled before any manipulation (basal conditions). Fish were euthanized with an overdose of 2-phenoxyethanol (1 ppt). Plasma samples were taken to determine changes on plasmatic secondary stress responses (cortisol, glucose, lactate, etc.). Liver was also excised to assess changes in the intermediary metabolism of amino acids, lipids and carbohydrates.

Tanks were well aerated every 90 min until oxygen full-saturation. Oxygen levels were monitored and remained above 5 ppm during the whole simulation periods. Temperature was also monitored and remained around 19 ± 1 ºC.

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Table. Metabolites in plasma and liver of *D. labrax* juveniles. Different lowercase letters represent statistical differences after stress. Different capital letters represent statistical differences after recovery and asterisks represent statistical differences between stress and recovery times. Hash-symbols represent statistical differences compared to the basal conditions ($p < 0.05$).

		3H Transport				6H Transport			
		Stress		Recovery		Stress		Recovery	
	Basal	CTRL	TecV2	CTR	TecV2	CTRL	TecV2	CTRL	TecV2
Plasma									
Glucose (mg/dL)	72 ± 7	101 ± 14 a #	71 ± 4 b	80 ± 5	74 ± 7	91 ± 6	105 ± 6 #	65 ± 3 **	67 ± 3 **
Lactate (mg/dL)	27 ± 3	23 ± 3 a	10 ± 1 b #	32 ± 3	29 ± 3 ****	18 ± 3 #	15 ± 3 #	23 ± 2	26 ± 3 * **
TAG (mg/dL)	325 ± 40	340 ± 63	389 ± 83	300 ± 24	282 ± 32	136 ± 16 b#	358 ± 67 a	268 ± 19 ***	268 ± 26
Proteins (mg/mL)	37 ± 1	36 ± 1 a	31 ± 1 b #	39 ± 2	40 ± 2 **	30 ± 1 #	34 ± 1	34 ± 2	37 ± 1
Amino acids (mg/mL)	0.8 ± 0.1	0.6 ± 0.0 #	0.7 ± 0.0 #	0.7 ± 0.0 #	0.6 ± 0.0 #	0.6 ± 0.0 #	0.6 ± 0.0 #	0.5 ± 0.2 # **	0.5 ± 0.0 # *
Liver									
Glucose (mg/g fw)	4.0 ± 0.2	3.8 ± 0.2	3.7 ± 0.4	5.3 ± 0.3 # ***	4.6 ± 0.2 *	5.0 ± 0.2 #	4.7 ± 0.3	4.8 ± 0.2	4.4 ± 0.5
Glycogen (mg/g fw)	21 ± 1.2	18 ± 1.2	18 ± 2.4	17 ± 1.0 A	12 ± 1.2 B # *	18 ± 2.3	19 ± 1.2 #	15 ± 2.2	17 ± 1.5
TAG (mg/g fw)	40 ± 2	44 ± 6	34 ± 6	63 ± 9	53 ± 8	26 ± 2 b #	51 ± 4 a	91 ± 16 # **	103 ± 11 # ***
Amino acids (mg/g fw)	8.0 ± 0.8	5.9 ± 0.3 #	5.5 ± 0.4 #	5.5 ± 0.2 #	5.8 ± 0.3 #	5.2 ± 0.2 #	4.7 ± 0.3 #	6.2 ± 0.2 # **	6.0 ± 0.3 # **

Results

Our results showed that both live transport simulations for 3 and 6 h, were effective to activate stress responses in the seabass juveniles (*D. labrax*). Furthermore, these responses were modulated by the addition of the sedative prototype TecV2 to the water.

After 3 h transport an increase on plasmatic glucose was observed, a common secondary stress response triggered by cortisol elevation (Schreck et al., 2016). However, this response was ameliorated by the use of TecV2, together induced a reduction on lactate and protein levels.

The 6 h transport induced a greater disturbance on stress parameters. At this point, non-sedated fish had overcome stress situation, so plasma hyperglycaemia was not detected. However, this response was observed on sedated fish after 6 h of transport, probably because of TecV2 sedation effect was lowering. Protein and triglycerides (TAG) levels were lower in non-sedated fish, so TecV2 was useful to maintain these energetic sources in plasma. Alternatively, amino acids levels did not return to initial values after recovery and values were even lower than those observed during stress. However, the reduction after recovery on amino acids levels was lower when TecV2 was used. Hepatic carbohydrate and lipid metabolism was also altered and an increase on TAG levels after 24 h was observed. These results are in concordance with the results of enzymatic activity determined.

In conclusion, TecV2 has the potential to be used as sedative for the live transport of seabass juveniles, especially for a 3 h transport, since it reduces energy mobilization.

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THE INCLUSION PROCESS OF A NATURAL ADDITIVE IN THE DIET MODULATES INTESTINAL MUCOSA AND GUT HEALTH IN THE GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

The inclusion of natural additives in aquafeeds is one of the most promising research areas to improve production and animal welfare in aquaculture. The essential oils of plants (EOs) as feed additives have multiple beneficial effects, such as growth promotion, appetite stimulation, immunomodulatory and antioxidant effects, etc., due to the bioactive molecules that present (Sutili et al., 2018). Furthermore, the use of these supplemented diets might affect both the gut structure and its functionality, improving diet digestibility (Firmino et al., 2021). For this reason, the study of intestine enzymes activities, such as proteases, is recommended to assess the digestive and absorptive capacity of fish. Besides playing a key role in the digestive and absorptive processes, the intestinal mucosa, and the mucus produced by goblet cells, also acts as an immunological barrier against pathogenic microorganisms. Therefore, the histological study of the intestinal mucosa is also relevant to understand the influence of dietary treatments on fish welfare and growth (Alarcón et al., 1998; Galafat et al., 2020).

This study assesses the effect of the inclusion process in the diet (before the extrusion or during the coating of pellets) of a phytogenic additive (PA), composed of garlic essential oil, thymol and carvacrol, on the digestive enzymatic activities and intestinal mucosa integrity of seabream (*Sparus aurata*).

Materials and methods

Seabream juveniles (*Sparus aurata*) (n=180; 57 ± 5 g) were randomly placed in 9 tanks of 300 L. Fish were fed for 78 days *ad libitum* using three different diets: i) Control diet (CTRL); ii) diet supplemented with PA during coating process of pellets (PA-Liq); and iii) diet supplemented with PA before extrusion process of the diet (PA-Sol). Growth performance was determined and intestine samples were collected to assess enzyme activity and histological parameters.

Total alkaline protease, L-Aminopeptidase, alkaline phosphatase, trypsin and chymotrypsin activity were determined spectrophotometrically. Intestine absorption rate was evaluated by a mucosal/submucosal surface ratio and the number of goblet cells was determined in order to verify whether the additive affected the gut health.

Results

Our results showed that the addition of PA to the diets did not have any negative effect on the growth parameters studied. Animals reached a final weight of 110 ± 10 g, with an average SGR of 3.3%. No differences were found on growth performance between both inclusion processes.

The inclusion of PA before the extrusion process increased the activity of L-aminopeptidase and alkaline phosphatase. These enzymes are located on the intestinal brush border and are often used as markers of intestinal integrity (Silva et al., 2010), consequently an increase of both activities might have contributed to improve the efficiency of digestive processes. However, a lower absorption ratio in the anterior portion of intestine was observed on fish fed with this treatment when compared to those fed with PA-Liq and the CTRL diets. The lower absorption ratio might indicate that the increased enzyme activity is due to compensatory mechanism. No differences were found among treatments on the posterior intestine segment. Additionally, the addition of PA-Sol reduced the goblet cells number, which corroborates that the extrusion process affects the properties of the additive and modulates gut health.

In conclusion, the inclusion of PA should be done during the coating of pellets to guarantee the beneficial effects of the natural additive.

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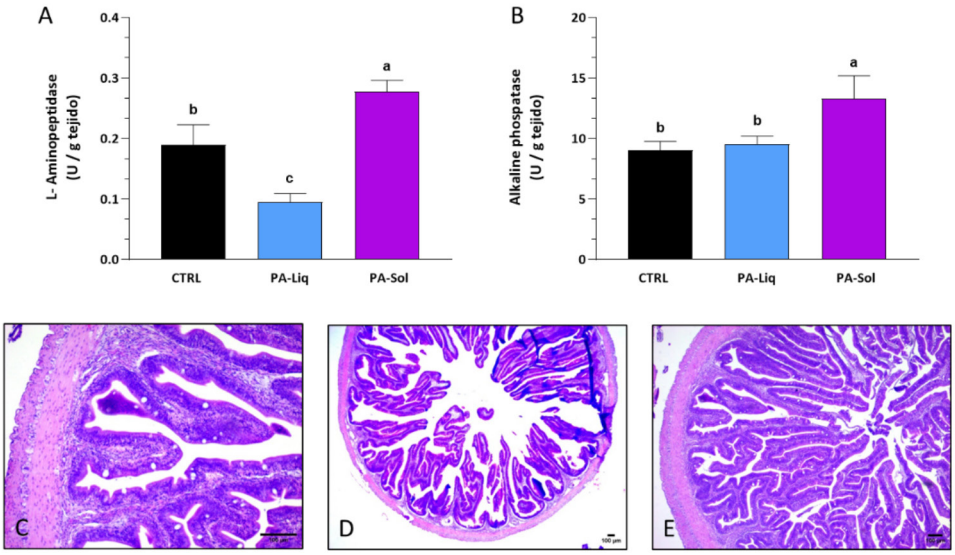

Figure 1. **A.** Intestine L-Aminopeptidase activity. **B.** Intestine alkaline phosphatase activity in *S. aurata* juveniles. **C.** Anterior intestine (AI) from control fish. **D.** AI from PA-Sol experimental group. **E.** AI from PA-Liq experimental group. Different lowercase letters represent statistical differences ($p < 0.05$).

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BOTANICAL COMPOUNDS INHIBITS SALMON PATHOGEN *Piscirickettsia salmonis* IN IN-VITRO STUDY

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Introduction

The illness with the biggest economic impact on Chile's salmon farming sector is Salmonid Rickettsial Septicemia (SRS) caused by the bacterium *Piscirickettsia salmonis*, which results in significant mortality rates during the last phase of their productive cycle at sea (Rozas & Enriquez, 2014). Antimicrobials, most frequently florfenicol, remain the major treatment and control option for this infection because currently and commercially available vaccinations have not shown the expected efficacy levels (SERNAPESCA, 2016). Botanicals are frequently employed in zootechnical feed additives for terrestrial animals, and they are gaining popularity as an alternative to using antibiotics for their antimicrobic and immunostimulants properties (Beltran & Esteban, 2022; Kuralkar & Kuralkar, 2021; Rossi *et al.,* 2020). To the best of our knowledge, there are not many research on aquaculture, and nothing is known about how they affect SRS control. The aim of this study was to evaluate the feasibility in using the active principles of Prototype α, a thymol-based blend of botanicals, as a mean to support the control of the seasonal outbreaks of SRS.

Materials and methods

In order to reach the aim of the study, Minimum Inhibitory Concentration (MIC) assays were performed. The antimicrobial activity of the compound was evaluated using the microdilution method in CASO broth (Vera *et al.*, 2012), in which the active principles of the blend were dissolved ranging from 10 to 5000 ppm. Wells with no addition of products and with ethanol equivalent to the highest concentration were used as control and vehicle respectively while wells without bacterial inoculum served as negative controls. The trial was performed in quintuplicate. The Chilean *P. salmonis* isolate PS005 belonging to the EM genogroup was inoculated at a final concentration of 10^7 CFU/mL, and the plates were incubated for 96 h at 18 °C. After incubation, bacterial growth was evaluated by absorbance measurement at 620 nm and the MIC was defined as the lowest concentration of the tested compounds that totally inhibited the growth of the bacterium. The data were analyzed with two-way ANOVA followed by the Dunnet's post hoc test, and differences were considered significant at $p \leq 0.0001$.

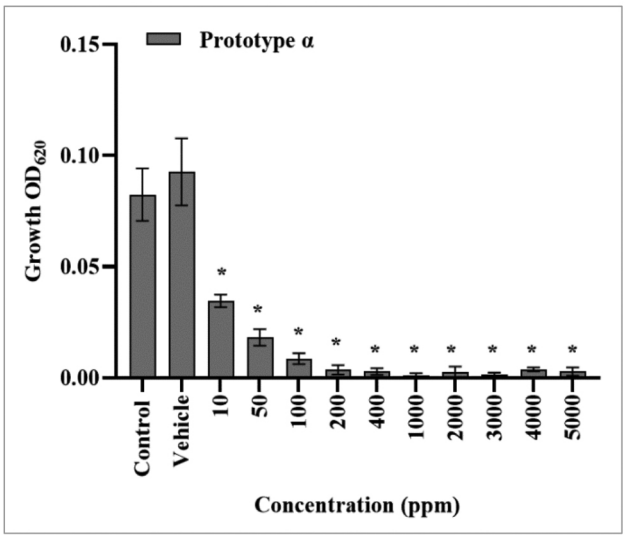

Figure 1 - Results of the MIC test for the thymol-based botanical blend, bars represent mean ± standard deviation (n=5). Asterisks indicate significant statistical differences.

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Results

The in-vitro effect of Prototype α's active principles on *P. salmonis* were evaluated in a MIC assay providing bacterial growth inhibition to a statistically significant degree starting as early as 10 ppm until proving a complete inhibition of the pathogen from 200ppm upward. Results are provided below in Figure 1.

Conclusions

Aquaculture is one of the fastest expanding sectors, contributing worldwide to food provision (FAO, 2022). Due to the limitations and concerns on anti-microbial resistance coming from consumers and institutions alike (FAO, 2023), it is one of the challenges of feed additive to support the aquaculture production by regulating harmful bacteria, promote growth and stimulate the immune system. These in-vitro results, coupled with many studies proving the health boost and growth enhancement of botanicals additive, show a promising new opportunity for these compounds and their use in support to SRS control.

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POTENTIAL OF THE INTEGRATED MULTI-TROPHIC AQUACULTURE IN THE ATLANTIC AREA: A SOCIO-ECONOMIC ANALYSIS OF THE CURRENT AQUACULTURE SECTOR AND RECOMMENDATIONS

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Introduction

According to the EU Blue Economy report (2023), The EU is the sixth largest producer of fishery and aquaculture products (behind China, Indonesia, India, Vietnam, Peru and the Russian Federation), covering around 2% of global production. However, overall production has been rather stable in the last few decades. The EU has slightly more than 56 100 active vessels landing about 3.9 million tons of seafood worth €5.8 billion; at the same time, the aquaculture sector reached a production of about 1.2 million tons worth €3.9 billion in 2020. Moreover, according to FAO, world production of aquatic animals was estimated at 178 million tons in 2020, a slight decrease from the all-time record of 179 million tons in 2018 and global consumption of aquatic foods increased at an average annual rate of 3.0 percent from 1961 to 2019, a rate almost twice that of annual world population growth (1.6 percent) for the same period. This aquaculture growth and intensive production causes a significant set of problems (i.e., creation of an anoxic zone, reduction in water quality, destruction of habitat, amongst others). In order to satisfy the demand, it is required to develop innovative, responsible and profitable cultivation methods to optimize the efficiency and mitigate negative environmental impacts. In this context, the Integrated Multi-Trophic Aquaculture (IMTA) farming is a promising concept to tackle some of these challenges, hence the EC has funded some projects on this subject to evaluate different aspects of the IMTA and its potential. Notably, the ASTRAL project focuses in IMTA farming as a possible alternative to develop a sustainable, profitable and resilient aquaculture, in this paper it will be presented a socio-economic assessment which highlights challenges and barriers of aquaculture and the IMTA possible solution approach.

Methodology

The methodology used was based on analysis involving ten countries: Norway, Spain, France, Portugal, Ireland, Scotland, Brazil, Argentina, South Africa and Nigeria. The two main sources of data were:

- the territory and regional knowledge (macro and meso economic approach) thanks to a strong bibliographic review per country,
- producers' knowledge (meso and micro economic approach) thanks to a survey and preliminary results of producers' one-to-one interviews; a total of 39 interviews were conducted, as follow:

Country	Nb of interviews	IMTA	Co-culture	Monoculture
Argentina	2	1	0	1
Brazil	4	1	1	2
France	4	4	0	0
Ireland	5	2	1	2
Nigeria	5	0	1	4
Norway	4	2	0	2
Portugal	5	2	2	1
South Africa	4	2	1	1
Spain	2	0	0	2
Scotland	4	1	0	3
Total	39	15	6	18

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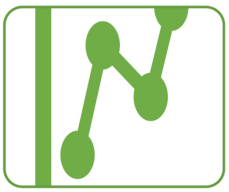
Collecting data faced two main obstacles:
• Most of the interviewees were not keen on providing economic data (confidentiality, lack of trust, no data available, etc.)
• IMTA development is quite recent in some countries. Most of IMTA projects are at experimental stage and don't have economic data to share.

Factors analysis impacting aquaculture



Species' growth rate

- Growth rate is higher with IMTA systems because of the feeding process and the algal effects (*mussel/scallop/seaweed or abalone/seaweed*), depending on the value chain,
- But this is only true for certain species and combination of species (depending on the trophic links)
- However, Complexity of regulations, often fragmented and per species

IMTA products as selling argument

- IMTA allows product diversification and could enable price premium (+10%),
- But public awareness is very low, products are subject to price volatility and new markets/channels need to be created to be able to sell at price premium - "*IMTA affects more the production side, rather than sales" (verbatim from an IMTA producer)*
- Diversification of products and, therefore, markets
- A « sustainable » systems (need to reduce consumption of resources such as space, food, etc.)
- The potential development of collaboration between players (between farmers and between farmers and academia)
- However, the social acceptance and public perception haven been fully validated

Return on investment

- IMTA NPV is always higher than that for monocultures and ROI is estimated to be 4 years for a small/family farm,
- But ROI can be much higher for a larger farm, which will have more investment costs to start an IMTA farm - "*Our ROI occurred after more than 10 years of production" (verbatim from an IMTA producer - 8 employees)*
- An accelerated innovation potential
- However, complexity to implement and deploy (business, techniques, production methods, adaptation)
- Not enough government support and public funds
- Competition from other aquaculture segments (monoculture)

Production & HR costs

- IMTA systems tend to reduce the production and HR costs,
- But these effects can only be seen on small farms with specific characteristics
- Positive: Nutrient recycling
- Reduced demand for feed from pelagic marine fisheries and terrestrial crops
- Lack of know-how to find the right economic model(s)
- Greater structure costs with a potential lower profitability in the short term

Environmental services

- IMTA allows to reduce the environmental impacts of such production,
- But no actual data are available to see the long-term effects of this production, leading to a lack of understanding of environmental impacts
- The ecosystem services could improve revenue opportunities; they remain to be better identified and quantified

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Recommendations

The following section aims to draw up the main recommendations that arise from our analysis and the interviews conducted.

These recommendations have been drawn for producers, the aquaculture industry organizations and/or the policy makers. For each entry, a sticker helps understand to whom the recommendation is addressed to:

Researchers/Academia | Producers | Aquaculture professional organisations | Policy makers

1 Develop pilots and demos

Economic demonstration is still premature and experimentation farms ongoing. More work is needed for IMTA systems to become a commercial reality, despite the large amount of research and field pilot work carried out to date. There is an urgent need to develop and optimize IMTA systems and to test new production methods. This implies that producers have time to dedicate to these projects, have been technically supported and benefit from public funding.

2 Encourage an active policy to make a change

IMTA implementation and growth will only be possible with the support of policy makers. There is a need to design an IMTA development plan in each country to answer to concrete needs, to fund synergies between producers and to operate a financial support policy which is pertinent and precise, to help producers shifting to IMTA. This work should be done in close link with researchers, producers and professional organizations.

3 Increase and organise the sharing of knowledge on IMTA

Co-culture and monoculture producers are generally open to this new approach but have not embarked on it due to the lack of knowledge and demonstration of the system's effectiveness and viability. It is now necessary to find the right way of dissemination and knowledge transfer of technical, economic and environmental aspects including experience exchange. Increasing links between researchers and aquaculture professional organisations is important to share with all producers.
Innovation is a key factor of success and is a great asset for IMTA systems.

4 Improve and increase availability of trainings

It is difficult for a manager with more than 50 employees to move from a standardised mono-specific model to a multidisciplinary model. It is important that the workforce get continuous training for the work to be done within the farm, especially regarding the complexity of the system. For the young generation and students, it is crucial to disseminate the approach and seize IMTA.

5 Estimate and monetise ecosystem services

It is crucial that the values of ecosystem services* are recognised, accounted for and used as financial and regulatory incentive tools, such as in the development of nutrient trading credits. However, no real system for recognising and rewarding ecosystem services is yet in widespread and robust usage. This awareness of ecosystem services should be targeted primarily at local stakeholders to facilitate aquaculture development.
* goods and services that humans can derive from ecosystems, directly or indirectly, to ensure their well-being (Millenium Ecosystem Assessment, 2005

6 Adapt regulations and administrative processes

A key aspect for IMTA growth will be to facilitate the implementation of experiments or diversifications on the concessions of professionals. A full place must be given to IMTA and its practices must not be blocked by the superposition of various regulations. Regulations must be made more readable, simpler and understandable by everyone.

7 Make IMTA more visible

Complementary to recommendation n°3, it is important that IMTA is recognized among policy makers, general administrations and the general public. A strong communication and promotional strategy has to be launched in order to show the benefits of IMTA for the sector and for consumers. Producers will get information and knowledge on the advantages coming from IMTA and consumers will get more insights on products' quality and environmental aspects.

8 Better address the commercialization strategy of IMTA products

The marketing of IMTA products is by definition diversified. A type of product corresponds to a specific market and marketing channel. For IMTA to be an economic advantage for producers, one can imagine the creation of an eco-label to highlight the environmental advantages of this type of production. Marketing through a local distribution channel is also to be preferred because it echoes the ecological philosophy of the IMTA. Producers need dedicated support on this aspect to reach new markets and sustainable growth/sales.

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THE IMPACT OF GENETIC SELECTION FOR FAST GROWTH ON THE EFFECIENCY OF GILTHEAD SEA BREAM (*Sparus aurata*) IN USING NOVEL DIETS

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Introduction

High inclusions of fish meals (FM) and oils (FO) in aquaculture feeds are not considered indispensable ingredients anymore. To meet future the demand for aquaculture products and to reduce completely the dependence on FM and FO, it is necessary to find new profitable and more sustainable sources of protein and lipids. Insect meals and single-cell proteins from bacterial or microalgal sources have been recently investigated and pointed out as promising future feedstuffs for the aquaculture industry due to their high nutritional potential for fish, as well as their low ecological impact. Added to novel nutritional strategies, the implementation of genetic selection programs can also be a complementary tool to improve the robustness of farmed fish and their plasticity to deal with nutritional innovations and challenging feeds with low FM/FO. Some studies reported that fish that are selected for fast growing on FM/FO diets display also higher growth on plant-based diets (Palti et al., 2006), while some others, reported a significant diet x genotype interaction, which means that fish that are selected for their fast growth on FM diets may not be the ones that grow faster when facing a challenging diet (Geay et al., 2011). Therefore, the aim of the present study, as part of the AquaIMPACT project (Genomic and nutritional innovations for genetically superior farmed fish to improve efficiency in European aquaculture; EU Horizon 2020, 818367), is to determine the effectiveness of genetic selection for growth in gilthead sea bream, in response to a challenging low FM/FO diet that aimed to partially replace FM by two emergent ingredients: insect meal from black soldier fly or single-cell protein from *M. capsulatus*. These diets also aimed to totally replace FO by a blend of poultry oil with a novel microalgae oil. The response of selected sea bream to the novel dietary interventions was assessed on fish productive parameters, fish proximate and fatty acid composition, the apparent digestibility coefficients of the dietary nutrients, as well as on fillet quality properties at commercial size.

Materials and Methods

Three diets were formulated to be isoproteic and isoenergetic to meet the nutritional requirements of gilthead sea bream juveniles. Control diet (C) contained 15% of FM and 5.9% of fish oil to mimic the composition of a current commercial diet, as well as completed with some vegetable meals and rapeseed oil as protein and lipid sources, respectively. Insect meal diet (INS) was included at 5% of the diet to replace 33.3% of the dietary FM. Single-cell protein was included at 10% of the diet and replaced 66.7% of the dietary FM. Fish oil was also totally replaced in these diets by a blend of poultry oil and Veramaris algal oil. Yttrium premix was added at 0.1% to both diets to further determine the apparent digestibility coefficients (ADC) of nutrients. The nutritional trial was carried out at the experimental facilities of the ULPGC. Gilthead sea bream from each experimental group (HG genotype vs REF genotype) and with an initial body weight of 49.91 g (average body weight), were randomly distributed in 12 experimental tanks, at a density of 45 fish/tank (3 tanks/treatment). Fish were initially allocated in cylinder-conical tanks of 500 L. Fish were manually fed until apparent satiation with one of the two experimental diets for 12 weeks (4 times a day, 6 days a week). Fish growth performance was monitored every 4 weeks until the end of the feeding trial and fish whole-body, fillets and liver samples were collected at the end of the trial for biochemical composition. Furthermore, fish faeces were collected after a digestibility trial to calculate the apparent digestibility coefficients analysis of the dietary protein and amino acids. Texture properties at 1 and 4 days post-slaughter, as well as the sensorial attributes of fish fillets, scored by a human panel, was also assessed.

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Results

Diet showed an effect on fish growth performance sooner (after 4 weeks of feeding) than genotype. INS and SCP diets led to a poorer growth performance in terms of body weight, length and/or SGR and TGC than C diet from 4 weeks of feeding and this difference was maintained until the end of the feeding trial (12 weeks). INS and SCP diets also significantly decreased fish FI compared with C diet, irrespective of the genotype, but showed no effect on FCR. In contrast, selected genotype increased fish growth in terms of body weight, total length and productive parameters like SGR and TGC, as well as optimized FCR, irrespective of the diet. No significant interactions between g x d were observed in any productive parameter evaluated after the 12-week feeding trial. HG genotype significantly increased the ADC values of protein and individual and total amino acids, except for tyrosine, whereas diet showed no effect on ADC values.

Genotype did not affect fatty acid profile of fish tissues, but SCP and INS diets increased the n-3 LC-PUFA content of fish fillets.

As expected, time post-slaughter significantly affected many texture properties of fish fillets, irrespective of the diet or genotype, with the values for fracturability, hardness elasticity, gumminess, and resilience of fish fillets decreasing with time. However, no significant effect of genotype or diet were noted in the texture properties of fish fillets, neither at 1 nor 4 days post-slaughter. Concerning the sensory properties of sea bream fillets, scored by the evaluation panel, no significant effects of diet, genotype or an interaction between g x d were observed for any of the sensorial attributes of fish fillets.

Conclusions

Overall, the results reaffirm the positive effects of genetic selection in improving sea bream productive key indicators, as well as support the use of insect meal and microalgal oil as replacers of FM and FO, respectively, in diets for selected sea bream, especially microalgae oil to increase n-3 LC-PUFA of fish fillets and improving the nutritional value of fish final products for consumers. However, the use of feed attractants might be useful to achieve similar growth as when a Control diet with FM is used, since the inclusion of insect meal and single-cell protein at 5% and 10% of the diet, respectively, might compromise feed intake.

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IMMUNOMODULATORY ROLE OF METHIONINE SUPPLEMENTATION ON MUCOSAL IMMUNE RESPONSE AND DISEASE RESISTANCE IN EUROPEAN SEABASS

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Introduction

The immunomodulatory role of certain amino acids is widely acknowledged. Apart from serving as building blocks in the synthesis of immune-related proteins, they also regulate crucial metabolic pathways associated with the immune response (Wu, 2013). Methionine is among the amino acids with recognized positive effects on immunity. Similar to the observed in other animals, dietary methionine supplementation has been shown to enhance the immune response and disease resistance in fish when faced with an inflammatory insult (Machado *et al.*, 2015; Machado *et al.*, 2018; Machado *et al.*, 2021). However, its role in mucosal immune machinery and response to pathogens requires further investigation. Therefore, this study aimed to investigate the effects of dietary methionine supplementation on mucosal immunity and disease resistance against *Tenacibaculum maritimum* in European seabass *(Dicentrarchus labrax)*.

Materials and methods

For 4 weeks, triplicated tanks of juvenile European seabass were given one of three experimental diets containing increasing levels of methionine supplementation: 0, 1 or 2% DL-methionine (referred to as CTRL, MET1 and MET2, respectively), resulting in methionine concentrations of 8.6, 18.5 and 29.2 mg/g dry matter, respectively. At the end of the feeding trial, blood, skin mucus, gut and head kidney were sampled and fish were bath-challenged with *T. maritimum*. The same tissues were collected at 48 hours post-infection, coinciding with the peak of mortality. The haematological profile was evaluated and blood smears were prepared for leucocyte counting and classification. Skin mucus samples were used to assess innate immune parameters, oxidative stress analysis was conducted on the gut and gene expression analysis was performed on the head kidney. The obtained results were subjected to canonical discriminant analysis (DA).

Results

A clear inflammatory response was observed in all experimental treatments, as evidenced by the separation between pre- and post-bacterial challenge points. While before infection fish fed different dietary treatments clustered together, inflamed fish fed experimental diets were significantly separated from one another. At 48h post-infection, the MET1 was the furthest group, whereas the CTRL and MET2 groups were relatively close to each other. The discrimination of the MET1 group was driven by the positive load of monocytes counts, IgM and total peroxidase in the skin mucus, as well as interleukin 8 (*il8*) and interleukin-1 beta (*il1β*) expression in the head-kidney. On the other hand, the variables that contributed the most to the separation of the MET2 and CTRL groups were haemoglobin and mean corpuscular haemoglobin concentration (MCHC).

Conclusion

While no significant differences were observed among dietary groups after the 4-week feeding trial, dietary methionine supplementation significantly influenced the immune response of the European seabass against *T. maritimum* infection. The great separation of MET1 from the other dietary groups suggests that there may exist an optimal or threshold level of methionine intake, beyond which the immune response may experience diminishing returns. These outcomes are consistent with previous literature findings (Shorter *et al.*, 2015) and highlight the importance of carefully considering the dosage of methionine supplementation.

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Nevertheless, in relation to the aim of investigating the effects of dietary methionine supplementation on mucosal immunity, the MET1 treatment seemed to positively impact the skin mucus immune-related mechanisms studied. Therefore, further research is currently underway to comprehensively understand its potential role in boosting mucosal immune function.

Acknowledgements

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SCREENING ANALYSIS OF THE PROBIOTIC BACTERIA *Phaeobacter inhibens* ON LARVAE OF MANILA CLAM (*Ruditapes philippinarum*), FROM GAMETE EMISSION TO METAMORPHOSIS

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Introduction

Manila clam (*Ruditapes philippinarum*) is one of the most economically important farmed aquatic species in Italy (EUROSTAT, 2023). Since its introduction in 1983, this species became one of the most exploited resources of the lagoons of the Northern Adriatic Sea, forming large natural benches that satisfied the request of juveniles by local farmers (Brusà *et al.*, 2013).

In the last few years, the natural recruitment of Manila clam juveniles decreases (Ponti *et al.*, 2017), so the hatchery-reared seed emerged as a source of juveniles to support the clam industry.

Infectious diseases represent one of the bottlenecks for the mass production of seed in hatchery, due to mortality peaks caused by *Vibrio* bacteria (Dubert, 2017).

Currently, the prevention of diseases outbreaks is preferred to treatments with antibiotics, in order to avoid the emergence of antibiotic resistant bacterial strains, so the administration of probiotic microorganism represents a beneficial practice to fight vibriosis in shellfish hatchery (Prado *et al.*, 2010).

The aim of this preliminary study is to verify the beneficial effects also on *R. philippinarum* larvae, starting from a test on survival of larvae treated with *Phaeobacter inhibens* DSM17395. *Phaeobacter inhibens* has been reported to produce antibacterial compounds and it has been verified to exerts a probiotic activity in other mollusk and fish larvae (Sohn *et al.*, 2016; D'Alvise *et al*., 2012).

Materials and methods

The marine bacteria *P. inhibens* DSM17395, identified as potential probiotics (Ruiz-Ponte *et al*., 1998) was grown in marine broth, at 25°C, with gentle rocking.

Once fecundation was carried out, larvae were maintained in flasks containing 200 ml SSW (Sterile Sea Water) at 24±1°C, 28 psu and daily fed with microalgae of the species *Isochrysis galbana* (var. T-ISO) and *Chaetoceros calcitrans*, dosed with a total concentration of 100.000 cells/ml.

Selected concentrations of the probiotic were 10^6, 10^5 and 10^4 CFU, referring to the most cited in similar work (Karim *et al.,* 2013; Prado *et al.*, 2009). Bacterial density was determined by measuring OD 600 through spectrophotometer. For each concentration, experiment was run in triplets, and in addition, a group of larvae were treated without the addition of probiotic, as control group.

SSW was periodically changed, together with the count of survived individuals, carried out through Sedgwick rafter cell counter. Then, survival rate has been calculated through the following formula (Prado et al., 2009):

$$Survival\ rate\ (\%): 100 \text{ x } \frac{(\text{No. of live larvae})}{(\text{Total no. of larvae})}$$

In the treated groups, the probiotic was dosed at the beginning of the test and in correspondence of water changes.

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Results

Preliminary results of the study are summarized in Fig. 1. Survival of larvae has been monitored from T0 (the day right after the emission) to T4 (ten days after the emission), when the metamorphosis event begins.

Among the three different treatments tested, the one involving the lower concentration of the probiotic, 10^4 CFU, is the one showing result similar to the control. In addition, at T3 and T4, 10^4 treatment results show a better survival rate.

Despite the decline in number of larvae through the time, this study shows that the survival rate of the treated groups is similar or improved when compared to the control ones (that should reflect the standard condition at which this species is farmed in the hatcheries).

Further results will be available for the oral presentation and will be focused on the effectiveness of the above-mentioned treatment.

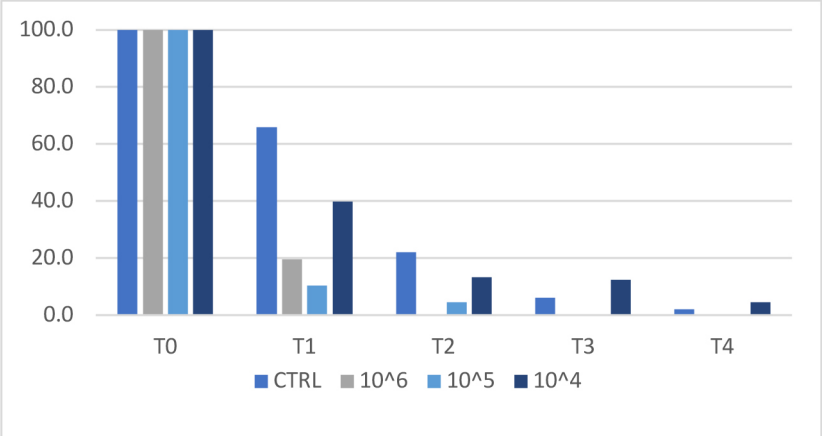

Fig. 1: Survival rate of larvae for each treatment. From T0, one day after the emission, to T4, ten days after the emission.

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SEAWATER CONDITIONED WITH CRUSTOSE CORALLINE ALGAE AS SETTLEMENT INDUCER FOR PATELLID LIMPETS

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Introduction

The fishery of the limpets *Patella aspera* and *Patella candei* (Patellogastropoda: Patellidae) in Madeira surpasses 100 annual tons and concerns about overexploitation of the stocks led to different legislative protective measures (Sousa et al. 2019). Recently, the research on the limpets' aquaculture achieved key methodological advances at hatchery level (Castejón et al. 2022a), being highlighted the potential role of the crustose coralline algae (acronym: CCA) to induce the limpet settlement (Castejón et al. 2022b). This study took a step further to explore the seawater conditioned with CCA as settlement inducer for *P. aspera* and *P. candei*.

Material and Methods

The adult limpets were captured during the breeding period (February to March 2022) and kept in captivity using an open system (20 ± 1 °C and 36 ± 1 psu). The production and culture of larvae used gametes obtained by dissection following the procedures described by Castejón et al. (2022a). The oocytes were matured artificially using a solution of NH_4OH at pH 9 during 20–30 min. Fertilization used 10^5 sperm cells ml^{-1}. Incubation and larval culture were done in glass beakers and plastic jars at 16 ± 1 °C. Incubation used 50 oocytes ml^{-1} and lasted 24h. Larval culture used 5 individuals ml^{-1} and lasted 48h.

The CCA were obtained from adult limpet shells of which the soft body was removed and the aperture was cleaned thoroughly following Castejón et al. (2022b). Then, the shells were broken into pieces that were kept in small containers with filtered seawater treated with UV (acronym: FSS) during four days to obtain the conditioned seawater (acronym: CSW).

Two assays were done for each limpet species (*P. aspera* and *P. candei*): 1) different CSW concentrations (ca. 17, 5, 2 and 0.7 mm^2 CCA · ml^{-1}) and 2) influence of frozen CSW (-24 °C) obtained from three different CCA communities (*Pneophyllum* sp., *Neogoniolithon* sp. 1 and *Neogoniolithon* sp. 2). In *P. candei*, the influence of different treatments applied to the CSW was tested: raw and autoclaved kept at room temperature (0, 24 and 48h), raw kept in the fridge 2–4 °C (24 and 48h), raw frozen at -24 °C (48h) and filtered (0 h). All the assays used FSS as negative control and CCA as positive control. Settlers were identified by the loss of the velum (metamorphics) and the teleoconch development (post-larvae). The ratio of metamorphics and the ratio of settlers (metamorphics + post-larvae) were calculated.

Results and Discussion

The ratios of metamorphics and settlers decreased with lower concentration of CSW in both *P. aspera* and *P. candei*, but the values were markedly lower in the former (Fig. 1A–B). Regarding the frozen CSW, only *Neogoniolithon* sp. 2 induced the settlement in *P. aspera*, while all the frozen CSW treatments induced the settlement in *P. candei*. In *P. candei*, all the treatments applied to the CSW (raw kept at room or fridge temperature, autoclave and frozen) induced the settlement independently of the timing after obtaining the CSW (0 to 48h); being the single treatment without effect the filtered CSW (glass microfiber 2.7 µm) (Fig. 1C).

CSW promoted *P. candei* post larvae settlement in at a ratio positively related to its concentration with the potential to be preserved frozen. In *P. aspera* an efficient settlement response required the physical presence of CCA. Altogether, these results showed inter-specific differences in the sensitivity to the soluble cues released by the CCA, highlighting that limpet settlement requires to be studied at species level.

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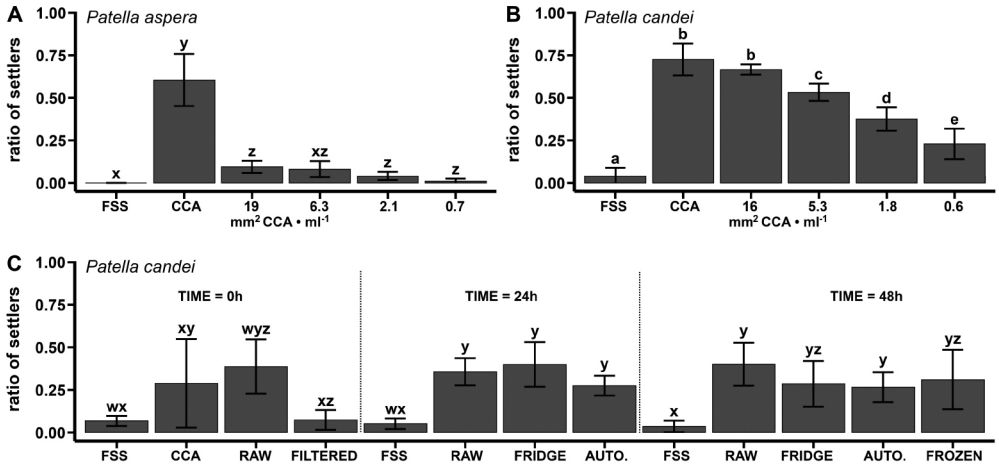

Figure 1. Influence of the CSW concentration (five replicates per treatment) in the settlement of two limpet species (A–B): *Patella aspera* (A) and *Patella candei* (B). C. Influence of the combination of different CSW treatments and time (six replicates per combination) in the settlement of *Patella candei*. Different letters indicated significant differences (a–e: Tukey's HSD, w–z: Games-Howell; $p < 0.05$).

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CORALLINE ALGAE, DIATOMS AND ACETYLCHOLINE AS SETTLEMENT INDUCERS OF THE TRUE LIMPET *Patella ordinaria* IN MADEIRA

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Introduction

The limpet *Patella ordinaria* Mabille, 1888 is a marine gastropod of high commercial interest. The settlement of competent larvae is one of the factors limiting the development of the limpet aquaculture, although success has been recently achieved using encrusting coralline algae as substrate (Castejón et al. 2022b). This research focused on testing alternative methods for settlement using neuroactive compounds tested as settlement inducers in other mollusk species such as γ-aminobutyric acid (GABA), 3-isobutyl-1-methylxanthine (IBMX), acetylcholine (ACH) and potassium chloride (KCl) (Alfaro et al. 2014) (Table 1).

Material and methods

Broodstocks were collected between November 2021 and April 2022. Competent larvae were obtained following Castejón et al. (2022a). Fertilization was performed at a concentration of 100 oocytes · ml^{-1} and 10^5 sperm cells · ml^{-1}. Larval culture was performed in 500 ml beakers under static conditions and at a concentration of 4 ± 1 larvae · ml^{-1}. Three assays were performed using no-substrate as negative control, coralline algae (CCA) as positive control, diatom biofilms of *Navicula salinicola* and different concentrations of different settlement inducers (Table 1). The larval stages identified were: metamorphosed specimens presenting velum loss and post-larvae with teleoconch growth. Experiments lasted between 12 and 13 days.

Results and discussion

Crustose coralline algae showed the greatest settlement response in all the assays, validating its great potential as settlement substrate and positive control for settlement studies in limpets (Castejón et al. 2022a, b). The diatom *N. salinicola* showed a variable settlement response among different assays dismissing its utility as a positive control or utility as a reliable settlement substrate. Regarding the neuroactive compounds, GABA, IBMX and KCl did not successfully induced settlement at the concentrations tested. In contrast, acetylcholine (ACH) at concentrations ranging from $5 \cdot 10^{-4}$ to $4 \cdot 10^{-3}$ M showed an increasing settlement response with increasing concentrations (Fig. 1). Acetylcholine was also reported to influence the settlement of the marine gastropod *Haliotis iris* suggesting its potential to modulate the settlement behavior and velum loss in mollusks (Alfaro et al., 2014). However, additional studies are required to elucidate why the settlement response induced by the neuroactive compounds did not result in an

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Table 1. Molar concentration (M) of the neuroactive compounds used in the different assays.

Assay	GABA	IBMX	ACH	KCl
1	$5{\cdot}10^{-7}$	10^{-7}; $5{\cdot}10^{-7}$; 10^{-6}; 10^{-5}	10^{-7}; $5{\cdot}10^{-7}$; 10^{-6}; 10^{-5}	$5{\cdot}10^{-3}$; 10^{-2}; $2{\cdot}10^{-2}$
2	-	10^{-5}; $5{\cdot}10^{-5}$, 10^{-4}	10^{-5}; 10^{-4};10^{-3}	$2{\cdot}10^{-2}$; $3{\cdot}10^{-2}$; $5{\cdot}10^{-2}$; $7{\cdot}10^{-2}$
3	-	-	$2.5{\cdot}10^{-4}$; $5{\cdot}10^{-4}$; 10^{-3}; $2{\cdot}10^{-3}$; $3{\cdot}10^{-3}$; $4{\cdot}10^{-3}$	-

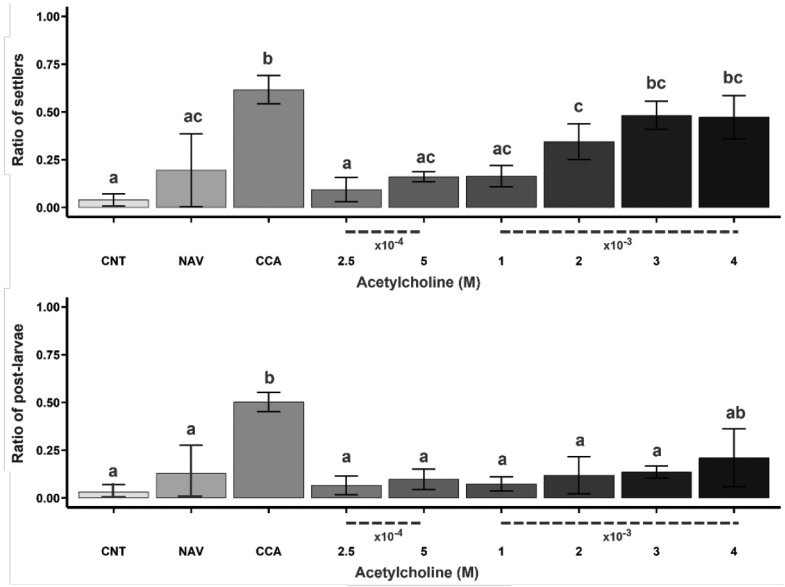

Figure 1. Ratio of settlers (metamorphics and post-larvae) and ratio of post-larvae in the different treatments. Bars indicate average ± SD. Different letters indicate significant differences (a–c: Tukey test; $p < 0.05$.)

DIETARY MICROPLASTICS EXPOSURE IN DIFFERENT LIFE-CYCLE STAGES: A STUDY ON ZEBRAFISH (*Danio rerio*) PHYSIOLOGICAL RESPONSES AND WELFARE FROM LARVAE TO JUVENILES

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Introduction

Microplastics (size < 5 mm; MPs) have been found in almost every environment including oceans and represents a widely diffused pollutant for aquatic organisms. Several studies have demonstrated the presence of MPs in marine animals, from plankton to higher trophic levels. Furthermore, sea-farmed aquatic species have been shown similar rates of MPs accumulation as those evidenced by wild specimens. More recently, the problem of MPs contamination in farmed fish has been found to also affect the land aquaculture sector. In this context, the main MPs source is represented by aquafeeds since conventional marine-derived ingredients used for their formulation derived from caught fish. Particularly, it has been shown that the concentration of MPs in fish meal is higher than that found in the raw materials since the processing procedures of the ingredients and packaging methods significantly contribute to increase the final amount of MPs in the aquafeed (polyethylene is one of the most widely used materials to produce "storage bags" for fishmeal). Dietary MPs contamination can have negative effects on farmed fish during different life-cycle stages. The larval development is one of the most critical phases because of the fast morphological and behavioural changes that increase the risk of mortality. During this phase: (i) possible obstruction of the gastrointestinal tract; (ii) a reduced predatory activity caused by an apparent feeling of satiety; (iii) a decrease in growth and swimming capacity; (iv) the activation of inflammatory responses in gut and other organs because of potential translocation processes can occur. However, despite the large number of publications on the presence of MPs in fish, not much is known about the long-term MPs exposure and the effects on the different life cycle stages and developmental phases of fish. In this regard, the present study aims to compare the effects of dietary MPs exposure in zebrafish (*Danio rerio*) larval and juvenile stages, monitoring MPs translocation among organ and tissues and the effects on fish growth and welfare through a multidisciplinary laboratory approach.

Materials and Methods

Five experimental diets were used in this present study. A control diet was prepared according to a commercially available standard diet for zebrafish (Zebrafeed, Sparos ltd, Portugal). The four experimental diets containing MPs were prepared by adding during the preparation of Control diet the fluorescent polymers A and B at two different concentrations, as follows: (i) 50 mg/kg feed of polymer A (diet A50); (ii) 500 mg/kg feed of polymer A (diet A500); (iii) 50 mg/kg feed of polymer B (diet B50); (iv) 500 mg/kg feed of polymer A (diet B500). The microbeads were purchased from Cospheric LLC (Goleta, CA, USA) and their features were: (i) polymer A: amino formaldehyde polymer, 1-5 µm of dimensional range, peak of emission at 636 nm when excited at 584 nm; (ii) polymer B: polyethylene, 40-47 µm of dimensional range, peak of emission at 607 nm when excited at 575 nm. After hatching, zebrafish larvae were initially reared in fifteen 20 L tanks (3 tanks per experimental group; 500 larvae per tank). After 20 days post fertilization (dpf), fish of each tank were transferred in 100 L tanks (3 tanks per experimental group). Zebrafish were fed the experimental diets two times a day (daily dose corresponding to the 3% of the body weight) from 5 to 60 dpf. The required amount of fish were sampled at both 20 dpf (in which whole larvae were collected for each analyses) and at 60 dpf (in which samples of liver, intestine, and muscle were collected from juveniles). For both larvae and juveniles, the following parameters were evaluated: (i) survival and specific growth rate (SGR%); (ii) the absorption of the fluorescent MPs microbeads at intestinal level and their potential translocation to liver and muscle through a Nikon A1R confocal microscope (Nikon Corporation, Tokyo, Japan); (iii) the MPs microbeads quantification in whole larvae or in the target organs of juveniles through chemical digestion followed by a vacuum filtration on 0.7 µm pore-size fiber-glass filters and consequent analyses of the filters through a fluorescence microscope (Zeiss Axio Imager.A2; Zeiss, Oberkochen, Germany); (iv) possible structural alteration of the intestinal epithelium and the hepatic parenchyma through the application of a series of histopathological indexes and stains; (v) the relative expression of genes involved in immune (*il1b*, *il10*, and *litaf*) and oxidative stress response (*sod1*, *sod2*, and *cat*) starting from total RNA extraction from whole larvae or liver and intestine samples for juveniles.

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Results and Discussion

No significant differences in survival and specific growth rates were detected among the experimental groups for both zebrafish larvae and juveniles. The ingestion of the two polymers used in this study was confirmed both by confocal microscopy and MPs quantification in the zebrafish larvae and juveniles. However, only the polymer A (size 1-5 µm) was absorbed at intestinal level in both larvae and juveniles. The presence of polymer A microbeads was detected in liver and muscle samples only in juveniles suggesting a time-related translocation from the gastrointestinal tract. Furthermore, the MPs quantification in both whole larvae and in intestine and liver samples of juveniles highlighted a dose dependent accumulation of polymer A. Regarding polymer B (size 40-47 µm), no absorption was detected, but the transit through the intestinal tract caused a reduction of mucosal folds length and an increase in goblet cells relative abundance in B50 and B500 groups, suggesting a higher intestine lubrication.

The absorption or the simple transit of both MPs in groups A and B did not cause inflammatory events at intestinal level nor alteration in the expression of immune markers in both larvae and juveniles. However, the accumulation of polymer A microbeads in liver samples of juveniles caused the upregulation of the oxidative stress markers.

Results of the present study suggest the presence of biological barriers against the ingested MPs in zebrafish that are related to the polymer size, dietary concentration, and time of exposure, able to reduce the number of MPs reaching the muscle which is the edible part of a fish. This result is extremely interesting for the aquaculture sector and needs further studies performed on finfish species.

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PHYSIOLOGICAL EFFECTS OF *Gymnodinium catenatum* AND *Skeletonema marinoi* BLOOMS ON THE OYSTER *Magallana angulata,* AN AQUACULTURE PERSPECTIVE

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Introduction

Bivalves are usually cultured in land tanks connected directly to estuarine and coastal waters. This type of aquaculture exposes the cultured species to possible toxic blooms that often affect these natural ecosystems. The effects of toxic algae on humans have been the subject of many studies. However, information on the effects of these toxic species on bivalve species have been less explored.

Gymnodinium catenatum is a dinoflagellate which produces a saxitoxin like compound, being responsible for paralytic shellfish poisoning. It has been shown to cause an increase in antioxidant enzyme activities and oxidative stress in some bivalve species after exposures of 6-12 hours. However, the effect of such contaminants to short-time exposures has not been extensively studied. Shorter exposure times are important to evaluate possible effects into aquacultures, as these types of systems usually allow to close the connection with external water, thereby reducing the exposure of the cultured animals.

Besides toxic algae, other species can negatively affect the reared species. Algae like *Skeletonema* spp. Are known to damage the gills, leading to several health complications in marine animals (Esenkulova et al., 2022). In addition to physical damage, *Skeletonema marinoi* produces polyunsaturated aldehydes (PUA) when exposed to grazing (Vidoudez et al., 2011) which are toxic to many marine species (Romano et al., 2011; Tosti et al., 2003). Nevertheless, *S. marinoi* is commonly used to feed bivalves in aquacultures (Guéguen et al., 2008).

It has been observed that antioxidant enzymes respond to several contaminants (Cereja et al., 2018; Dias et al., 2019) including algae toxins (Cao et al., 2018) and thus can be used as a physiological stress indicator for to toxic algae exposure.

The aim of this study was to assess the physiological effect of short-term exposure to *G. catenatum* and *S. marinoi* in cultured *Magallana angulata.*

Material and methods

Oysters were collected at an aquaculture farm in the Sado Estuary, Portugal and carried to MARE facilities where they were allowed to acclimate in 70L tanks. During the acclimatization period, all oysters were fed with $\approx 2*10^9$ cells L^{-1} of a mixture of *Tetraselmis sp.* and *Phaeodactylum sp.* For the exposure treatments, both *Gymnodinium catenatum* and *Skeletonema marinoi*, were obtained from the algae culture collection of the Lisbon University (ALISU).

In the experiment, $\approx 2x10^7$ cells L^{-1} of *Tetraselmis sp.* for the control group, $\approx 4x10^7$ cells L^{-1} of *S. marinoi* and $\approx 1x10^4$ cells L^{-1} of *G. catenatum* for the exposure treatments were added to three tanks, each containing 6 oysters, and allowed to filter for two hours. Afterwards, the six organisms of each treatment were opened by cutting the adductor muscle. The gills, the digestive gland and the adductor muscle were removed and stored at -80 ºC.

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Subsequently, the samples were homogenized in PBS buffer saline solution, centrifuged (15 min, 10,000×g at 4°C) and the supernatants used to quantify superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), acetylcholinesterase (AChE) and total ubiquitin (UBI) and the data was analysed using a PerMANOVA.

Results:
The results showed significant differences in both treatments: tissues and treatments. Gills were the tissue showing higher biomarkers while the adductor muscle had the lowest levels. Regarding the comparison between treatments, the control treatment presented higher levels of the analysed biomarkers. *S. marinoi* treatment presented higher CAT and GST than *G. catenatum* treatment.

Discussion:
The higher biomarker levels determined in the control treatment were hypothesized to be a consequence of reduced metabolic rates in the two other treatments, although such relation must be confirmed in future studies. The higher CAT and GST activities determined in the *G. catenatum* treatment in comparison to the *S. marinoi* treatment was hypothesized to result from detoxication process. These results suggest that *G. catenatum* may affect the physiology of *M. angulata* even in short-term exposures and that live *S. marinoi*, which is usually used as aquaculture feed, may also impact the *M. angulata* physiology, probably due to the production of PUA. Further studies must be performed to confirm this possibility and if such effect is also observed when using dead *S. marinoi* as PUA production may differ.

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PROJECT "NEWPACK - A NEW GENERATION OF PACKAGING MATERIALS" – INNOVATIVE STRATEGIES TO REDUCE PLASTICS WASTE AND ASSOCIATED ECOLOGICAL IMPACTS IN COASTAL AND AQUACULTURE REALMS

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Introduction

From electronics to food containers and fashion accessories, plastics are indissociable from our daily lives. Designed to have high durability and resistance, the use of plastics in the food packaging industry has grown to unprecedented and unsustainable levels in the last decades. Despite they are usually meant to have a single use, to date, packaging plastics are not fully biodegradable and their complete elimination during waste treatment is a rather complex process (involving economical, societal, logistical, and territory planning), often resulting in their unintentional introduction in natural environments and landfills (Walker and Xanthos, 2018; Karbalaei et al., 2019). Hence, the presence of plastic polymers (e.g., polyethylene and polyethylene terephthalate, PET) derived from packages (and other materials) in the aquatic environment is a major ecological concern of our times. The ongoing inability to efficiently manage the huge amount of plastics generated and subsequent environmental introduction compromises water quality in coastal areas, as well as, the welfare and survival of organisms (e.g. fish) that accidentally ingested them.

Despite being usually associated with fishing activities, plastic pollution is a worldwide problem whose impacts also affect the aquaculture context, because: i) extensive and semi-intensive aquaculture facilities are often settled in areas subjected to high anthropogenic pressures and, thus, can present substantial levels of plastic pollution (Lusher et al., 2017); ii) recent studies have reported considerable levels of microplastics in fishmeal, due to the environmental contamination of wild small pelagic fish species that are used to prepare feed meals and oils; and iii) the aquaculture industry itself is also an important contributor to the global plastic usage and subsequent environmental pollution (Skirtun et al., 2022, Tian et al., 2022).

To respond to such issue, several research and technological innovation actions have recently attempted to create "eco-friendly" solutions, such as the development of materials/packages that are partly or entirely made from bio-based materials (e.g., sugar-based polymers, Ferreira et al., 2019, Li et al., 2023). Yet, they still present significant toxicity for aquatic environments and different biodegradability depending on the polymer, not representing a true ecological alternative (Babaremu et al., 2023, Zimmermann et al., 2020).

Within this context, NEWPACK is an innovative vertical project within the VIIAFOOD Agenda of the Recuperation and Resilience Plan in Portugal that aims to create a new generation of packaging materials - *sustainable, from renewable raw materials, with functional preservation activity for packed food products and with the benefit of being an intelligent packaging, which allows providing information on product conservation.* If such novel packaging material is unintentionally introduced into the marine environment and accidentally ingested by fish, it will not be dangerous but a nutritional asset upon metabolization.

Material and methods

Focusing on plastic reduction and technological innovation specifically within the vegetable oil packaging industry, the NEWPACK project encompasses 6 different and interconnected tasks, including the:

1. Revision of the state-of-the-art, with a special focus on the reported ecotoxicological impacts of conventional plastics and their additives, as well as, of recently developed and alternative bioplastics in aquatic biota;
2. Selection of suitable sugar-based candidate monomers (building blocks) to be used in the production of the new biopolymer, taking into account compound toxicological aspects, physical-chemical attributes (and related handling/synthesis easiness) and production costs;

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3. Conceptualization of a new recyclable and biodegradable biopolymer unharmful and nutritious to marine fish, that efficiently replaces the use of conventional plastics within the food packaging industry;
4. Optimisation and standardization of a multicompartment *in vitro* methodology that mimics, in a more realistic way, marine fish digestion process. The achievement of such milestone will constitute an important landmark in the field of "Live Animal Sciences" in general, and in Aquaculture Research in particular, as it will constitute a "Replacement" strategy, an alternative to *in vivo* studies that, in the future, will certainly reduce the number of animals used in nutrition studies, which are crucial to the expansion of the aquaculture sector;
5. Development and successful market implementation of cutting-edge smart labeling technology applied to the food packaging industry to assure products' traceability throughout the whole production and value chain, preventing product quality deterioration, incorrect discard of food packages, and the subsequent plastic waste generated.

Dissemination of results among different stakeholders at national and international levels, to raise awareness regarding the impacts of plastic packages on the environment, the use of more sustainable alternatives already available to replace conventional packaging and validation of tools to improve traceability throughout the food chain, as well as, the disposal and treatment of plastic waste.

Expected outcomes:
NEWPACK project is aligned with the European Green Deal, aiming to provide a relevant contribution towards the end to wasteful packaging, by promoting the "reuse and recycle" policy and use of sustainably produced materials in one of the most plastic polluting industries, i.e., the food packaging industry. It is also expected to contribute to Goal 14 – Life below water - of the UN Sustainable Development Agenda, that aims at ensuring the conservation and sustainable use of the oceans and marine resources and has the specific target of preventing and significantly reducing marine pollution of all kinds from land-based activities, including marine debris, by 2025. The innovative concept and environmentally responsible nature of NEWPACK project will certainly have various tangible impacts at scientific, technological, ecological, economical and societal levels.

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INFLUENCE OF MICROALGAE SUPPLEMENTATION ON GUT MICROBIOTA OF GROWING *Sparus aurata*

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Introduction

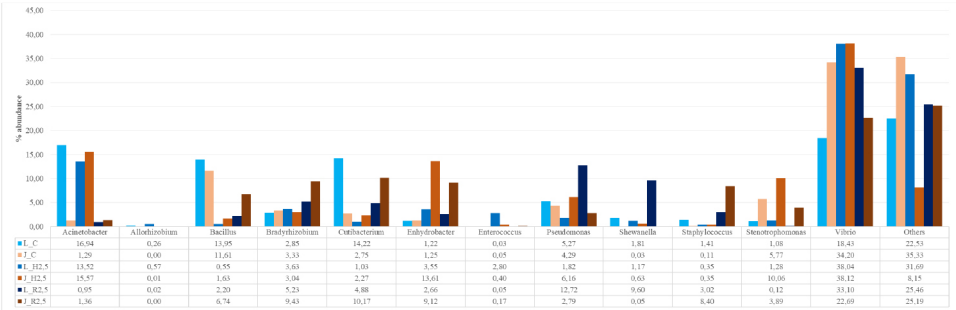
Diet plays a crucial role in causing intestinal dysbiosis, leading to disruptions in the intestinal barrier and increased permeability. This condition facilitates the entry of harmful substances and microorganisms into the bloodstream, directly and indirectly contributing to the development of bacterial pathologies and physiological imbalances in the body. Consequently, the analysis and study of the intestinal microbiota have become significantly important for designing and implementing new diets or supplements in aquaculture. By studying the diet and gut microbiota at different life stages of *Sparus aurata*, this research has the potential to provide valuable information on how to promote a healthy digestive tract and improve aquaculture production in the future.

In this context, *Nannochloropsis gaditana* are microalgae rich in beneficial lipids, antioxidant compounds, and amino acids, which have shown to improve growth and product quality in *Sparus aurata*. The objective of this study is to investigate the changes in the microbiota during the transition from larval to juvenile *S. aurata* when fed a diet supplemented with microalgae in both raw and hydrolyzed forms for a period of 45 days.

Material and methods

N. gaditana was cultured in tubular photobioreactors at the pilot plant (EU-H2020 SABANA facilities) of the Universidad de Almería (Spain). Subsequently, enzymatic hydrolysis of raw algal biomass was carried out as reported in Ayala et al. (2020). An inclusion level (25 kg-1 w/w), and two microalgae formats (raw and enzymatically hydrolysed) were considered in 0.2 to 0.4 mm microdiets.

Therefore, diets were designed as R2.5 for raw microalgae, H2.5 for diet containing enzymatically hydrolysed biomass, and a microalgae-free diet was used as control (C). The feeding trial was carried out at randomly distributed in 15 tanks and fed daily at 5% of their biomass and sampled at 45 days of feeding trial.



	Acinetobacter	Allorhizobium	Bacillus	Bradyrhizobium	Cutibacterium	Enhydrobacter	Enterococcus	Pseudomonas	Shewanella	Staphylococcus	Stenotrophomonas	Vibrio	Others
L_C	16,94	0,26	13,95	2,85	14,22	1,22	0,03	5,27	1,81	1,41	1,08	18,43	22,53
J_C	1,29	0,00	11,61	3,33	2,75	1,25	0,05	4,29	0,03	0,11	5,77	34,20	35,33
L_H2.5	13,52	0,57	0,55	3,63	1,03	3,55	2,80	1,82	1,17	0,35	1,28	38,04	31,69
J_H2.5	15,57	0,01	1,63	3,04	2,27	13,61	0,40	6,16	0,63	0,35	10,06	38,12	8,15
L_R2.5	0,95	0,02	2,20	5,23	4,88	2,66	0,05	12,72	9,60	3,02	0,12	33,10	25,46
J_R2.5	1,36	0,00	6,74	9,43	10,17	9,12	0,17	2,79	0,05	8,40	3,89	22,69	25,19

Figure 1. Relative abundance (%) of dominant microbiota genera in larvae (L) and juvenile (J) *Sparus aurata* individuals fed control (C), 2.5% raw and (R), and 2.5% hydrolyzed *N. gaditana* (H) supplements

(Continued on next page)

A total of 80 intestinal content samples were subjected to DNA extraction using the precipitation saline protocol. Subsequently, the 16S rRNA gene of these samples was sequenced using the Illumina® MiSeq platform (Illumina, San Diego, CA, USA) with pairwise sequencing (2 × 300 bp) at the Ultrasequencing Service of the Bioinnovation Centre of the University of Malaga (Malaga, Spain). However, due to quality issues, only 35 larvae (L) and 19 juveniles (J) of Sparus aurata could be used for the microbiota analysis.

The bioinformatic data processing involved a workflow based on the DADA2 library of R, utilizing the SILVA 138 database with 99% clustering. The Shannon index was calculated to assess microbial diversity. Additionally, informative bar charts were generated to visualize the taxonomic distributions. The significance of the results was determined based on the Shannon index, using a t-student's p-value of <0.05. Taxonomic comparison was conducted using the R package DESeq2 ($p<0.05$).

Results and discussion

All sequences exhibited Phred quality values higher than 20, indicating reliable results. The analysis of alpha diversity using the Shannon index revealed no significant differences across conditions or treatments.

In this study on *S. aurata*, the predominant *phylum* was Proteobacteria, followed by Firmicutes and Actinobacteria. However, in larval individuals, the percentage of Proteobacteria was lower, with a higher proportion of Firmicutes and Cyanobacteria. Furthermore, the presence of Bacteroidota was absent in treated larvaes. On the other hand, in juveniles, the percentage of Proteobacteria was higher, especially in those fed with H2.5, where it reached around 90%. Notably, the *phylum* Bacteroidota was present in juveniles fed with the raw diet, but it was not observed in the other treatments studied.

Regarding genera (Figure 1), the presence of Acinetobacter in larvae (L_C and L.25) significantly differed from its occurrence in juvenile individuals. Similarly, *Shewanella* was more prominent in larvae compared to juveniles. Additionally, *Shewanella* and *Pseudomonas* exhibited significant differences in the R2.5 treatment in larval individuals. In the case of juveniles, there was a significantly higher abundance of *Vibrio* in the control group. *Pseudomonas* showed a significantly higher occurrence in the J_H2.5 group compared to L_H2.5, while *Stenotrophomonas* was more abundant in the J_R.5 group than in L_R2.5. Importantly, no significant differences at the genera level were observed in juveniles among the treatments.

The treatment of larval individuals fed the raw diet at 2.5 exhibited the most substantial changes, both compared to other treatments at their respective stages and, in comparison to juveniles, fed the same diet. These data suggest the larval stage is more sensitive to this treatment, leading to a more pronounced shift in the microbiota. However, the supplementation with *N. gaditana* at 2.5 did not affect the microbial composition in juvenile individuals. In summary, this study on *S. aurata* revealed significant differences in the microbial composition between larval and juvenile individuals and among different treatments.

Acknowledgments

This research was funded by MINECO-FEDER (grant # RTI2018-096625-B-C33 and grant # RTI2018-096625-B-C31, AquaTech4Feed (grant # PCI2020-112204) granted by AEI within the ERA-NET BioBlue COFUND, and SABANA project (EU-H2020, grant # 727874).

EVALUATION OF BIOLOGICAL EFFECTS OF A WINE INDUSTRY BY-PRODUCT ON SEA BASS *Dicentrarchus labrax*

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Introduction

The present study was developed as a part of a currently developing research project "Potential use of wine and beer bagasse as sources of functional ingredients and nutrients in feeds for aquaculture" (UBAGALAC, P20-00923) jointly funded by the Junta de Andalucia and the EU. This project focus on the applications of wine by-products in fish fed and their benefits on health, and physiology of marine fish. Among by-products of interest in the project, like grape pomace, specific extracts like oligo-procyanidins from grape seeds, known for their strong antioxidant properties as well as their bioavailability, could be potentially benefic supplements to marine fish diets, and were investigated in this study.

Material & Methods

The objective of this preliminary study was to test different biological effects of a wine by-product concentrated extract specifically rich in oligo-procyanidines (Olpheel® Anti-Ox, OAO, Laboratoires Phodé, France) in feeds for juvenile European sea bass (*Dicentrarchus labrax*), on three different aspects: the oxidative status of fish, functionality of intestinal microbiota, and oxidation of fillets after fish sacrifice and one week storage. A total of 120 juvenile sea bass with average body mass of 46.35 ± 0.12 g were equally divided into 6 under controlled environmental conditions of salinity (37 ‰), temperature (19°C) and photoperiod (10L:14D). Experimental feeds (control diet and a diet supplemented with 80 ppm of OAO) were prepared using a lab-scale extrusion machine. Daily ration was offered till visual satiety distributed in 4 daily meals ensuring that the amount offered in each experimental unit was fully ingested. No mortality was recorded in any experimental group. The feeding-trial lasted 5 weeks. The normality of the data was performed using the Shapiro–Wilk test, and homoscedasticity analysis was conducted using the Brown–Forsythe test. Statistical analysis of the data was carried out by one-way or two-way ANOVA, followed by Fisher's LSD test where appropriate. The significance level was established at $p < 0.05$.

Results

A significant effect of OAO on the microbial profile was evidenced as a reduced biodiversity (a lower number of functional groups) when compared to that of fish fed on the control diet, as well as by a higher functional richness (a higher intensity of the response that could be related to a higher number of specific OTUs (Operational Taxonomic Unit). Significantly higher levels of superoxide dismutase (SOD) were measured in the groups of fish receiving OAO compared to the control, with 7.38 ± 0.63 U SOD/mg SP in that treatment compared to 6.80 ± 0.34 in the control diet (Tab.1). The significantly higher levels of SOD can be the net result of an increased expression of genes coding such enzyme. Regarding oxidation in stored fillet of the fish, a protective effect against oxidation was evidenced in samples of fish fed on diet including OAO when compared to those fed on the control diet (Fig.1). At day 2, significant differences were observed as 457,47 pmol MDA/mg protein were found in muscles of the control fish, compared to 261,23 pmol MDA/mg protein for fish fed the OAO diet (Fig.1).

Those results together suggest that incorporating a wine by-product concentrated extract, Olpheel® Anti-Ox, to sea bass diets, could improve their antioxidant status and fillet quality. The low significancy of differences among different experiments in this preliminary study suggest that dosage of the product was potentially low and could be increased. Further studies should then be carried out with higher dosage, and to further understand the effect of such a product on diversity and functionality in microbiota and its consequences.

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Liver	Control	OAO
U SOD/ mg SP	6.80 ± 0.34 [a]	7.38 ± 0.63 [b]
U CAT/mg SP	107.88 ± 17.77	114.65 ± 16.44
U GPx /mg SP	6.19 ± 2.10	5.09 ± 1.99
picomol MDA/mg SP	344.42 ± 145.14	361.68 ± 178.66

Table 1. Oxidative status measured in liver of fish receiving the different experimental diets. Values are presented as mean ± SD. Values not sharing a common letter differ significantly with $p < 0.05$.

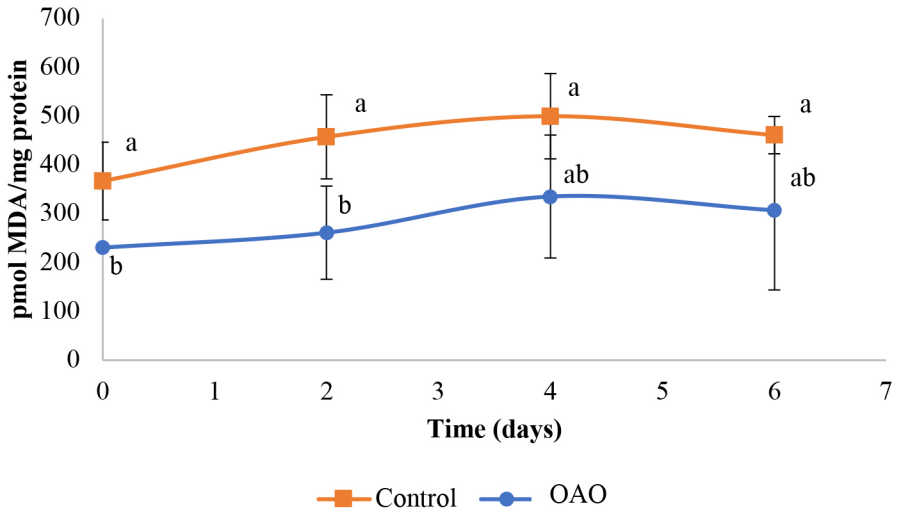

Fig.1 Muscle thiobarbituric acid-reactive substances (TBARS) content in fillets of *D. labrax* fed on experimental diets over cold storage (4 □C) at times: 0, 2, 4, 6 and 8 days. Values are presented as mean ± SD. Values not sharing a common letter differ significantly with $p < 0.05$.

RANKING FISH DIETS BY USING A RAINBOW TROUT *(Oncorhynchus mykiss)* ARTIFICIAL INTESTINE PLATFORM

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Introduction

The expansion of aquaculture fed species strengthens the need for sustainable fish nutrition and requires the development and testing of novel ingredients to be used in fish feeds. The Fish-AI consortium (H2020-FETOPEN project) is developing an artificial intestine platform based on Rainbow trout (*Oncorhynchus mykiss*) intestinal epithelial cells to evaluate the nutritional and health values of feed ingredients while reducing the use of animals in feeding trials. In this study, three test diets were digested *in vitro* and presented to rainbow trout (RT) intestinal epithelial cells and cell monolayers in order to evaluate the metabolic and health effects of feed ingredients on epithelial cells as well as their effects on the functional epithelium barrier.

Material and Methods

RT intestinal cells used in this work were derived from the proximal (RTpiMI) or distal (RTdiMI) region of the intestine (1). RT reference diet, a diet with high soymeal inclusion and a diet with high feather meal inclusion (Skretting) were digested *in vitro* by incubating the feed with RT crude enzymatic extracts from the stomach and the intestine in a static system and in a sequential manner (3). Protein hydrolysis was determined based on free amino groups and by protein electrophoresis. To assess the cell viability upon the exposure to the digested feeds, cells were seeded in 96-well plates (25,000 cells/cm^2) and after 3 days in culture were exposed to various digestate concentrations (6%, 12%, 25% and 50%) for 24 h. After the treatment, cell viability was determined by Alamar blue fluorescence, an indicator of the metabolic status of the cells and by CFDA-AM, which also reveals the integrity of the plasma cell membrane. To study the effect of the digests on the intestinal epithelial monolayers, cells were seeded on ThinCert inserts (Greiner BioOne, 0.4 pore size) at a density of 250,000 cells/cm^2, maintained in culture until a functional monolayer was formed (2) and then incubated with different concentrations of the digests (6%, 12% and 25%) for 72 h. To evaluate the effect on the integrity of the cell barriers, transepithelial electrical resistance (TEER) was measured before and after the incubation with the digests. In addition, permeability assays were performed after the treatments by using three different fluorescent markers: β-Ala-Lys (AMCA) (BioTrend), BODIPY FL C-12 (Thermo Fisher) and Dextran-568 10 kDa (Thermo Fisher).

Results

The exposure of RTpiMI or RTdiMI cells to the three diets for 24 h induced a concentration-dependent detrimental effect ($P<0.05$.) on cell viability for all the tested feeds. However, no significant differences ($P>0.05$) were observed between diets, and both cell lines displayed similar responses. Interestingly, when cell monolayers were exposed to the different digested feeds for 72 h, the diets enriched with soy meal or feather meal induced a significant decrease ($P<0.005$) in TEER values at both, 12% and 25% concentrations. This was in contrast to the reference diet, which showed similar TEER values ($P>0.05$) to cell monolayers incubated with L15 medium. In accordance with these results, the permeability to β-Ala-Lys-AMCA, BODIPY FL C-12 and 10 kDa dextran was significantly increased ($P<0.05$) in a concentration- and diet-dependent manner. While 6% of the digests did not induce any alteration on the permeability to the markers analyzed, at 12%, diets enriched with either soy meal or feather meal induced a significant increase ($P<0.05$) in the permeability to all the fluorescent molecules, indicating a disruption of the integrity of the cell barrier. At 25% digesta, the negative effect of diets enriched with either soy meal or feather meal on the monolayer integrity was stronger, with the reference diet also demonstrating a significant adverse effect ($P<0.05$).

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Conclusion

High levels of feather meal or soy meal in a RT diet had no apparent effect on the metabolic status of RT intestinal cells, nor on the integrity of the plasma cell membrane, as compared to a reference RT feed. However, an epithelial cell membranal monolayer's integrity was affected significantly following an exposure to digestates of both soymeal and feather meal rich diets. This study further demonstrates that both, TEER and the permeability to fluorescent molecules are efficient means to discriminate and rank different diet formulas using RT intestinal cell barriers. Thus, representing an important advancement in the development of the Fish-AI platform as an *in vitro* screening tool to predict the nutritional and health properties of novel fish diets.

Acknowledgements

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THE IMPACT OF NUCLEOTIDE SUPPLEMENTATION ON THE PERFORMANCE AND IMMUNE RESPONSE OF DEPLOYMENT-SIZE BALLAN WRASSE (*Labrus bergylta*)

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Introduction

Nucleotides have a wide variety of functions beyond their most recognized role as the basic building blocks of RNA and DNA. They provide and mediate energy metabolism, are key components of cell signalling, and are key enzymatic cofactors (Cosgrove, 1998), to name a few. While organisms can produce nucleotides without dietary input (Cosgrove, 1998), this process is metabolically expensive and direct provision may allow fish systems to react faster to challenges (Ringø et al., 2012). Therefore, nucleotides are generally considered semi-essential nutrients (Li and Gatlin, 2006) and beneficial during periods of rapid growth or high metabolic demands, such as larval stages or disease (Li and Gatlin, 2006). Taking their lead from human nutrition (Cosgrove, 1998; Li and Gatlin, 2006), aquaculture research has begun to explore the potential of nucleotides to be used as feed additives (Li and Gatlin, 2006). A wide range of nucleotide-based products have been used for a variety of fish species and have been shown to optimise cell proliferation, promote growth (Hossain et al., 2016), enhance immune response (El-Nokrashy et al., 2021) as well as improve gut integrity and function (de Cruz et al., 2020). However, the mode of action of nucleotides on these various functions has in many cases yet to be fully elucidated. The deployment of ballan wrasse (*Labrus bergylta*) from the hatchery to the net pen is a period of high stress and mortality, with increased exposure to environmental stressors and disease. Working closely with industry partners, this study will explore the use of nucleotides as health additives in developing functional feeds to support ballan wrasse during this period.

Materials and Methods

Ballan wrasse (38.4 ± 9.4g) were randomly distributed into twelve 350 L flow-through tanks (100 fish per tank). Tanks were allocated to one of four experimental diets: A control diet following the current standard commercial formulation for this species with no added nucleotides (NT0) and three treatment diets with increasing levels of nucleotide supplementation (NT1, NT2 and NT3) using the commercial product Rovimax NX (DSM Animal Health and Nutrition). Each condition was conducted in triplicate with fish fed the experimental diets to satiation for 8 weeks. At the end of the trial, samples from different tissues were collected for histological, vertebral health, molecular biology analysis as well as plasma. In order to examine the robustness of the fish after feeding the supplemented feeds, the fish were subjected to an immune challenge by intraperitoneally (IP) injecting lipopolysaccharide (LPS) of *E. coli* as pathogen-associated molecular patterns (PAMP) at the end of the experiment. Fish were sampled 24 hours post-LPS injection, with plasma and head kidney collected. Operational welfare indicators (OWI) were also scored at the end of the nutritional challenge and after the challenge.

Results and Discussion

Fish performance was generally good, with fish increasing weight on average 1.6 times compared to the start of the trial. After 8 weeks of feeding the experimental feeds, fish fed the non-supplemented feed did not display significant different body weight compared to fish fed nucleotide-supplemented feeds. No difference was also found in terms of survival, being 99.2 ± 0.7 % on average. The fish showed good OWI scores, though not significantly different among the experimental fish. No significant difference was found in the fish haematocrit, the hepatosomatic index, and liver histology at the end of the nutritional trial.

The preliminary results seem to indicate that nucleotide available from the non-supplemented commercial formulation covered the nutritional requirements for the general performance of ballan wrasse. Further results will be presented, including molecular markers' response to the immune challenge, to ascertain the benefits of nucleotide supplement for the ballan wrasse subjected to an immune stressor, in order to prepare them for the cage transfer.

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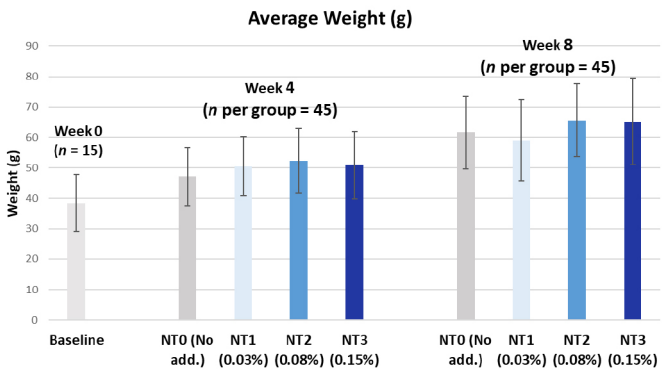

Fig 1. Mean weight (g) of the experimental fish at the beginning and after 4 and 8 weeks of feeding without nucleotide supplementation (NT0), and with nucleotide supplementation of 0.03% (NT1), 0.08% (NT2), and 0.15% (NT3). The error bars represent the standard deviation.

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THE CHALLENGE TO PROMOTE IMTA IN A COUNTRY WITH LOW MONOCULTURE PRODUCTION. THE ARGENTINE CASE

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Introduction

Aquaculture is an essential activity contributing to food security, providing 88 million tonnes of aquatic food in 2020 (FAO 2022). Aquaculture continues growing rapidly, with an average annual growth rate of 6.7% between 1990-2020. Nevertheless, 92% of the global production is concentrated in only 20 countries, while 95 out of 170 countries with aquaculture activity produce less than 10,000 tons per year, as is the case of Argentina (Cai et al. 2022). 90% of aquaculture production in Argentina focuses on only two freshwater fish species (*Oncorhynchus mykiss* and *Piaractus mesopotamicus*), while marine aquaculture has been historically a marginal activity.

In this context, the aim of this work was to gather existing and generate new information relevant to promote and develop marine IMTA in Argentina and analyse the potential of low trophic native species to diversify the current production concentrated in fed aquaculture.

Material and Methods

We evaluated the potential of 12 native species of the Beagle Channel, located in the extreme south of Argentina (54°S), for IMTA production. The species were 2 fish, 2 crabs, 1 octopus, 2 sea urchins, 2 bivalves, 2 algae and 1 halophyte, covering the different roles in a theoretical IMTA: Fed aquaculture Organic Extractive Aquaculture (sedimented POM feeders), Organic Extractive Aquaculture (suspended POM feeders), and Organic Extractive Aquaculture (DIN feeders). Besides, several activities were carried out to design strategies to develop IMTA, including assessment of environmental conditions, regulations, markets, and social perception, among others.

Results and Discussion

We detected two groups with potential to be part of an IMTA system: 1) Red sea urchin (*Loxechinus albus*) reared in lantern integrated with blue mussel (*Mytilus chilensis*) reared in longlines. The other proposed system consists in whitebait (*Galaxias maculatus*) integrated with halophytes (*Salicornia magellanica*) in an open land-based system.

The market study showed that blue mussel is one of the most important species, while Salicornia (included in the algae category) is little offered. From the interviews with the restaurant managers, it was detected that there is interest in incorporating sea urchins, and they are still unaware of the gastronomic value of whitebait (Figure 2).

One of the strengths detected is that potential producers, decision-makers, and society, have an idea about the meaning of the IMTA concept.

Regarding the limitations found, it is worth mentioning the lack of aquaculture facilities to develop projects on a pilot scale, and a great distrust on the part of society towards aquaculture projects due to their potential environmental impacts and the lack of government capacity to monitor this activity.

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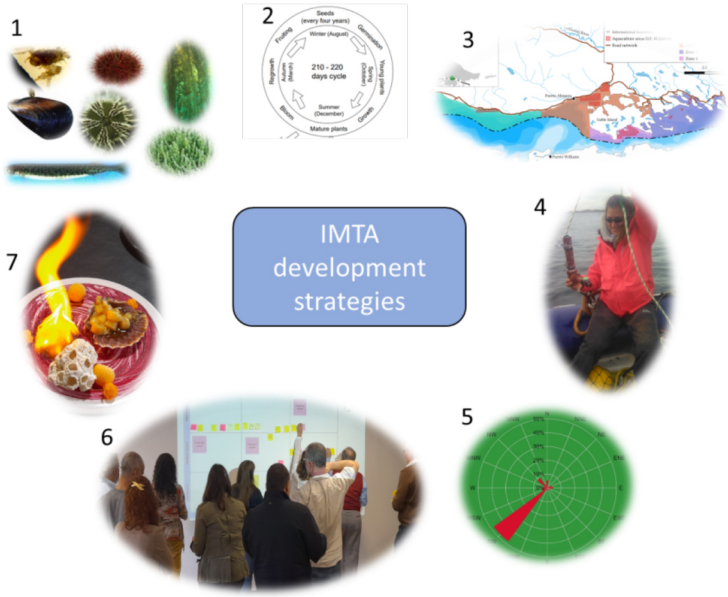

Figure 1: Some of the activities carried out in Argentina to boost IMTA development. **1)** Species selection in terms of biological potential, monoculture experiences and technical knowledge. **2)** Design of potential productive cycles of native species. **3)** GIS development gathering information about environmental data, infrastructure available, other uses, etc. **4)** Record of environmental data in areas of interest. **5)** Analysis of atmospheric data (e.g., wind speed and direction). **6)** Participatory workshops with stakeholders to understand needs, opportunities, and weaknesses. **7)** Market study and analysis of the gastronomic value of native species.

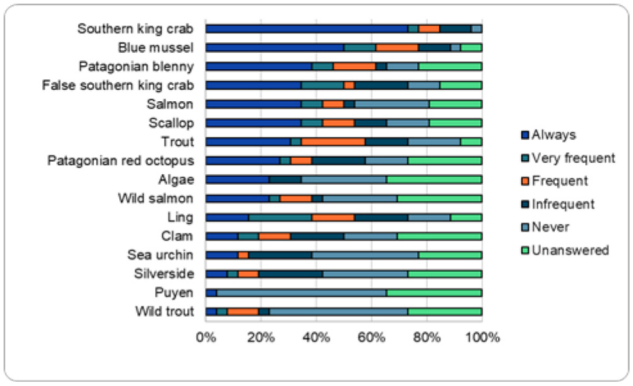

Figure 2: Species offered by local seafood restaurant and their importance in terms of offer frequency.

Acknowledgements

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"THE ROLE OF CERTIFICATION & ACCREDITATION IN SUSTAINABLE DEVELOPEMNT OF SEAFOOD INDUSTRY"

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The Technological advancement in the field Aquaculture and seafood processing necessitated the regulation, to have food safety uniform to all markets of the world. Sustainable and monitored development of the industry will enhance optimum production and enhanced food safety. To enable the process certification and accreditation play very important role, to achieve uniform product specification and quality

MARKET RESEARCH ANALYSIS FOR POSSIBLE EXPANSION OF CYPRUS AQUACULTURE

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Introduction

The market for seabass and gilthead seabream in Cyprus is considered to be saturated, with the wholesale market leaders dominating the market and utilizing their extensive retail networks to maintain their market share positions. In addition, local producers are facing competition from Greek aquaculture farms, which have been able to quickly and easily send fresh products to Cyprus at lower prices since last year. Furthermore, new local aquaculture farms will face significant increases in infrastructure costs, which potential government subsidies may not fully cover. As a result, it is advisable for prospective new aquaculture companies to develop business plans that prioritize exporting production overseas rather than relying on local consumption. To this end, a market research study was conducted to explore the possibility of exporting to neighboring countries such as Israel, Egypt, Jordan, and Lebanon. As fresh fish is favored by the consumers, the swift transportation and low freight costs create an ideal setting for potential new export markets. The market research analysis also explored the possibility of exporting two new species for Cyprus aquaculture, meagre and red porgy.

The primary objective of the study was to determine the market size per country, identify the dominant market leaders and key players, and understand the essential product category features according to consumer preferences such as fish species, size, quality, process level or product configuration, and distinctive characteristics. Additionally, the study outlines the sea transport procedures for importing fish into each country and documents any potential taxes, charges, and fees. Finally, the report presents business strategies for Cyprus aquaculture firms based on a comparative evaluation of the four countries included in the study.

Results

Israel, although a small country in terms of population, has a high per capita gross domestic product and a significant yearly fish consumption rate. These factors position Israel as a potentially attractive market for Cypriot fish farms to export seabass. However, the Israeli market for seabass is relatively small, with an annual consumption of approximately 6,500 tonnes. Additionally, the wholesale price of seabass is attractive for Cypriot fish farms. The wholesale price of seabream does not favor exports.

Egypt, on the other hand, is a significantly larger market for fish products, both as a producer and an importer. However, the country's large-scale aquaculture projects have led to low wholesale prices for the species under study, making it a challenging market for Cypriot aquaculture farms to penetrate. In addition, Egypt plans to increase its exports and reach self-sufficiency in fish production, potentially becoming a strong competitor for Cypriot exports.

Jordan is a small market with a low but steadily increasing fish consumption rate. Local production is minimal due to the country's very short coastline, and the low per capita gross domestic product has resulted in moderate wholesale prices for seabream and seabass. While this market may not be ideal for Cypriot fish farms at present, the increasing trend in fish consumption suggests a possible future market, especially if prices continue to rise.

Lebanon, too, is a small market with low yearly fish consumption. Political instability in the country has led to a constant drop in per capita gross domestic product and changing consumption preferences. While the wholesale retail price for seabass is high, demand is low. In contrast, Lebanon can import seabream at extremely low prices from Turkey, making it an unattractive market for Cypriot aquaculture farms.

According to the market research analysis, it has been revealed that neither meagre nor red porgy are feasible for export due to a number of factors.

(Continued on next page)

Table 1: Comparative fact sheet of the Israel, Egypt, Jordan and Lebanon.

COUNTRY	POPULATION	GDP (per capita in US dollars)	Yearly consumption per capita	SEABREAM		SEABASS		MEAGRE		RED PORGY		Local Industry growth potential	bureaucratic and other barriers for imports	Political stability	OTHER
				Demand	AVG WHLS [1]	Demand	AVG WHLS	Demand	AVG WHLS	Demand	AVG WHLS				
ISRAEL	9 million	$51430	26kg	↑	€5.80	↑	€7.50	↓	N/A	↓	N/A	MODE RATE	NO	YES	Exports of Seabass
EGYPT	111 million	$3876	24kg	↑	€4.46	↑	€0.84 (1kg)	↑	€4.71	↓	€~2	HIGH	YES	YES	N/A
JORDAN	10 million	$4405	5.8kg	↓	€5.74	↓	€6.56	↓	€3.62	↓	€3.88	LOW	NO	YES	Cheap Turkey Imports
LEBANON	6.9	$4186	8.7kg	↓	€3.13	↓	€7.93	↓	€6.98	↓	€2.70	LOW	NO	NO	Cheap Turkey Imports

Table 1 summarizes the major findings for the four markets under study. Red color represents *Not Favorable* conditions, green color represents *Favorable* conditions, while light blue represents *Moderate* conditions.

Acknowledgement

The Open Sea Aquaculture in the Eastern Mediterranean Project (OS Aqua), co-financed by the European Regional Development Fund and the Republic of Cyprus through the Research and Innovation Foundation (Grant No. INTEGRATED/0918/0046).

FEED FOOD COMPETITION AMONG DIFFERENT TILAPIA FARMING SYSTEMS

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Reducing feed-food competition in aquaculture and livestock is a way to decrease the environmental impacts of the food system and to free up large areas of arable lands to potentially produce more food [1]. Previous studies showed that almost 50% of the feedstuff used in aquafeeds are causing feed-food competition [2]livestock and aquaculture production, focusing on feed use and the availability of by-products and residues. We then analysed the potential of replacing food-competing feedstuff—here cereals, whole fish, vegetable oils and pulses that account for 15% of total feed use—with food system by-products and residues. Considering the nutritional requirements of food-producing animals, including farmed aquatic species, this replacement could increase the current global food supply by up to 13% (10–16%. Indeed, fed aquaculture utilizes fish resources that could have been eaten directly by humans, but are used to grow more desirable species due to market demands. Furthermore, the quality of soybeans, maize, wheat, and other agricultural crops used in aquafeed, may or may not live up to food-grade qualities, but they are indirectly competing for land that could be used to produce food-grade crops. In general, food-competing feedstuffs have lower environmental footprints than farmed aquatic or terrestrial animals fed these resources [3], hence the interest to feed farm animals mainly with by-products that humans cannot or do not want to eat.

Tilapia is an aquaculture species of global importance, with a high contribution to food supply, especially in Asia and Africa. It can be cultured in various production systems (pond, cages, tank, raceway, RAS), with a varying need for external feed inputs and feed conversion efficiencies. Depending on the systems, and locations, the feed used may contain high-quality ingredients potentially competing with human food and/or non-food competing by-products. The total human-edible yield of tilapia can also be variable, as this fish can be consumed in various ways from the use of the fillet only, to the consumption of the whole fish, depending on fish size, processing technologies, and culture. All these factors may influence the food in / food out ratio of the tilapia farming systems leaving unclear which are net consumers of human edible nutrients, and which are net producers.

This study aims to quantify and compare the net contribution to human food production of contrasted tilapia farming systems from various world regions. To do so, we calculated the human-edible protein conversion ratio (HePCR) i.e., the quantity of human-edible protein in the feed divided by the quantity of human-edible proteins in the animal products, for different tilapia farming systems. These tilapia case studies were taken from peer-reviewed Life Cycle Assessment studies and covers the broad range of rearing systems that can be used in tilapia farming. Overall, this study discusses how the feed, the farming system and the valorization of fish by-products can influence feed-food competition in tilapia farming systems.

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FOOD SAFETY ON BIVALVES AND HOLOTHURIANS CO-CULTIVATED WITH FISHES IN MEDITERANEAN IMTA AQUACULTURE

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Introduction

Integrated Multitrophic Aquaculture (IMTA) has been suggested as an innovative method of aquaculture development (Mansour et al, 2022) that ensures sustainable development in agreement with the EU directions for Blue Growth and Blue Economy. Methodology of co-culture fishes, bivalves and holothurians have been developed in Mediterranean waters (Chatzivasileiou et al 2022). Although, studies on IMTA project have promising results in Mediterranean waters (Chatzivasileou et al 2022), food safety of IMTA organisms has not been studied extensively. According to the Greek and European law it is not yet allowed to cultivate and commercialize organisms cultured in IMTA systems due to lack of specific IMTA food safety protocols. In the context of Innovated Development of Marine Aquaculture (IDMA) project after the co-cultivation of fishes, bivalves and holothurians, an investigation of food safety parameters was implemented. The results are expected to facilitate the European Food Safety Authority and the Greek Food Authority to compose safety protocols for IMTA products and consequently to permit the development of commercial IMTA systems.

Material and Methods

In this study, three Mediterranean species were co-cultured in three operating fish farms in the Aegean with different trophic conditions. It is defined as the cultivation of two or more aquatic species from different trophic levels in the same area in order to mimic the energy flow in natural ecosystems (Chapin T. et al 2004) The co-cultivated species were Mediterranean mussel (*Mytilus galloprovincialis*), rayed pearl oyster (*Pinctada imbricata radiata*), and sea cucumber (*Holothuria polii*) as described by Chatzivasileiou et al (2022). After a year of cultivation, edible tissue samples were collected for the co-culture species, in order to find out if the co-cultivated organisms are appropriate for consumption. The food safety analysis included the determination of antibiotics (oxytetracycline, florfenicol), metals (Hg, Pb), dioxins and related substances (PCBs, PCDDs, PCDFs, PBDEs, DDT, DDE, HCB, PFAs, and lastly biotoxins (DSP, PSP, ASP). All the analysis was conducted in samples of IMTA cultured sea cucumber, mussels and oysters as well as samples for a typical mussel farm and oyster and sea cucumber natural population.

Results

European committee has established limits on oxytetracycline and florfenicol concentrations for safe consumption of food products (100μg/kg for mussels and oysters, Regulation EU 37/2010). Our results showed that the concentrations of the two antibiotics were under the permitted limits for all samples regardless the type of mussel cultivation. Metals concentrations in this study were below the food safety limits defined by the European committee (0.5 mg/kg WW for Hg, and 1 mg/kg WW for Pb, Regulation EU 4661/2001). The permitted limits of dioxins and dioxins like substances is 3.5 pg/g for TEQ PCDD/PCDF, 6.5 pg/g TEQ PCBs, 75 ng/g for PCBs, 50 mg/kg for DDT, for HCB and for PBDEs (Regulation EU 1259/2011 and 2019/1021). In this study, the concentrations of organic pollutants were below the safety limits for IMTA mussels and oysters. Lastly, the permitted limits of marine biotoxins DSP, PSP and ASP are 160 mg/kg, 800 mg/kg and 200 mg/kg respectively (Regulation EU 853/2004). Our results showed that the biotoxins concentrations were very low and under the safety limits for all mussels and oysters' samples.

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Conclusions

Until now the products of an IMTA aquaculture couldn't commercialize, cause the lack of knowledge regarding the safe consumption of the IMTA organism. These results showed that bivalves and holothurians cultivated in a fish farm area are safe for human consumption, and IMTA aquaculture is a safe method to co-cultivate species from different trophic levels. Aquaculture wastes were not contaminating the co-culture species with antibiotics, toxins or metals. That fact makes IMTA aquaculture a promising method to cultivate more species in the same farm

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CIRCULARITY ASSESSMENT OF IMTA INFRAESTRUCTURE IN THE CONTEXT OF ASTRAL PROJECT

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Introduction

In the context of the Circular Economy Action Plan (European Commission, 2020) and as part of the growing economy sectors, aquaculture may play a relevant role within the circular and green transition.

Together with the transition to a circular economy, aquaculture of low-trophic species has been pointed out as an important industry for sourcing alternative proteins and facilitating the sustainability of food systems (Farm to Fork Strategy, 2020). Although these aquaculture systems are overall recognised by its lower environmental impact (Strategic guidelines, COM/2021/236 final), the infrastructure elements used are often composed of non-biodegradable fossil-based plastics. In this sense, there is an increasing interest on prospecting innovative sustainable solutions for the low-trophic –aquaculture sector to increase the circularity of infrastructure elements.

ASTRAL is a European project that aims at developing and providing innovative techniques and species combination to improve Integrated Multi-Trophic Aquaculture (IMTA). Within ASTRAL, a circularity assessment is being carried out to provide evidence-based metrics to evaluate how the new aquaculture systems performs in the context of the circular economy. Particularly, for the specific case study in Scotland, focused on the cultivation of seaweed and bivalves, we have identified the major drivers to increase the circularity in infrastructure.

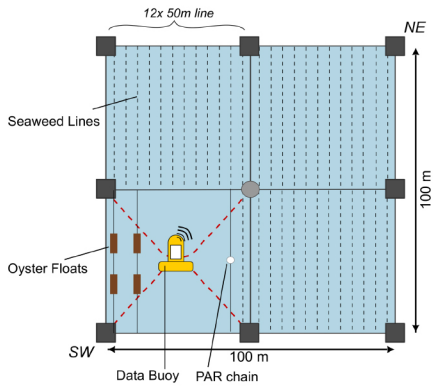

Fig. 1: SAMS infrastructure configuration site.

The study's circularity calculation has been based on the Material Circularity Indicator developed by the Ellen MacArthur Foundation. It considers utility (U), virgin feedstock (V) and lifespan (L).

The study firstly addresses of infrastructure elements for Baseline and IMTA scenario.

i. MCI Mooring Structural (improved U and L)
ii. MCI Surface Structural (improved V, U and L)

$$\bar{x} = \frac{MCI\ Mooring\ Structural + MCI\ Surface\ Structural}{n}$$

Secondly, the assessment addresses the role of IMTA through the improved U when utility is defined as the total capacity to develop biomass.

Each infrastructure element is associated with an MCI parameter, which has been improved following the implementation of the IMTA differentiating between the three circularity principles (reduce, reuse, and recycle).

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The infrastructure elements have been assessed individually to quantify the circularity improvements of IMTA:

i. Anchor line: Increasing the biomass production due to the IMTA species, the utility of the element is increased.
ii. Seaweed Growing Line: Manual cleaning of remaining biomass and seeding twine before being reused for next deployment.

Results

The first approach has shown an increase of circularity by 20% related to the anchor line element, and by 68% for seaweed growing line element.

Secondly, the implementation of IMTA at SAMS site has increased the total biomass by 9%, reflected as a 6,7% increase in the Material Circularity Indicator.

Conclusions

When assessing the circularity individually for each infrastructure element and after the implementation of best practices, the circularity is increased based on the specific parameter that has been modified.

Positive results are shown as well if the circularity is assessed under a general approach, considering the higher amount of biomass produced, increasing the utility of infrastructure due to the shared purpose in IMTA.

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HEALTH-PROMOTING ADDITIVE PROVIDES DUAL PROTECTION AGAINST ECTOPARASITES IN FISH: CORNIFICATION AND COMPLEMENT SYSTEM ACTIVATION

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Introduction

Ectoparasite infections represent a significant challenge in fish aquaculture, with the fish skin immune system serving as the frontline defense and comprising multiple mechanisms to protect against microbial invasion. Incorporating health-promoting additives as a preventive approach has enhanced skin mucus defense mechanisms and reduced parasite prevalence. This study aimed to examine the modulatory effects of a phytobiotic-based additive, APEX® BRANCHIA (Adisseo), on fish skin defense against ectoparasitic diseases- The Guppy-*Gyrodactylus* infection model and a shotgun proteomics approach were applied to evaluate the additive's impact on fish skin.

Material and methods

Forty individually housed guppies (*Poecilia reticulata*) were initially provided with a diet containing the functional additive for 14 days. Subsequently, each guppy was inoculated with two *Gyrodactylus turnbulli* parasites, and the additive-supplemented feed was continued for an additional 17 days. Parasite numbers on each fish were assessed every 48 hours, and based on infection susceptibility, fish were classified into responsive, resistant, and susceptible groups. Skin samples were collected on days 13 and 17, corresponding with peak immune activities and the end of infections, respectively. Differentially expressed proteins in the skin tissue among the various susceptibility groups were identified using nano LC-MS/MS.

Results

This analysis elucidated the mode of action of APEX® BRANCHIA in relation to fish response to infection, revealing two primary strategies for combating parasite infection. The first strategy involved skin cornification in susceptible fish, wherein parasite burdens peaked on day 13 and subsequently declined sharply by day 17. Cornification is a programmed cell death process generating a dense barrier of dead cells, effectively impeding parasite entry. The second strategy was employed by resistant fish, which consistently displayed low parasite levels throughout the study. In these fish, the additive was found to activate the complement system, initiating a series of proteolytic events that facilitated the elimination of invading pathogens. In conclusion, the dual protection mechanism delivered by APEX® BRANCHIA is likely a key mechanism to reduce the severity of infection observed in field testing.

METABOLIC RESPONSE OF GREENSHELL™ MUSSELS (*Perna canaliculus*) IN RESPONSES TO VARIABLE TEMPERATURES, OXYGEN LEVELS AND ANAESTHETICS — IMPLICATION FOR SEAFOOD LIVE TRANSPORT

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Introduction

Live transport has great potential to maintain the quality of cultured seafood (Fotedar & Evans, 2011). The live transport approach, however, involves different handling processes, which in mussels could cause stress and mortality (Nguyen et al., 2020). By inducing metabolic depression before transport, live mussels may be less sensitive to stress caused by handling processes associated with live transport. In this study, conditions that trigger metabolic depression were explored using a commercially important green-lipped mussel, *Perna canaliculus*, as a model species, by measurement of cardiac activity.

Materials and methods

Mussels were exposed to different treatments i.e., temperature (4°C, 6°C, 8°C and 14°C (control)), oxygen level ($0.5 mgO_2L^{-1}$, $1 mgO_2L^{-1}$, $3 mgO_2L^{-1}$ and $8 mgO_2L^{-1}$ (control)) and anaesthetic concentration ($MgCl_2$; $0 gL^{-1}$ (control), $30 gL^{-1}$, $40 gL^{-1}$ and $50 gL^{-1}$) for two hours. During the exposure, eight mussels from each treatment were sampled at two time points (i.e., 30 minutes and 120 minutes after the exposure started) respectively for measurement of heartbeat (bpm) for 20 minutes.

Results

Linear mixed-effects models showed that temperature, oxygen level and $MgCl_2$ concentration, and the exposure duration had an interactive effect ($p < 0.05$) on mussel HR (Figure 1). HR of mussels at low temperatures was depressed by 50–100%, whereas HRs of mussels exposed to $MgCl_2$ decreased by 36–97%. Low oxygen levels only dropped mussels' HRs by 1.5–51%.

Discussion

Depression of metabolic rate was most pronounced in the temperature and $MgCl_2$ treatments. Reasons explaining this finding are body temperature of ectothermic organisms depends on the environmental temperature, which governs the biochemical reactions and, subsequently, metabolism and performance within thermal tolerance windows (Cheng et al., 2018). Decreased body temperature due to dropping environmental temperature could reduce ectotherms' enzyme activity level and thus reduce metabolism, which could be indicated by depressed physiological rates such as respiration and heart rates (Schulte, 2015). Bivalves can also depress their metabolism by shifting from aerobic to anaerobic metabolism to tolerate prolonged hypoxic conditions (Stevens & Gobler, 2018), which was also demonstrated by depressed HRs of *P. canaliculus* at $0.5 mgO_2L^{-1}$ over exposure time. Immersion in $MgCl_2$ solution could relax the heart and adductor muscles of *P. canaliculus*, resulting in reduced HRs as Mg^{2+} blocks Ca^{2+} from entering the cell, which obstruct the release of acetylcholine to initiate muscle contraction (Azizan et al., 2021; Namba et al., 1995).

By comparison, low temperature and immersion in $MgCl_2$ solution both successfully induced metabolic depression of mussel *P. canaliculus*. Studies on conditions in the combination of low temperature and $MgCl_2$ should be explored to see if they will further suppress mussels' metabolism.

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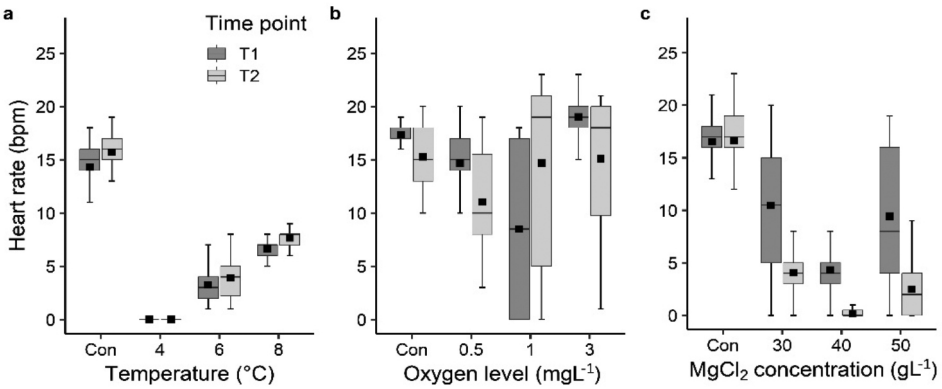

Fig. 1 Heart rates of mussel, *Perna canaliculus*, sampled at different time points (i.e., T1 and T2) respectively exposed to various (a)temperatures, (b)dissolved oxygen levels and (c)$MgCl_2$ concentrations. The solid line and dark square represent the median and the mean respectively (Con = control, i.e., for temperature (14°C), dissolved oxygen ($8mgO_2L^{-1}$) and $MgCl_2$ ($0gL^{-1}$) treatments).

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SEA-MONITOR: A GENOMIC DIAGNOSTIC SUITCASE BASED ON NANOPORE SEQUENCING

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With the development of high throughput sequencing techniques and dedicated bioinformatics tools, molecular epidemiology has experienced a spectacular boom in recent years and has demonstrated its usefulness in human health for the early detection of emergencies and the understanding of transmission and circulation dynamics of many infectious diseases. Aquaculture is a sector particularly sensitive to infections where flocks are regularly subjected to epizootics causing considerable ecological and economic damage. Currently, the sector lacks methods for early detection, prevention and control of diseases. To address this need, we have developed a tool and methodology to accurately and rapidly identify pathogens that could cause mortality in livestock. As a proof of concept, the SEA-MONITOR project has provided a concrete solution for real-time monitoring of marine mollusc pathogens to significantly improve their prevention and control. The concept is based on the development of a suitcase containing all the laboratory equipment necessary for genomic diagnosis. More precisely, this mini-laboratory is based on the use of Oxford Nanopore Technologies (ONT) portable sequencing technology, which allows to obtain high quality genomic sequences in near-real time. The solution proposed also includes the development of an analysis pipeline allowing the transfer of raw sequencing data to an *adhoc* calculation server and the automatic analysis of these data to quickly provide an accurate and complete diagnostic report to users. Tests performed in laboratory as well as in the field by our partners have enabled the identification and sequencing of viral and bacterial pathogen genomes within a few days. Results of the SEA-MONITOR project have demonstrated that genomic monitoring and diagnosis represent a promising opportunity for a better management of marine mollusc diseases.

THE EFFECTS OF ENVIRONMENTAL ENRICHMENT ON COGNITIVE FUNCTION AND STRESS RESPONSE OF JUVENILE ATLANTIC SALMON (*Salmo salar)*

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Introduction

To achieve high standards of animal welfare, animals must be raised in healthy conditions in terms of pathogen presence and nutritional requirements, be free from discomfort or pain, free from distress or fear, and in conditions suitable for the development of natural behaviours (Mellor, 2016) In this regard, efforts are being made to not just avoid negative welfare states, but promote positive welfare, by providing environments that allow animals to fully develop their cognitive abilities and express natural behaviours. This can be achieved by providing environmental enrichment that can enhance positive welfare conditions. However, positive welfare is a complex concept, which depends on the biology of each species, the stage of development and even the individual's own personality (Mellor, 2016). It is therefore necessary to study in detail the behaviour of different organisms in relation to the application of protocols or techniques that result in the improvement of their quality of life. In the present study we focus on the impact of structural environmental enrichment (SEE) on molecular parameters indicative of cognitive capacity, neuronal activity and stress response in juvenile Atlantic salmon (*Salmo salar*).

Material and methods

Groups of fish (1.59±0.41 g; 5.43±0.46 cm) were randomly distributed in 8 (700L) conical tanks (n=375fish each) and keep in a Recirculating Aquaculture System (RAS) for 12 weeks, being fed ad libitum with a 24h L photoperiod. Four of the eight tanks included structural environmental enrichment (SEE), through the addition of artificial plants attached to a PVC mesh. To assess the effects of SEE on the acute stress response, fish were exposed to a stress event at the end of the 12-week challenge period; a 5-minute net chase, a husbandry practice known to induce stress in fish, was used as a stressor. Fish were sacrificed with an overdose of MS222 and the brains were immediately dissected and placed in a bath of RNA-latter. 3 brain samples of each tank were obtained 3 days before the stress event, and 6 brain samples per tank 30 minutes after the stress event. Absolute quantification of gene transcripts was performed to evaluate cognitive function (*bdnf*, *syt*, *pcna* and *ndf1*), neuronal activity (*cfos*), stress-axis, (*crfb1*, *gr1* and *gr2*) and cellular stress response (*hsp70* and *hsp90)* Total RNA was extracted by using TRIZOL-reagent method. 2µg of total RNA was used to obtained cDNA, using (RT2 First Strand Kit, QUIAGEN). Transcript copies of target genes were determined by real-time quantitative PCR (q-PCR) using the Biometra TOptical thermocycler. Analyses were performed on 1 µL of diluted cDNA (1/20) using the Luminaris HiGreen qPCR Master Mix (ThermoFisher Scientific), containing 100 nM of each primer. Experimental transcripts levels were quantifying by using standard curves of previous cloned and sequenced target genes. All data are shown as mean number of RNA transcripts ± standard error of the mean (SEM). Statistical analysis was carried out using two-way ANOVA, having as main factors (enrichment and stress) followed by turkey's t-test. Values of $p < 0.05$ were considered as statistically significant. Matrix correlation analysis were made between the levels of each target gene. All experimental protocols described here followed ethical guidelines and complied with UK government regulations. Procedures were approved by AWERB 2021 0281 3001

Results

Preliminary results indicate significant increase in *bdnf* and *ndf1* in salmon with structural environmental enrichment (SEE), $F_{(1,68)} = 5.379$, $p = 0.0234$ and $F_{(1,68)} = 4.250$, $p = 0.0431$, respectively, meanwhile there was no significant effect of acute stress ($p = 0.4163$ and $p = 0.2751$). However, the acute stress events promote a significant increase in the expression of *hsp90*,($F_{(1,68)} = 4.556$, $p = 0.0364$) and *cfos (*$F_{(1,68)} = 4.300$, $p = 0.0419$), regardless of enrichment condition $p = 0.4769$, and $p = 0.8767$, respectively. Correlations analysis shown that there is high consistency between the levels of the *ndf1* and *bdnf* $p < 0.0001$ for the same individuals, Pearson index = 0.708. Also between *ndf1*and *hsp90* $p = 0.0293$, Pearson index = 0.2570 and *bdnf* and *hsp90* $p = 0.085$, Pearson index = 0.3078. In particular, in these last two pairs, there was a high correlation between fish with enrichment $p < 0.0001$, disappearing among fish that were not subjected to enrichment. $p = 0.2704$ and $p = 0.1199$, respectively.

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Discussion

There is a growing body of evidence indicating that *bdnf* and *ndf1* are associated with neurogenesis, neuronal maintenance, neuronal survival, plasticity, and neurotransmitter regulation in fish (Luccini, 2018). In addition, previous studies in mammals point to a strong relationship between the transcription factor ndf1 and the stimulation of nerve growth via bdnf/trkb pathway (Lai et al., 2020). In our experimental conditions we observed that fish reared under a SEE displayed higher levels of *bdnf* and *ndf1*, suggesting an increase in neuron proliferation in those individuals. Moreover, the high correlation displayed between the expression of both genes support the interaction proposed before. Therefore, the results obtained demonstrate that SEE has a positive impact on the expression of genes related to neurogenic processes which translate onto higher cognition capacity (Lucon-Xiccato et al., 2022).

On the other hand, we observed that the acute stress event did not have a significant impact on the expression levels of genes related to cognitive function or stress axis, but we have detected an activation of the cellular response to stress, evidenced by higher levels of *hsp90* and *cfos*, indicating a neuronal integration of stress event-related signals. There is considerable evidence indicating that a coordinated interaction between hsp70/hsp90 enhances the binding capacity of glucocorticoid receptors, increasing their sensitivity and affinity to stress related hormones (Kirschke et al., 2014), with cortisol (the main stress-related hormone) having been shown in teleost fish to promote the binding of hsp70 to the glucocorticoid receptor (Basu, et al., 2003). In this regard, we found a significant correlation between neurogenesis and cellular stress response-related genes in SEE-reared fish, suggesting that fish exhibiting higher cognitive abilities also develop a high level of hsp90. This finding may be indicative of higher sensitivity to stress events, thus indicating a superior competence of these fish to integrate stress-related stimuli as a stress coping mechanism.

Acknowledgements

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SALINITY INDUCED CHANGES IN THE PROGRESSION OF WATER, ION AND NUTRIENT FLUXES ALONG THE GASTROINTESTINAL TRACT OF ATLANTIC SALMON SMOLT (*Salmo salar*)

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Introduction

Anadromous fish species have a life cycle that begins in freshwater (FW), followed by smoltification as juveniles, which allows them to migrate to seawater (SW). Smoltification allows the FW fish to prepare for the SW environment (McCormick, 2012; McCormick et al., 2013; Prunet et al., 1989). Different organs are involved in osmoregulation during smoltification: intestine, gills, kidney and skin, but the main one responsible for water and ion fluxes is the intestine (Sundell and Sundh, 2012)to absorb ions to replace water lost by osmosis in SW. During smoltification, the drinking rate increases, in the intestine the ion and fluid transport increases and is further elevated after SW entry. In SW, the intestine absorbs ions to create an inwardly directed water flow which is accomplished by increased Na+,K+-ATPase (NKA. To maintain body homeostasis, water and ion flux occur in and out the gastrointestinal tract (GIT) (Larsen et al., 2014). Increasing water salinity is known to increase drinking rate in fish. Drinking behavior has been widely investigated in marine, euryhaline and freshwater teleost fish (Wood, 2019)and is a site of temporal and spatial variations in pH, HCO3−, and PCO2 (CO2 dynamics. However, only a minority of those studies have considered the interaction between drinking and feeding (Bucking et al., 2011; Eddy, 2007; Kristiansen and Rankin, 2001; Ruohonen et al., 1997; Usher et al., 1988; Wood and Bucking, 2010)4-6 h after abrupt transfer from freshwater to seawater. In unfed fish the rate steadily increased to 6.46 ml kg-1 h-1 from 4 days post-transfer, apparently in response to increasing plasma ion concentrations. Drinking rate was independent of gut food content but was significantly higher in feeding fish (7.94 ml kg-1 h-1. Furthermore, it is unclear whether the increased drinking rate during feeding is intended to aid chyme liquefaction in the stomach or to maintain the osmotic homeostasis in the body. We hypothesized that the ingestion of seawater would decrease the dry matter in the stomach, especially at high salinities. Therefore, this study assessed the impact of water salinity on the progression of water, ion and nutrient fluxes along the gastrointestinal tract of Atlantic salmon smolt (*Salmo salar*) fed a commercial-like diet.

Materials and methods

The experiment was performed with a mixed sex population of Atlantic salmon (*Salmo salar*) smolts (n = 480) ready for seawater transfer. The fish were randomly allocated to 16 tanks with 30 fish per tank. Four different salinities, 0, 10, 20 and 35 ppt, were used in quadruplicate tanks for each salinity level. Fish were fed a commercial-like diet twice a day until apparent satiation for 8 weeks and feed intake was monitored through collection of feed spill. The final sampling was carried out during two days (days 56-57). Yttrium oxide (Y_2O_3) was used as an inert marker to measure digestion kinetic and water/ion fluxes in the gastrointestinal tract (GIT). Chyme was collected quantitatively from four segments of the GIT: proximal, middle and distal intestine, and was analysed for pH, osmolality and dry matter (DM). Furthermore, the kinetics of water, ion, crude protein, and protease activity were measured along the GIT. Blood was collected from the caudal vein to measure blood pH and plasma osmolality. All parameter were tested for the effect of salinity by regression analysis as well as one-way ANOVA.

Results

Drinking rate increased with salinity from 0.78 to 4.11 ml/kg/h between 10 and 35 ppt salinity. Between 0 and 35 ppt, chyme dry matter decreased by 1.7% and 4.8% in the stomach and proximal intestine, respectively. Chyme pH was not affected by water salinity in the stomach, but it increased linearly ($p < 0.001$) with salinity in all intestinal segments. Chyme osmolality increased linearly ($p < 0.001$) with salinity in the stomach and it decreased in all intestinal segments. Water fluxes were similar among salinities in the stomach, but they increased nearly fivefold (6.2 versus 27.3 ml g^{-1} ingested DM) in the proximal intestine between 0 ppt and 35 ppt. An efflux of monovalent ions (Na^+ and K^+) increased linearly ($p < 0.001$) with salinity in the proximal intestine. An efflux of divalent ions (Ca^{2+} and Mg^{2+}) increased curvilinearly ($p < 0.001$) with salinity in the middle intestine. Plasma osmolality and ion levels increased with salinity. Crude protein digestibility and protease activity decreased significantly with water salinity in the intestine.

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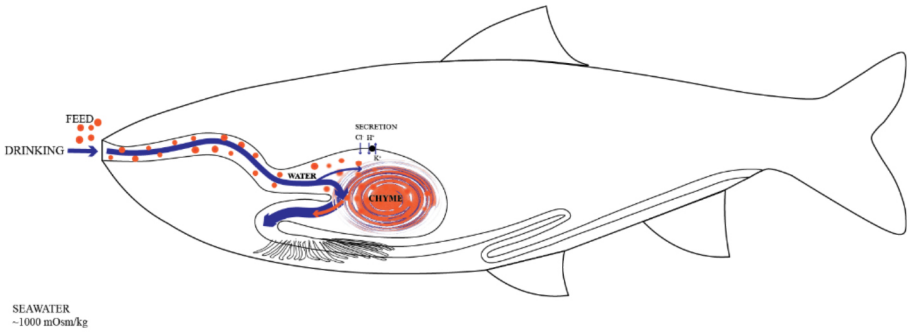

Figure 4. Water and chyme dynamic in the stomach of seawater acclimatized Atlantic salmon (*Salmo salar*).

Discussion

In contrast to our hypothesis, the DM of the chyme in the stomach was stable across all salinities, slightly decreasing (1.6%) between 0 and 35 ppt. Furthermore, the drinking rate calculated and the osmolality of the chyme in the stomach increased with salinity without affecting the chyme DM. Therefore, our results show that drinking rate increased with water salinity but the influx of water in the stomach chyme was minimal compared to the chyme in the proximal intestine at higher salinities. Moreover, water influx in the proximal intestine was followed by a re-absorption of water in the middle intestine. As a result, ingested water had an effect on chyme characteristics and digestion kinetics in the intestinal segments rather than the stomach, where the ingested food appears to stay longer (higher chyme DM). At higher salinities, the majority of the ingested water is bypassing the stomach and moving to the proximal intestine more rapidly compared to the chyme in the stomach (Figure 1). In conclusion, our results suggest that the exogenous water entering the GIT does not really mix with the chyme in the stomach. Therefore, we propose that ingested water by the fish is primarily used for osmotic processes rather than chyme liquefaction in the stomach.

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IMAGE-BASED FISH IDENTIFICATION – THE TOOL FOR COMMON FISH SAMPLING

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Introduction

Optimizing fish production in intensive aquaculture can lead to increased production with respect to fish welfare. The new technologies enable to development of the Precision Fish Farming (PFF) (Fore et al. 2017) concept, whose aim is to apply control-engineering principles to fish production, thereby improving the farmer's ability to monitor, control and document biological processes in fish farms. Individual fish identification is one of the tasks necessary for precision fish farming.

The widespread and popular methods of fish identification are tagging and marking (PIT, RFID or VIE tags), which are invasive methods. It was shown that image-based fish individual identification could substitute fish tagging (Bekkozhayeva 2021, Schraml 2020, Bekkozhayeva 2022 and Cisar 2021). All the studies demonstrated long-term stability and high accuracy of image-based identification but only under controlled laboratory conditions. In this study, we implement image-based individual fish identification for real-time usage during the standard fish sampling procedure. The software used the ordinary web camera and was tested on rainbow trout identification with an accuracy of 100%.

Materials and methods

Rainbow trout Oncorhynchus mykiss (Fig.1 (right image)), a commercially important fish species, was used in our study. The size of the fish was around 13.7 ± 9.1 cm 32.8 ± 6.0 g. This species has a skin dot pattern on the body (juvenile pattern), which was used for the individual identification procedure. Those dots are different in size and location, which is expected to provide high discriminative power for individual identification within a close group of fish (tank). Fish were bought at the fish farm in Rybářství Litomyšl s.r.o., Czech Republic. Finally, 1602 individuals were used in the experiment. Three pictures of the lateral view of the fish were collected for each individual at different angles and positions. An image of the right side of the fish was captured for all fish. The average resolution belonging to the one fish was 820 x 180 pixels.

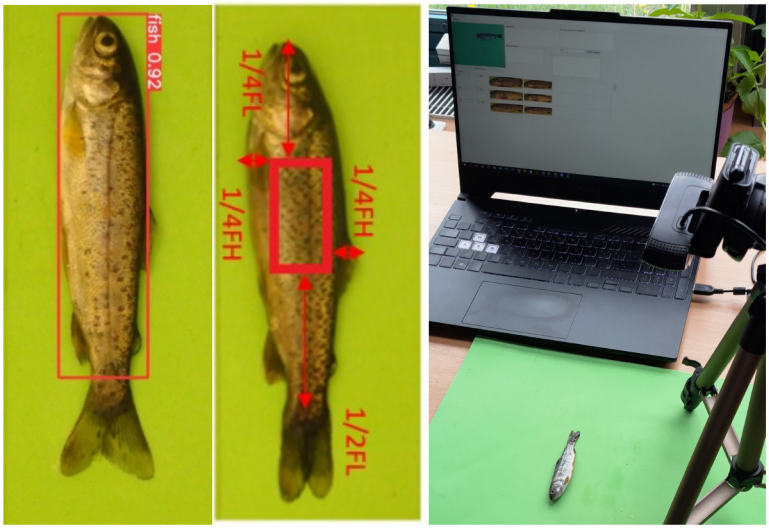

Figure 1. Left – fish detected by the CNN, centre -localization of ROI based on the fish height FH and fish width FL. right – fish sampling using the standard web camera, the software on the computer takes the video stream, detects and identifies the fish in real-time.

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The fish detection is based on the CNN with YOLOv8 architecture. YOLOv8 is one step bounding box detector. The CNN was trained using 187 manually labelled images of the fish randomly selected from 1602 fish. The original implementation from Ultralytics was used. The CNN was trained using Google colab environment. The trained CNN was then used in C# .net software developed by the authors for fish identification. The example of detected fish is in Fig. 1.

The CNN performs the detection of the fish bounding box without the tail. The region of interest (ROI) used for identification is extracted from the central part of the fish. The area is defined by the percentage of fish width and height. The ROI is parametrized using a Histogram of oriented gradients descriptor to calculate the fish feature vector. The identification is implemented as nearest neighbour classification, where the distance of the unknown fish is measured to all images in the database. The unknown fish is identified as the fish with the smallest distance. Two images of each fish were used as reference database and one image for each fish was used to test the identification accuracy.

Results

The accuracy of fish detection using the CNN approach was 100%. The accuracy of individual identification using nearest neighbour classification was 100%. The time of fish individual detection using CPU only (no GPU acceleration is needed) was 500ms. The time of fish identification of one fish within 1602 individuals was 800ms.

Conclusion

Individual fish identification is one of the keystones of the emerging concept of Precision Fish Farming. It was already proved by several studies that individual fish identification based on the fish skin pattern could substitute invasive fish tagging. In this paper, we developed the software for real-time fish individual identification using an ordinary web camera to provide support for fish sampling. The software was tested on 1602 individuals of juvenile rainbow trout and reached an accuracy of 100%. The software can be used by researchers or by fish farmers to speed up non-invasive fish sampling. The adaptation for other species is simple and needs just training CNN detectors for the new species.

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THE EFFECT OF BROODSTOCK DIETS AND FATTY ACID COMPOSITION ON THE REPRODUCTION OF BURBOT (L*ota lota*) IN RECIRULATING SYSTEMS

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Introduction

The burbot (*Lota lota*), a nocturnal and benthic gadoid freshwater fish commonly referred to as 'the freshwater cod', holds significant promise for the diversification of European inland aquaculture. Its delectable white flesh makes it an esteemed food fish among consumers.

Burbot exhibits a unique reproductive behavior, spawning only once a year when temperatures dip below 4°C. Current research efforts pertaining to burbot reproduction heavily rely on wild breeders captured shortly before the spawning season or those maintained in semi-natural conditions. Addressing the reproduction gap between hatchery and wild breeders is crucial to develop a well-informed strategy that ensures the consistent production of high-quality eggs and larvae within culture systems. This understanding is essential for the advancement of burbot aquaculture and the overall success of the industry.

For successful commercial production of burbot, it is imperative to establish a self-contained Recirculating Aquaculture System (RAS) for broodstock, as access to wild spawners is limited due to their scarcity and biosecurity concerns. An important step in the successful operation of a RAS for broodstock is to improve our knowledge of nutritional requirements, particularly fatty acid content, which has been poorly documented in burbot.

Material and methods

Burbot (*Lota lota*) used in this experiment were sourced from two commercial hatcheries (AquaLota, Belgium and LotAqua, Germany) during early fall 2020. The selected broodstock were originally reared from eggs obtained in January 2017 and were subsequently raised from larvae to sexually mature individuals under intensive RAS conditions. Fifty 5-year-old individually PIT tagged burbot broodstock with average bodyweight 1932,86 ± 756,51 gram were utilized in this study, comprising 30 females and 20 males. The broodstock were evenly distributed into two separate tanks (15 females and 10 males per tank), each with a capacity of 1800 liters.

These tanks were connected to an intensive RAS with photothermal manipulation to mimic natural conditions. At the time of the trial, the broodstock were second time spawners. The diets existed of a commercial compound dry feed specially formulated to enhance fish reproduction (Lansy breed Performance, Inve Aquaculture NV, diet A) and defrozen forage fish (diet B), commonly used in fish farms. Feeding quantity was corrected on dry matter basis at a level of 0,5% of biomass per tank per day.

At the start of the trial, fish were carefully examined to stage the oocytes. The oocyte staging process was performed by adapting the method as described for *Perca fluviatilis* (Żarski et al., 2017) which categorizes oocyte stages into stages I to VI. Females in stage VI were checked daily for ovulation. Fish, which were in stage I – IV, were injected with sGnRHa. The fish were subjected to strip spawning, and milt was collected from three randomly selected males to fertilize the oocytes of each female at a ratio of 1,5 mL of milt per 100 gram of eggs. To provide a uniform fertilizing ability for all egg batches, 0,5 ml of milt was collected from each of three males. The incubation process took place in a 96-well plate per female, each well containing one fertilized oocyte. The incubation temperature was maintained at 3,0± 0.2°C throughout the incubation period.

Various parameters were recorded during the study: fertilization rate, survival rates on 3, 6, and 20 days post-fertilization to assess developmental potential and hatching rate. Broodstock-related parameters such as body weight and length, condition factor, relative and absolute fecundity were also recorded. A sample of oocytes from each female was obtained and kept at -80°C to perform FAME analysis.

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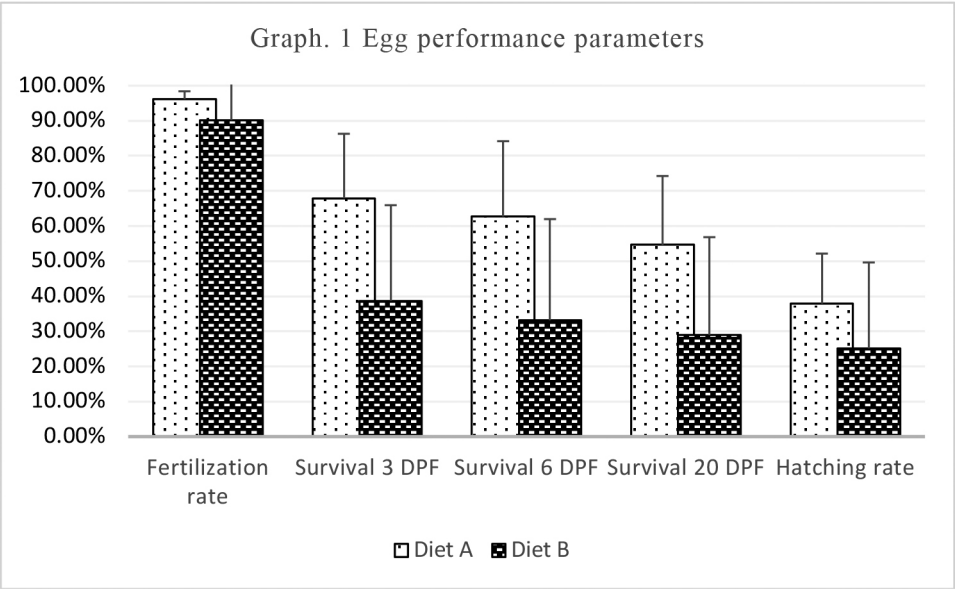

Results

No significant effect of diets was found on the fertilization rate, survival at 3, 6 and 20 days post fertilization and hatching rate. In most cases, though not always, the fatty acid content of the feed is reflected in the oocytes. This is true, for example, for ARA and DHA, but not for LA. There were no differences in terms of relative fecundity or body condition factor. One interesting finding was that diet B led to a spawning period of only 4 days, whereas diet A required a total of 12 days. This indicates that the diet given to the broodstock influenced the speed and synchronization of the final oocyte maturation process.

Conclusion

This study was a first preliminary investigation on the impact of two diverse broodstock feeds on the reproductive performance of burbot. While interpreting these findings requires prudence, it is pertinent to note that both dry feed and forage fish-fed broodstock exhibited similar spawning qualities. However, the effect of the fatty acid composition of broodstock diet on larval performance has not yet been studied in burbot but needs to be done to distinguish the suitability of the feed. High fertilization rate of the oocytes and good embryonic development doesn't necessarily imply good larval performance and qualitative fingerlings later on.

Further research should be performed to find a totally adequate feed for reproduction in burbot broodstock.

BUILDING ARMOUR FROM WATER:BICARBONATE UPTAKE FOR CALCFICATION DIRECTLY POST MOULT IN THE WHITELEG SHRIMP *Penaeus vannamei*

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Introduction

Whiteleg Shrimp production is one of the most valuable and fastest growing sectors in aquaculture producing 5.5 million tonnes in 2019 (FishStatJ FAO, 2021). This industry is expanding across the world into indoor closed-system production where culture conditions can be carefully controlled. Moulting is a key process for growth in crustacea, with their exoskeletons typically made up of chitin, calcium carbonate and other hardening elements. Immediately post moult shrimp cease feeding, and their soft shell leaves them vulnerable to predation, cannibalism and disease. To complete hardening sufficient quantities of these hardenning elements are required in the culture environment, with their depletion being sub-optimal for exoskeleton hardening efficiency, potentially exacerbating vulnerability in closed aquaculture systems. Our hypotheses are two-fold, firstly that alkalinity in the production environment is a limiting factor for exoskeleton hardening rate immediately post-moult, and secondly, light cycle is a key factor influencing shrimp moult timing.

Materials and Methods

We exposed individual animals to one of three light regimes (12:12, 4:20 and 2:22 dark/light), whilst continually recording their behaviour, to identfy moult time in relation to the light/dark conditions. Additionally, we measured post-moult calcification, measruing bicarbonate uptake rate in animals immediately after they had moulted,, derived from a one-point titration for alkalinity as a proxy for bicarbonate concentration.

Results

Here we present data on moult timing in relation to light dark cycle, in additon to the uptake rate of bicarbonate and other calcification-related variables at 2, 22 and 24 hours post-moult from shrimp placed in alkalinities ranging from 100 to 2,000 µmol at 20 psu. From this we can report the maximum uptake rate of bicarbonate and the alkalininty at which uptake is most efficient. The data also enable us to identify cumulative uptake of bicarbonate for the hardening process. This provides a bicarbonate budget for Whiteleg Shrimp moulting, as well as evidence for bicarbonate concentration thresholds for successful post moult exoskeleton hardening, vital for improving the health and welfare of indoor intensive Whiteleg Shrimp farming.

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TURBOT LARVAE PERFORMANCE IS SENSITIVE TO DIETARY PROTEIN AND LIPID SOURCES

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Introduction

Turbot (Scophthalmus maximus) is a flatfish species farmed in Southern Europe. Production of high-quality turbot juveniles is paramount to have consistent and profitable aquaculture production. However, there are still some bottlenecks especially at early development stages that could improve hatchery phase cost-effectiveness and improve quality of juveniles: variable survival and growth rates, high dependence on live feeds and high malformations incidence in some batches. In the last decade turbot aquaculture has experienced very little R&D efforts while other flatfish species such as Senegalese sole experienced significant developments, including microdiet quality (Pinto et al., 2018). The knowledge transfer from such R&D developments is perceived as a good strategy to further develop early feeding and nutrition of turbot larvae. Therefore, this work presents two trials which were conducted to evaluate the effect of several microdiets with varying protein and lipid sources on the growth performance and survival of turbot early life stages, in order to create a tailored microdiet.

Material and methods

The first trial aimed at testing different dietary protein sources to investigate an eventual preference in turbot larvae. As such, three microdiets were produced by cold-extrusion and contained increased levels of a specific ingredient: Fish, Squid and Krill meals. Turbot larvae were reared according to standard procedures in triplicate tanks from 23 to 50 DAH (Days After Hatch). Microdiets were supplied using automatic feeders providing 5 meals per day with 1 hours stopping time in between meals, resulting in a 15-hour feeding period. The second trial, aimed at testing microdiets with different lipid sources on the growth performance of turbot larvae. Three diets were also produced by cold extrusion and contained a combination of specific ingredients: Diet 1 (fish oil and algal oil), Diet 2 (fish oil and algal meal), Diet 3 (algal meal). Turbot larvae were reared from 30 to 66 DAH, under the similar conditions as the first trial. Performance parameters evaluated at the end of both trials included wet weight, survival, relative growth rate (RGR) and feed conversion ratio (FCR).

Results

At the end of the first experiment, turbot fed on Squid meal -rich diet presented significantly lower wet weight when compared to turbot fed on the fish meal and Krill meal -rich diets (Figure 1). Furthermore, the Squid group also showed the lowest survival (53.8%) when compared to the other groups (>67.7%). No significant differences were found in RGR (11.9 to 12.3% day-1) and FCR (0.9-1.3). At the end of the second experiment, turbot fed on diet 3 showed significantly lower wet weight than turbot fed on diet 1 (Figure 1). No significant differences were found in RGR (9.0 to 9.5% day-1) and FCR (2.7-3.0).

Discussion and Conclusion

Overall, results clearly show that turbot has a preference for microdiets containing increased levels of Krill or fish meal, whilst squid meal rich diets can have an unfavourable effect on performance. The concept of testing the effect of different protein sources on growth performance was also object of study in other flatfish species including Senegalese sole and Atlantic halibut in this same project. Whilst sole did not show a preference between krill meal, squid meal, fish meal and vegetable protein rich diets (RGR varying from 6.3 to 8.6 % per day and survival from 67 to 83%, unpublished data), Atlantic halibut fed on the Squid meal rich diet showed significantly lower wet weight at the end of the trial (RGR 3.02-3.77% and survival >77%, unpublished data). Moreover, even the optimal diet for sole was giving sub-optimal results for turbot larvae.

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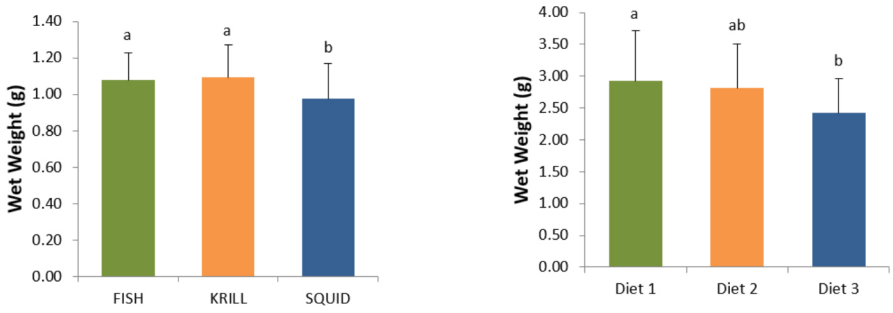

Figure 1. Wet weight of turbot from first trial (left) fed diets rich in different protein sources – Fish (fish meal), Krill (krill meal) and Squid (squid meal) – and Wet weight of turbot from the second trial (right) - Diet 1 (fish oil and algal oil), Diet 2 (fish oil and algal meal), Diet 3 (algal meal). Results are expressed as means ± standard deviation. Different lowercase letters indicate significant differences between the dietary treatments ($P<0.05$).

As for lipid sources, results of the present paper show that a mix of fish oil and algal oil relate to improved growth performance of turbot larvae, hence being preferable to the inclusion of algal meal alone in the diets. All the reported results ultimately show that turbot have improved growth performance when fed microdiets with specific ingredients and customized formulations. Furthermore, different flatfish have different feeding behaviours and distinct dietary preferences which supports the concept of tailoring microdiets to maximize fish performance and help to produce better quality juveniles.

Acknowledgements

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SCREENING THE EFFECTS OF β-GLUCANS AND SULFATED POLYSACCHARIDES FROM ALGAE IN FISH GUT HOMEOSTASIS

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Introduction

Algae are aquatic organisms that share some beneficial properties with those of land-based plants. They hold an interesting number of bioactive compounds known to have health-beneficial effects. When used in aquaculture feeds, these compounds will reach the gut mucosa and elicit a response that might support gut homeostasis and stability required for good fish performance. β-glucans and sulfated polysaccharides are carbohydrates that, due to their chemical structure, can elicit a defensive response coupled with a stronger regulation of epithelial permeability. These features are essential to support the response to insults in fish under culture conditions. In recent years, there has been a strong effort in obtaining these compounds from algae to access natural sustainable functional ingredients for fish health management. The aim of this work was to screen the functional effects of algae-derived β-glucans and sulfated polysaccharides as promoters of the intestinal mucosa defensive system and homeostasis, based on an *Ex vivo* model.

Material and Methods

β-glucans from *Euglena gracilis* and a mix of sulfated polysaccharides from macroalgae were tested in *Sparus aurata* intestinal explants. Each ingredient was tested in two dosages (low and high), and the responses were compared with the ones from β-glucans from other sources (e.g. yeast) and to insult-like compounds (e.g. LPS). Intestinal explants were processed following in-house standardized protocols, and after a 5-hour incubation with the ingredients, samples were collected and processed for response analysis. Here, the assessment was based on the molecular response triggered in the intestines following a panel that included genes related to immune (e.g. *cox2*, *IL8*) and antioxidant response (e,g, *cat*, *gpx*), but also related to epithelium integrity (e.g. *tjp*, *cldn12, ocl*). Relative gene expression was assessed and provided the basis for ranking the potential of algae-derived ingredients, comparing with responses after incubation with ingredients from other sources.

Results and Discussion

Algae-derived β-glucans promoted a stimulation of the intestinal mucosa comparable with others, and this was mostly highlighted through a differential expression of immune-related genes, and to less extent to epithelial integrity-related genes. A dose-response was not clear for these compounds, whereas, for the sulfated polysaccharides, a higher dosage prompted a stronger response. The latter induced an upregulation in genes related to epithelial integrity and permeability regulation (i.e. *tjp*, *cldn12*) and also in the acute phase response-related gene (i.e. *cox2*). Overall, these algae-derived ingredients have potential as intestinal mucosal health boosters, and further studies will be performed to further understand the beneficial outcomes of the triggered responses. This study highlighted the potential of these algae-derived ingredients as functional ingredients with specific applications in aquafeeds.

Acknowledgement

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MEETING THE CHALLENGES OF IMPROVED TRACEABILITY OF STURGEON PRODUCTS IN TRADE

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Introduction

This joint presentation aims at an intensified dialogue between farmers and conservationists about the shortcomings of the current labelling and enforcement approach for sturgeon products and best strategies to facilitate and improve the traceability of legal caviar.

As a result of the legal protection of wild sturgeons in trade, a massive increased production of sturgeon products from aquaculture was observed over the past 20 years, providing the only legal source for sturgeon products in trade. Nevertheless, wild sturgeons are still poached, and their meat and caviar enter the market illegally and mostly unnoticed. To safeguard the last surviving sturgeon stocks as well as to support the legally operating industry, effective and reliable traceability systems throughout the trade chain are essential. However, current CITES regulations are not sufficient or adequately enforced to detect and stop illegal trade.

An overview of the current gaps in the traceability chain (no marking of individuals, no documentation of transported fish, no labelling on domestic markets, labelling for international trade easy to falsify, no authenticity controls) will be presented.

To support enforcement activities, several experimental approaches are currently available and in continuous development, which include different methods of genetic analysis for the identification of species and interspecific hybrids, analysis of fatty acids for the distinction of animals from aquaculture or from natural environment and isotopic analysis for the geographical traceability of the samples. All these methods have considerable application potential but also have limits and margins of uncertainty which will be discussed in a very general way.

The adoption of strategies that allow efficient traceability and transparency of legal caviar would help limit illicit activities, by limiting the possibilities of using aquaculture to greenwash poaching products. In this perspective, we want to draw attention to two possible strategies that could be adopted to maximize traceability efficiency, facilitate commercial controls and reduce the associated costs.

Genetic references sample storage: As a possible way to minimize any kind of technical ambiguity we would like to explore the possibility of establishing tissue banks for temporary storage of tissue samples from each animal used for caviar production. These banks should be centralized at the national level and supervised by a public authority. Each tissue can be easily collected during caviar processing and would be identified by a code also reported on the boxes of caviar along any repackaging. This would allow the genetic analysis upon request, only of those tissues with the code corresponding to the caviar to be checked. The analyzes would be very simple as it would be a matter of confirming the genetic identity. The approach, however, could be subject to various fraudulent circumvention possibilities which should be the subject of collective discussion to identify the most effective control solutions.

Blockchain database approach through genetic characterization: The second approach is also based upon the genetic analysis of individual profiles of fish based upon SNPs panels, allowing individual assignment of fish to a lineage of broodstock maintained at a farm. The difference to the previous approach is the fact that the test is performed upon identification of sex of an individual, when the fish is marked with an individual code (PIT) that is stored in a blockchain database and that is used to document movements of the fish between farms, providing a record of the performance/location of the fish and is passed on to the products that derive from the individual. The blockchain database is stored centrally, can be used on three different levels of detail: by the farmers to follow up on the stock development, as a documentation for CITES purposes, as well as and for consumer information upon rearing conditions.

The applicability of the methods, along with improved approaches for labelling as a prerequisite for increased consumer safety and enforcement agency information are to be discussed and the advantages for the caviar trade are to be identified in a discussion round, aiming at the identification of preferences and concerns by the industry.

A NEW SET OF FEED ADDITIVES TO PROMOTE FISH FEED INTAKE AND WELFARE IN AQUACULTURE: A COMPARATIVE STUDY ON ZEBRAFISH (*Danio rerio*) LARVAL AND JUVENILE STAGE

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Introduction

One of the main goals of modern aquaculture is the formulation of sustainable aquafeeds, mainly based on alternative protein sources respect to the conventional marine-derived ones. The first widely used alternative was represented by vegetable ingredients which, however, have led to negative side-effects on fish growth and welfare, especially considering many carnivorous species. However, the improvement of processing methods for vegetable meals production as well as the introduction of new and more sustainable alternatives (e.g., processed animal proteins, insect meal, and single-cell proteins derived from cultures of different microbial sources), have partially solved these drawbacks. Nevertheless, the use of alternative ingredients often results in lower diet palatability that can consequently impair fish zootechnical performance, water quality, and the farm's economy. In fact, not ingested feed remains in wastewater outflows and thus can contribute to the eutrophication of aquatic ecosystems, affecting the company's economy as well. To improve aquafeed palatability, marine-derived feed attractants are regularly included in aquafeeds. Particularly, meals derived from shrimp, anchovy, and squid are well-recognized feed attractants, but their use on large-scale is still limited by different factors. In fact, they still rely on fisheries activity that poses sustainability concerns and fluctuations in the product availability. Furthermore, their attractive effect is highly variable depending on raw material composition, freshness, and processing methods. The current alternative solution is represented by a limited number of molecules, such as a mixture of free amino acids, nucleosides, and nucleotides, or substances such as betaine and taurine, that can be used as feed attractants. Particularly, amino acids have been widely studied for their attractive properties, although the limitations and disadvantages of their use as feed attractants are well-known. Therefore, a novel, standardized, and sustainable alternative to marine-derived feed attractants is represented by synthetic ones, obtained through standardized processes. Existing literature indicates that few specific studies exist on this topic, which instead represents a great opportunity for the aquaculture sector.

The aim of this study was to identify new different synthetic feed additives, widely used in other industrial sectors, testing them on zebrafish (*Danio rerio*; ZF) to assess their potential role as feed attractants. ZF represents a well-validated model for aquaculture nutritional studies and allows to investigate possible dietary effects on all its life cycle stages, in a relatively short time.

Materials and Methods

A behavioural screening test was conducted on ZF larvae to select three positive and a negative commercial feed additives (To Be Pharma S.r.l., Stant'Egidio alla Vibrata, Teramo (TE), Italy) within a *plethora* of 40 ones. The selected feed attractants (positive: B, C, D; negative: E) were then included in a commercial diet for ZF (Zebrafeed, Sparos ltd, Portugal) to be tested from the larval to the juvenile stage. Particularly, the commercially available standard diet for ZF was used as Control, while five experimental diets were obtained by adding the previously selected feed additives to the Control diet (diets B, C, D, and E). All feed additives were added at 1% (w/w) concentration using a micropipette, coating the pellet by mixing homogeneously. ZF larvae obtained from broodstock AB strain were divided to set up seven experimental groups: Control, group A (Control diet + feed additive basic solvent), group B (Control diet + feed additive B), group C (Control diet + feed additive C), group D (Control diet + feed additive D), group E (Control diet + feed additive E), and group F. This last group was fed the three attractive diets (B, C, and D), each administered singularly in a weekly rotation scheme since it has been demonstrated that teleost, in response to a repeated stimulation, develop olfactory receptors habituation to the same stimulus. ZF were daily fed the experimental diets (3% of the body weight) from 5 to 60 days post fertilization (dpf). The required number of fish per tank were sampled at 21 dpf to evaluate dietary effect of feed additives administration during larval development. The remaining fish from each tank were gently transferred to bigger tanks, fed on the same diets and, at 60 dpf, were euthanized to evaluate the long-term dietary effect of feed additive administration on juvenile stage. For both

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larvae and juveniles the following analysis were performed: (i) growth performances calculating the specific growth rate (SGR%); (ii) histological analyses to evaluate eventual alterations or the occurrence of inflammatory conditions in both the intestinal tract and the hepatic parenchyma; (iii) molecular analyses to evaluate the relative expression of genes involved in fish growth (*igf1*, *mstnb*), appetite regulation (*ghrl*, *npy*, *lepa*), stress response (*gr*), immune response (*il1b*, *il10*, *tnfa*), and brain reward system (*drd1b*, *drd2a*, *drd3*).

Results and Discussion

Fish welfare was not affected by all the feed additives tested, while considering SGR%, significant differences ($p < 0.05$) were detected among groups, supporting the fact that the positive feed additives here tested stimulated fish feed intake and thus growth. All these results are well supported by both histological and molecular analysis on fish welfare (immune-response, stress response, gut/liver anatomical architecture) and appetite regulation and showed some interesting analogies or differences between the larval and juvenile stages which will be presented during the research discussion. As regards the analysis on gene expression involved in reward analysed in brain, results evidenced a different activity among groups of the dopamine receptors, the main neurotransmitter involved in the pleasure sensation, suggesting a pivotal role of the tested feed additives in their activation/inactivation. In conclusion, the positive feed additives administration promoted higher feed intake, acting on the fish brain reward system without impairing welfare and represents a very promising result for the aquaculture industry since a faster and complete feed intake by the fish has both ecological and economic benefits for the sector.

DAILY RHYTHMS OF THERMAL PREFERENCE IN THE BLACK BULLHEAD CATFISH *Ameiurus melas*

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Introduction

The ability to coordinate internal biological processes and behavioural response with cyclical environmental variations is crucial for health and survival and has been widely demonstrated from the simple form of bacteria to more complex organization of vertebrate (Bell-Pedersen et al., 2005). As a result, the evolution has selected the existence of endogenous circadian clocks which are an important adaptation to life on a rhythmic planet. Daily cycles of light and temperature are powerful environmental cues and they strongly influence ectotherms' behaviour, physiology, and habitat distribution (Chapman et al., 2020). Moreover, the light-dark cycles and the daily variations in temperature are directly linked: highest temperature during the day (thermophase) and lowest temperatures during night (cryophase).

Fish, being ectothermic animals, display a thermal preference because water temperature affect and modify their performance by influencing their growth and survival rate.

This study sought to verify that the presence of daily rhythms of thermal preference using an automated system that maintain a horizontal temperature gradient. In fact, in nature, fish experience gradients in water temperature on a variety of spatial scales, e.g., in relation to depth and horizontal position. The species of interest of this study was the black bullhead catfish *Ameiurus melas* which is nocturnal and may have commercial interest.

Materials and methods

According to previous studies (Rey et al., 2015a; Vera et al., 2023), we built 3 multi-chamber tanks in which fish are freely to move across the chambers. An automated system based on Arduino microcontroller (Elegoo Mega R3) recorded the temperature in each compartment and regulated the activation of water heaters in order to maintain a horizontal thermal gradient (from 18 ºC to 26 ºC).

The fish (n=8/system) were subjected to 14:10 h light-dark cycle (LD) which was subsequently reversed to a 10:14 h dark-light cycle (DL), concluding with the constant dark condition (DD) to ascertain whether rhythms are controlled by an endogenous circadian clock (Herrero et al., 2003). The experiment lasted a total of 27 days.

Animals were randomly fed once a day during the night phase and the food was evenly distributed in all chambers. During the DD period the fish were fasted. The light-on time was designated Zeitgeber Time 0 h (ZT 0 h). All experimental phases lasted 10 days except the DD phase which lasted 7 days. Daily temperature preference was established by video analysis of animal behaviour using Ethovision XT software (Noldus, the Netherlands). The videos were recorded by 3 different camera modules connected each to Raspberry Pi (Raspberry Pi 4 model B), remotely controlled for avoiding disturbance to fish during the experiment. Behavioural observation was videorecorded by a custom written Python script based on picamera package (ver 1.13) created for this purpose (RTS-Fish).

Results

We are still analysing the videos and afterwards we plan to analyse the rhythmicity using the El Temps chronobiology software (v.1.291, Prof. Díez Noguera, University of Barcelona, Spain). In agreement with previous works (Vera et al., 2023), we expect to find a daily rhythmicity in thermal preference. The thermal preference could be related to daily changes of environmental temperature that fish experience in the wild, higher temperatures during the day and lower temperatures at night. However, being a nocturnal species, we would expected a reversal scenario in which *A. melas* may select higher temperatures during the night when it is most active and cooler temperatures during the day when it is in its resting phase.

The results may suggest an important cue to design husbandry protocols where fish are kept in captivity (e.g., fish farms).

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FEEDING THE FUTURE: THE POTENTIAL OF POLYCHAETE MEAL FOR EUROPEAN SEABASS FUNCTIONAL DIETS

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Introduction

Common practices in aquaculture production often include procedures as fish manipulation for triages and transportation, which can act as stressors. Stressed fish are more susceptible to infection, potentially leading to massive losses for aquaculture produces. Hence, stress mitigation strategies, including the development of new functional feeds are on demand. Due to its high protein content and biochemical composition, polychaeta meal (PM) is emerging as a viable alternative to fishmeal and potential functional ingredient to modulate fish immunological system and/or stress response.

The overall aim of this study was to evaluate the potential of PM as a functional ingredient in European seabass diets by assessing its impact on intestinal health, metabolic and immune status, and hepatic oxidative response towards a stress challenge.

Materials and Methods

A growth trial with European sea bass (*Dicentrarchus labrax*) juveniles (14.5 g) was performed using four isoenergetic (22% dry matter, DM), isoproteic (51% DM), and isolipidic (17% DM) diets. A fish meal-based diet (FM, control) was compared with three experimental diets including 2.5% (PM2.5), 5% (PM5) and 10% (PM10) of spray dried PM (*Alitta virens*), to replace 10%, 20% and 40% of FM, respectively. All experimental diets were extruded (Nofima, Norway). Homogeneous groups of fish (initial body weight: 14.5g) were distributed by twelve 160 L fiberglass tanks and fed the experimental diets to satiety, three times a day (9h, 12h, and 16h), for 93 days. Triplicate groups were established per diet. The fish were subjected to a 12-h light/12-h dark photoperiod regime and kept in a recirculating saltwater system (salinity 35‰, 22 ± 1 ºC). Upon trial conclusion, fish were weighted and measured and a small part of the posterior intestine was fixed in phosphate-buffered 4% formalin, for histologic evaluation. The remaining fish underwent an acute stress challenge: 1-minute air exposure followed by 5-minute overcrowding. After 1 hour of recovery, plasma and liver samples were collected. Plasma metabolite levels, immune parameters and liver oxidative status of stressed fish were compared to those of non-stressed fish (n = 18).

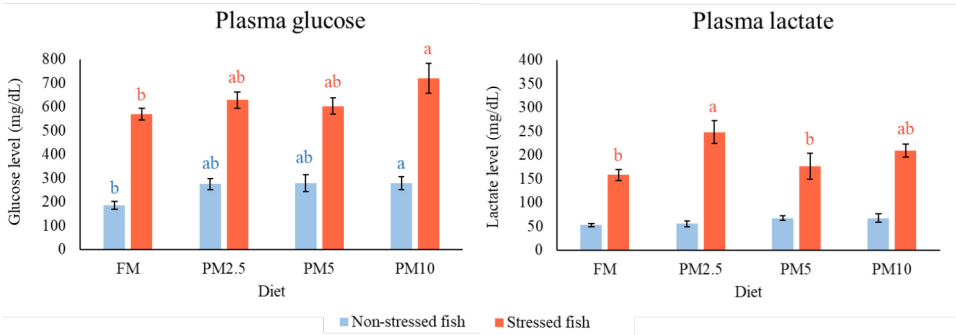

Figure 1. Plasma glucose and lactate of stressed and non-stressed European seabass juveniles fed the experimental diets.

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Results

All diets equally promoted growth and ensured high feed efficiency. Posterior intestine health status was assessed by histologic analysis. No statistically significant differences were found among dietary groups concerning submucosa lymphocytes and granulocytes presence and lamina propria granulocytes. However, lamina propria lymphocytes presence was reduced in fish fed PM2.5 and PM10. PM impacted plasmatic metabolic status. Plasma glucose levels were significantly higher in fish fed PM10 compared to fish fed FM, independently of stress (Figure 1). Both the diet and stress condition affected the hepatic redox status. In non-stressed fish the basal GST activity was significantly higher in fish fed PM diets compared to those fed the CTRL, but no differences were observed in stresses fish. Likewise, the glutathione content was highest in fish fed PM2.5 and PM5 before stress.

Upon stress, a significant increase was observed in all biomarkers analyzed, regardless of the dietary treatment. Cortisol, lysozyme and peroxidase levels were not affected by diet. Although basal plasma lactate levels were similar between diets, levels after stress were significantly higher in fish fed PM2.5 compared to fish fed FM (**Figure 1**). On the other hand, no differences were observed in the hepatic redox status among dietary treatments.

Discussion and Conclusion

Our results suggest that dietary replacement of fishmeal with PM can modulate European seabass intestinal health and acute stress response, although dietary impact appears to be limited to metabolic (glucose and lactate alterations. In the present work, PM10 fed fish exhibited higher glucose levels, independently of stress. Plasma glucose is commonly used as a sensitive indicator of stress in fish. However, the observed increase in this parameter, particularly in fish fed PM10 diets, may be attributed to an increase in gluconeogenesis to provide energy to meet the higher metabolic demands of these fish, independently of stress condition. Cortisol and lactate did not follow glucose increasing trend, supporting the idea that increased glucose in fish-fed PM may be linked to metabolic alterations not related to stress. Furthermore, the metabolic alterations triggered by PM in the hepatic redox status before experiencing stress, including increased activity of glutathione and GST, remain to be explored.

Acknowledgments

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DISSOLVED OXYGEN CONSUMPTION AND REOXYGENATION: FACTORS THAT AFFECT THE SURVIVAL OF THE SHRIMP *Penaeus vannamei* IN BIOFLOCS SYSTEMS

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Introduction

Implementing biofloc technology (BFT) has allowed an increase in shrimp culture densities, but at the cost of raising the requirements for dissolved oxygen (DO) in the water of production units, becoming one of the biggest risk factors in aquaculture production. Because BFT systems rely on technologies such as blowers and hydraulic pumps to generate and replenish oxygen in the culture water, the risk of energy outbreaks, generator, or pump failures could lead to a potential drop in oxygen levels, with resulting mortalities in the cultured organisms. The high oxygen consumption in BFT systems depends on several factors such as the density of cultured organisms like shrimp, salinity, and water temperature. Temperature, for example, acts directly on the solubility of gases in water and regulates the metabolism of the animal by increasing or decreasing its consumption over time. Salinity also directly affects the oxygen solubility in water, when salinity increases the dispersion decreases, and when it decreases the portion of DO in water increases. Due to several factors, the oxygen concentration in the water can be depleted, which requires emergency strategies to overpass a lack of aeration, affecting shrimp survival. For this reason, we ran several experiments to test the factors affecting oxygen consumption in biofloc systems and the effect of different re-oxygenation strategies on *P. vannamei* survival.

Material and Methods

The experiments were performed in 20L plastic tanks with individual aeration supplies, placed in thermostatic baths for temperature maintenance throughout the experiment, using total suspended solids (TSS) at 500mg/L, all treatments with three replicates. Dissolved oxygen levels were initially measured in all tanks, and then the air supply was cut off and again measured at 20-min intervals until levels reached hypoxia. Then the survival was evaluated by counting the number of dead animals after exposure to stresses. First, we tested high (30º and 36° C) and low (16º and 22°C) temperatures at different densities (150 and 600 cam/m^3), using individuals with an average weight of 15g, to determine the consumption time and survival. Then, we determined the DO consumption time in a *P. vannamei* culture and survival using different salinities (5 and 30‰) and different stocking densities (150 and 600 cam/m^3). Finally, we determined the effect of different re-oxygenation methods (hydrogen peroxide and manual aeration) on the survival of *P. vannamei* shrimp in BFT systems. The re-oxygenation was performed in 4 different ways: Abrupt (RA - total reinsertion of DO up to 5 mg/L in less than 20 min), Gradual (RG - DO rising 1 mg/L every 20 min), with hydrogen peroxide (RPH - 7ml/m^3) and with hydrogen peroxide powder (RPP) in the amounts indicated by the manufacturer (SwBio). Survival was observed for 3 days after reoxygenation

Results

The experimental units at a density of 600 reached critical levels faster than those at 150. There was also an increase in the time required for the system to reach the hypoxia scenario considering the temperature of the experimental units. The temperature directly affected survival, which reduced the animal's metabolism, directly influencing energy output and productivity.

In the salinity trials, the stocking density was the most significant factor in DO consumption. The densities of 600 cam/m^3 took about 1h40min at 30‰ and 2h at 5‰ to reach hypoxia, consuming about three times faster the DO than the treatments with a density of 150 cam/m^3 that took 4h20min at 30‰ and 5‰. At low salinity, outside its isosmotic point, osmoregulatory stress combined with hypoxia leads *P. vannamei* to a high mortality rate, at a salinity of 5‰ and density of 600 cam/m^3, survival was only 29.1%, thus demonstrating that this combination of stress factors is highly detrimental to production.

(Continued on next page)

		Temperature			
	(shrimps. m^{-3})	36°C	30°C	22°C	16°C
Oxygen consumption	**150**	50	120	300	910
	600	20	46	120	230
Survival	**150**	0%	100%	89%	88,8%
	600	0%	39%	61%	75%

Table 1: Recommended reaction time limit (minutes) to avoid the DO in the culture reaching values < 2.0 mg/L and survival after exposure to hypoxia until reaching DO values < 2.0 mg/L at different temperatures and under different culture conditions.

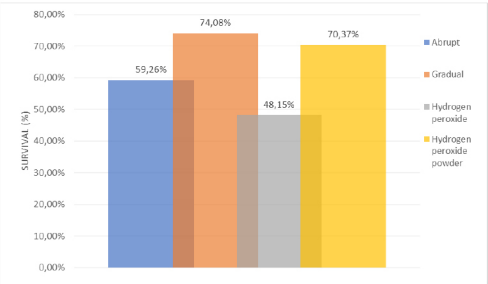

Figure 1: Survival of *P. vannamei* after exposure to hypoxia and re-oxygenation with different re-oxygenation methods after 72 hours of recovery.

In the re-oxygenation trials, survival was largely affected by critical oxygen depletion, showing significant differences between treatments. The animals can maintain themselves in the basal state to prevent mortality as long as there is no other problem associated with DO deprivation. The lack of oxygen combined with re-oxygenation is a stressful factor for the animals and may affect their ability to maintain homeostasis and metabolism, directly affecting survival and may have caused mortality when these organisms were exposed to combined hypoxia/re-oxygenation stress.

Conclusion

Based on our results, we recommend determining a strict protocol for dissolved oxygen management in shrimp biofloc culture systems. Monitoring and maintaining adequate TSS concentrations, animal culture density, and water temperature is also critical. We also emphasize the importance of not letting dissolved oxygen levels go under the value of 2 mg/L. Our data and observations from both experiments showed that the density of the cultured animals and the biofloc in the BFT system can directly influence the consumption of dissolved oxygen in the water, and consequently affect the production performance of the shrimp.

All references are available from the corresponding author.

MIXTURE OF PROCESSED PROTEIN FROM BLACK SOLDIER FLY AND YELLOW MEALWORM LARVAE AS PROTEIN SOURCE IN DIETS FOR EUROPEAN SEABASS *Dicentrarchus labrax*

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Introduction

The rapid growth of aquaculture requires an increase in aquafeed production, and consequently in raw materials availability. Currently, a change in dietary ingredient basis is already taking place and future aquafeed formulations will rely on novel ingredients, including insects (Glencross et al., 2023), allowing a reduced and strategic use of fishmeal (FM). Over the last years, research on the impact of the dietary inclusion of black soldier fly (BSF, *Hermetia illucens*) and yellow mealworm (YM, *Tenebrio molitor*) larvae meals on the growth performance and feed efficiency of European seabass (*Dicentrarchus labrax*) has increased significantly, but little is known about the inclusion of a mixture of processed BSF and YM protein in diets for this fish species that include low FM levels (Maulu et al., 2022). Thus, this study aimed to evaluate the potential of a processed mixture of BSF and YM protein to partially replace FM in practical diets for European seabass, focusing on the effects on fish growth and feed efficiency.

Materials and Methods

A practical diet (15% FM, 9% processed animal protein sources, and 55% vegetable protein sources) with 46% protein and 19% lipids on a dry matter (DM) basis was formulated and used as a control diet (CTRL). Three other isoproteic, isolipidic, and isoenergetic (23 kJ/g DM) diets were formulated to include 0.5%, 4.3%, and 8.4% of an insect-based protein mixture (50% processed protein from BSF larvae and 50% processed protein from YM larvae), replacing 3% (IM3), 25% (IM25) and 50% (IM50) of FM, respectively. Homogeneous groups of 75 fish (12.9 ± 1.3 g) were distributed among 12 fiberglass tanks of 250 L within a saltwater recirculation system (22 °C, 35‰ salinity, 16 L min^{-1} flow rate, 12 h light/12 h dark photoperiod), and each diet was randomly assigned to triplicate groups of fish, which were fed until apparent satiety three times daily, for 75 days. By the end of the trial, all fish were individually weighed (g) and measured (total length, cm), and the amount of feed (g) eaten per group was registered. Then, growth performance and feed efficiency parameters, as well as body somatic indexes, were calculated.

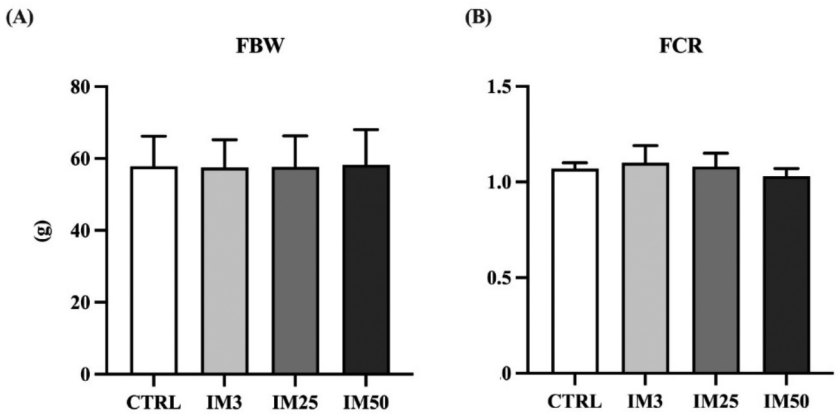

Figure 1 – (A) Final body weight (FBW) and **(B)** feed conversion ratio (FCR) of European seabass juveniles after 75 days of feeding with the experimental diets.

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Results

After 75 days of feeding, no statistically significant differences were observed among the dietary groups concerning the final body weight (57.5-58.2 g – **Figure 1A**), weight gain (44.6-45.3 g), daily growth index (2.0 g day^{-1}), feed conversion ratio (1.0-1.1 – **Figure 1B**), and voluntary feed intake (1.8-1.9% day^{-1}). Fish hepatosomatic and viscerosomatic indexes also remained identical among the dietary groups (1.6-1.7% and 7.5-8.2%, respectively).

Discussion and Conclusion

The results of the present study indicate that a mixture of processed protein from BSF and YM larvae can replace, at least, up to 50% of the FM in practical diets for European seabass, without compromising fish growth, feed efficiency, and somatic indexes. Altogether, these results evidence the great potential of this insect-based processed protein mixture as a FM substitute. Future work will focus on the impact of this mixture on diet digestibility and seabass metabolic status. The functional potential of this ingredient will also be explored.

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Acknowledgments

The research leading to these results has received funding from the InFishMix project (PT-INNOVATION-0094) funded by Iceland, Liechtenstein and Norway through the EEA and Norway grants. Financial support from FCT to CIIMAR within the scope of UIDB/04423/2020 and UIDP/04423/2020 is also acknowledged.

NoviFEED - NOVEL TOOLS FOR INTELLIGENT FEEDING MANAGEMENT IN RAS MEAGRE (*Argyrosomus regius*) FARMING

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As seafood demand increases globally, aquaculture production is growing fast and becoming the main source of seafood, but not without facing some sustainability issues. Most challenges in the aquaculture sector today are related to the sustainable use of resources, such as space, water, energy and feed, with the latest probably being the most important resource used in the aquaculture industry.

Feed will directly influence production performance and efficiency but can also indirectly affect water quality if there's a high feed loss or increased production of faeces, resulting in an excessive organic load in Recirculating Aquaculture Systems (RAS).

SPAROS has been developing nutritional IT tools based on mathematical modelling (FEEDNETICS™ and FiT feeding tables™) to support feeding management. These tools are already being commercialized for gilthead seabream, European seabass, rainbow trout, Atlantic salmon and Nile tilapia, and one of the objectives of the current study is to extend them to corvina (meagre). For this, data collected from the scientific literature were processed and used to calibrate the models for corvina. The models were tested with independent datasets and will be further validated for SEAentia's pilot RAS using data that are being generated in the context of the present study.

Based on the previous knowledge gathered from other fish species, this study set out to gather ground-truth measurements (feed intake and growth) of corvina in real operational conditions, for the purpose of generating data to validate the models. To achieve this, a trial is being carried out at SEAentia's pilot RAS, with 2 batches being grown with IBW of 0.03g and 50g and fed *ad libitum* to apparent satiety. All parameters that may affect the factors of interest (e.g., dissolved oxygen, temperature, salinity, pH, photoperiod) and other variables that may correlate with the responses of interest (e.g., behavioural patterns) are being measured and recorded.

This trial is currently ongoing, and we only have preliminary observations, although we expect to have all the data from the trial collected and analysed on time to be presented at the workshop and the updated abstract included in the book of abstracts.

Acknowledgements
This work is part of the NoviFEED project (http://www.sparos.pt/projects/novifeed), financed by Iceland, Liechtenstein and Norway, through EEA grants, in the scope of the program Blue Growth, operated by Directorate-General for Maritime Policy (DGPM), Portugal, under reference PT-INNOVATION-0099.

TRANSFORMING OBSTACLES INTO OPPORTUNITIES IN THE DEVELOPMENT OF RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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RAS are in the planning, investment, and development stages throughout the World. RAS have tremendous potential to play a pivotal role in the radical transformation of aquaculture to become a major global food source. However, there are identifiable risks arising. Aquaculture's modern industrial history is repeating itself in many countries as RAS developments are being denied or delayed due to adverse decisions by less-than-fully informed government agencies and well-funded public interest groups. Both use less than comprehensive, updated, science-based information to deny permits. Both accelerate community opposition to RAS. The GESAMP (2008) Risk Analysis and FAO (2010) Ecosystem Approach to Aquaculture (EAA) are valuable frameworks for use worldwide to move RAS developments forward. These frameworks integrate aquaculture developments into the wider, interlinked, community and bioregional social-ecological systems to promote greater equity and resilience. They account for the full range of stakeholders, spheres of influences, community and educational development contexts, and their interlinkages. Using GESAMP and the EAA, RAS companies can pro-actively engage communities to identify win-win situations. However, companies need to accelerate their investments to build the expertise and capabilities of grass-roots, aquaculture community organizations to ensure their long-term stability to address the known social-ecological concerns of communities considering incorporating RAS into their futures:

Accelerated non-product investments in these approaches would advance RAS developments in rural communities worldwide so that these high potential systems can contribute more significantly to the urgent needs for the global transformation of food systems as envisioned in the United Nations Sustainable Development Goals (SDGs).

Systemic Concerns of Communities	Win-Win Scenarios for Innovations
Additional Nutrient Pollution	*Blue-Green Bioeconomies *Integrated, Discontinuous Aquaponics *Watershed Management *Coastal/Bay Management
Additional Exploitative Economies Incompatible with Rural Communities	*Rural Community Development *Scaling Out Strategies *Business Integrations *Tourism, Art *Development of Local and Export Value Chains

OCEAN FOOD SYSTEMS AND HYBRID SEAFOOD PRODUCTION: TRANSDISCIPLINARY CASE STUDIES OF EELS, SALMON AND LOBSTER

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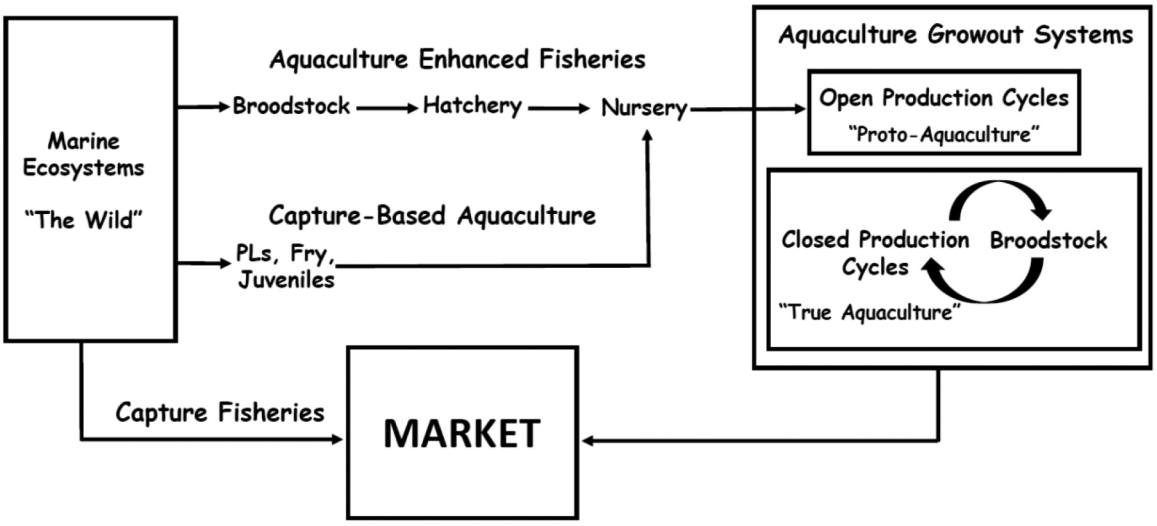
Capture fisheries and aquaculture are researched, planned, and managed throughout the world as if they are independent entities. This ignores their complex and evolving interdependencies. Global attention on "blue foods" is focused on restoration of capture fisheries and sustainable expansion of aquaculture. Such a binary approach does not fit the current realities, opportunities, and innovations in ocean food systems (OFS), and does not integrate knowledge across the professions. Three OFS typologies of American and spiny lobsters as fed fisheries, salmon aquaculture as aquaculture-enhanced fisheries, and eel aquaculture as a capture-based aquaculture.

Typologies illustrate productive, valuable, and evolving OFS which have great potential for accelerating innovations greater than those of capture fisheries and aquaculture alone. OFS increase production and social benefits of blue bioeconomies but are disruptive as they require radically changed science, education, management, and development policies.



INFLUENCE OF GENISTEIN & GENISTIN CONTENT FOUND IN SOYBEAN MEAL AND SOY PROTEIN CONCENTRATE ON GROWTH PERFORMANCE AND ANTIOXIDANT & LIPOGENIC BIOMARKERS IN GILTHEAD SEABREAM *Sparus aurata*

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Introduction

Nowadays, soybean meal (SBM) or soy protein concentrate (SPC) are the most used plant-based protein source ingredients in commercial fish feeds as alternative to partially replacement of fish meal-based diets in carnivorous fish such as the gilthead seabream *S. aurata*. Both SBM and SPC components contain phytoestrogens such as isoflavones the most abundant of which are the aglycone genistein and its β-glycoside conjugate genistin. The glycoside (genistin) is in greater amounts than the aglycone genistein in SBM and SPC. Literature evidence suggests that the biological activity of soy phytoestrogens does not depend upon the glycoside form. In mammals, for example, hydrolysis of the glycoside is necessary for their absorption and activity in the organism [1, 2]. Studies in fish with exogenous (synthetic) genistein-enriched diets have shown variable metabolic effects on fish depending on the genistein dose administered [3.4]. Besides, there is good evidence that exogenous supplemented genistein in the diet affects lipids metabolism as well as fish overall growth rate, produces estrogenic effects and alters fish antioxidant ability [3,4,5]. This study aims to estimate the impact of genistein and genistin on the growth performance and on the antioxidant and lipogenic mechanisms (enzymes activities) of gilthead seabream *S. aurata* via feeding on SBM-based and SPC-based diets and applying specific and indicative biomarkers.

Materials and Methods

Genistein (GE) & genistin (GIN) were quantitatively determined in the methanolic extracts of SBM and SPC ingredients by a spectrophotometric method [6]. Three isoproteinic (49.75% ± 0.44%) and isolipidic (17% ± 0.13%) diets were formulated and produced at the IMBBC-HCMR (Anavyssos). The diets were designated as: a soy-free diet (Control), a 20% soybean meal diet (D-SBM) and a 20% soy protein concentrate diet (D-SPC). Three groups of *S. aurata* with an average initial weight of 27 g were fed on the diets in triplicate groups for two months. The amount of food consumed was calculated during the whole experimental period. At the end, all fish were weighted. Livers were excised, placed in liquid N_2 and stored at -80°C till analysis. The specific activities of the antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), selenium-dependent glutathione peroxidase (Se-GPx)] and the lipogenic enzymes [fatty acid synthase (FAS), malic enzyme (MA), glucose 6-phosphate dehydrogenase (G6PDH)] were determined. All the enzymes activities were expressed in nmoles/min/mg protein. Statistical analysis was performed with the SPSS 13.1.

Results and Discussion

GEN and GIN content was significantly higher in SBC (~621μg/g) than in SPC (~1.6 μg/g). The lower content in SPC could be attributed either to lower content or to the extraction method that was with methanol. Growth indicators are shown in table 1. The antioxidant and lipogenic biomarkers are indicated in figures 1 & 2, respectively.

Conclusions

Findings suggest that soy-based diets with elevated dietary genistein & genistin content such as SBM-based diet could significantly increase weight gain and enhance lipogenesis in *S. aurata*. Contrary, SBM-based diet could lead to noticeably dysfunction in eliminating ROS production, while SPC-diet could improve antioxidant capability.

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Table 1. Growth performance indicators of *S. aurata*.
(different letters significantly differ at p<0.05 level)

	Control	D-SBM	D-SPC
Weight gain (g)	93.77 ± 2.08 **a**	101.78 ± 1.98 **b**	97.57 ± 2.32 **a**
FCR	1.11 ± 0.07	1.10 ± 0.04	1.09 ± 0.03
SGR	2.31 ± 0.03 **a**	2.41 ± 0.02 **b**	2.36 ± 0.03 **b**
DFC (daily feed consumption)	2.18 ± 0.16	2.22 ± 0.08	2.18 ± 0.09
FEED EFFICIENCY	0.90 ± 0.06	0.91 ± 0.04	0.91 ± 0.03
Total Feed (g)	3,120.67 ± 257.36	3,317.27 ± 116.50	3,201.50 ± 172.39
Hepatosomatic Index (HSI)	1.5 ± 0.1 **ab**	1.7 ± 0.2 **a**	1.3 ± 0.1 **b**

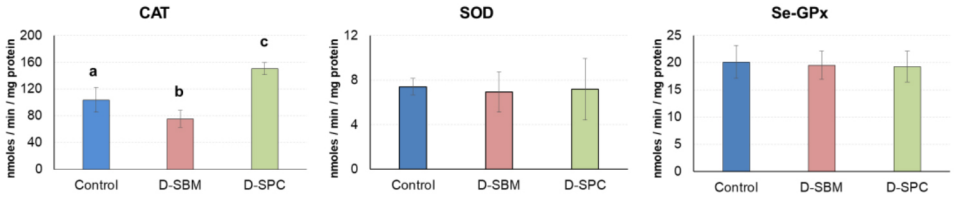

Fig. 1. Antioxidant enzymes activities in the liver of *Sparus aurata*.
(small letters indicate significant difference at p < 0.05 level)

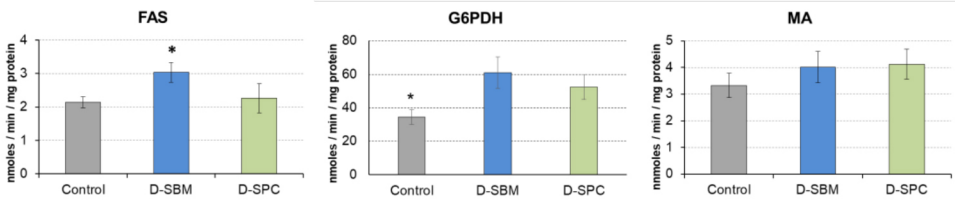

Fig. 2. Lipogenic enzymes activities in the liver of *Sparus aurata*.
(asteriscs indicate significant difference at p < 0.05 level)

Acknowledgements
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EFFECT OF SALINITY DROP ON SUSCEPTIBILITY TO WSSV INFECTION IN *Litopenaeus vannamei* SHRIMP USING A PER OS CHALLENGE MODEL

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Introduction

In shrimp farms, salinity drop in the water due to excessive rainfall has been mentioned to be a risk factor for WSSV outbreaks (Thuong et al., 2016a). It was hypothesized that when WSSV is introduced into the rearing water and a sudden lowering of the salinity occurs, this could lead to an uptake of water through the nephropores into the antennal gland, as shrimp attempt to regulate their haemolymph osmolarity and urinary ion excretion. Once the cells of the antennal gland become infected, the virus infection can spread further into the body. An experimental WSSV immersion challenge mimics a natural water-borne WSSV transmission. Thuong et al. (2016a), performed an experiment in which shrimp were immersed in sea water containing $10^{5.5}$ SID_{50} mL^{-1} of WSSV. Subsequently, these shrimp were subjected to a salinity change from 35 to 5 g l^{-1}. After 5 hours, the salinity was restored to 35 g l^{-1}. The mortality due to WSSV infection was 53%. There was no mortality in the control group without a salinity drop. This suggested an important role of a salinity drop in the WSSV infectivity during an immersion challenge. However, WSSV is also reported to be transmitted through consumption of infected tissues (Wang et al., 1999). In the current study, we examined the effect of a salinity change on infection and mortality during a per os WSSV challenge, because it simulates natural WSSV infections through cannibalism. By testing these conditions, we aim to investigate if salinity change is also a risk factor for WSSV infection during an oral WSSV challenge in *L. vannamei*. These results could then be used in future work to further elucidate WSSV transmission dynamics.

Materials and Methods

Virus stock production: specific pathogen free (SPF) *Litopenaeus vannamei* were imported as postlarvae (PLs) from the United States of America (USA). Shrimp were housed in artificial seawater at 20 ppt salinity and 27°C ± 1°C. They were injected intramuscularly with the WSSV Thai-1 strain (Escobedo-Bonilla et al., 2005). WSSV positive solid inoculum was prepared from the resulting infected carcasses.

WSSV challenge and salinity drop: The inoculum was used to infect PL76 shrimp through oral route. Briefly, during the experiment, shrimp were randomly divided into three challenge groups (A, B, C) consisting of three replicates of 10 shrimp. Ten shrimp were assigned to a first negative control (Mock1- 15 ppt drop). Another group of ten shrimp served as the second negative control (Mock2 – 30 ppt drop). Shrimp were housed individually in 10L tanks. Shrimp from groups A, B, and Mock1 were acclimatised to 20 ppt salinity, while shrimp from groups C and Mock2 were acclimatised to 35 ppt salinity. The oral infection trial followed a procedure adapted from Van Thuong, et al. (2016b). Group A remained at a salinity of 20ppt during and after the oral WSSV challenge. Individual shrimp from groups B, C, Mock1, and Mock2, were transferred into seawater with a 5ppt salinity. Groups A, B, and C received WSSV positive inoculum, while Mock1 and Mock 2 received negative solid inoculum. After a period of 5 hours, the salinity in the individual tanks from groups B, and Mock1 was restored to 20ppt, while the salinity in the tanks of groups C and Mock2 was restored to 35ppt. The animals were observed in the following days and the experiment ended when no mortality was observed for 48hours. WSSV infection presence or absence in the tissues of collected shrimp was confirmed by qPCR. The survival/mortality data were analysed statistically using the Log-rank (Mantel-Cox) test.

Results

At the end of the challenge trial, cumulative mortality rates in the WSSV-challenged groups A, B, and C were respectively 21%, 33% and 40%. The differences in mortality rates showed a trend between group A, that was not subjected to a drop in salinity, and group C, that was subjected to a 30ppt drop (from 35 to 5 ppt) (p-value = 0.0952). In the two control groups, Mock1 and Mock2, that were subjected to a salinity drop of respectively 15 and 30ppt, all shrimp survived. WSSV infection was confirmed by qPCR in a sample of the dead shrimp. WSSV was absent in sampled survivors and negative controls.

Discussion and conclusion

The results of the experiment showed that the probability or risk of infection in the population increased when the animals were subjected to a salinity drop during an oral WSSV challenge. This result was similar to the results obtained by Thuong et al. (2016a) during their WSSV immersion experiments with the same change in salinity. It suggests that salinity change could indeed be a risk factor for WSSV infection in the field, where natural WSSV transmission occurs both by water-borne or cannibalism routes. De Gryse et al. (2020) argued that this could be explained, because sudden salinity drop during, e.g., heavy monsoon rains, aggression, establishment of social dominance, and feed intake (cannibalism) are conditions where frequent urination, and thus frequent opening of the nephropore, takes place. Subsequently, this could create a window of opportunity for WSSV invasion, making entry via the antennal gland possible (de Gryse et al., 2020).

Acknowledgements

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LARVAL GROWTH AND DIFFERENTIAL SURVIVAL IN THREE NATURAL POPULATIONS OF EUROPEAN SEA BASS *(Dicentrarchus labrax)* EXPOSED TO FOUR THERMAL REGIMES

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Introduction

With global warming, the surface temperature of the Mediterranean Sea has increased by 0.4 °C per decade from 1985 to 2006 (Nykjaer, 2009) and is projected to continue increasing by 2.2 to 3.4 °C until 2080 (IPCC, 2021). Therefore, it is crucial to understand the impacts of this warming on the larval growth of the European sea bass, *Dicentrarchus labrax*, which is very important for both fisheries and aquaculture. In this study, we investigated the larval growth and differential survival of three natural populations: Atlantic (AT), Western Mediterranean (WM) and Eastern Mediterranean (EM) in different temperature conditions.

Materials and Methods

The production of the three populations was achieved using three different full factorial design with 30 sires per population and 6, 14 and 13 dams for AT, WM and EM populations, respectively. Until 19 days post-hatching (dph), the populations were reared separately in 2 tanks per population at 13 °C. From 19 dph, the populations were mixed in equal proportions and placed in four thermal environments that mimic the temperatures encountered in the Atlantic (*eAT*, annual average = 13.8 °C, min = 10.4 °C, max = 18 °C), the western Mediterranean Sea (*eWM*, annual average = 16.6 °C, min = 12.6 °C, max = 22.5 °C), the eastern Mediterranean Sea (*eEM*, , annual average = 21.7 °C, min = 16.8 °C, max = 27.6 °C) and a temperature generally applied in aquaculture (*eAQUA*, 16 °C until 70 dph, 23 °C from 70 to 120 dph and then an eastern Mediterranean temperature regime). Each thermal regime was replicated in 4 tanks. Twenty larvae per tank were sampled in each regime at six time points corresponding to equivalent developmental stage from an average notochord length of 8.8 mm to an average fork length of 53.5 mm. Larvae were photographed, weighed, and their photos were analyzed with ImageJ to measure the notochord length (until the fourth sampling) and then, the fork length. The population of origin of each larvae was recovered by parentage assignment using 96 SNPs with the APIS software(Griot et al., 2020). At around 10 grams, 2000 fish per regime were genotyped and assigned to their parents to evaluate the survival of each population in each thermal regime.

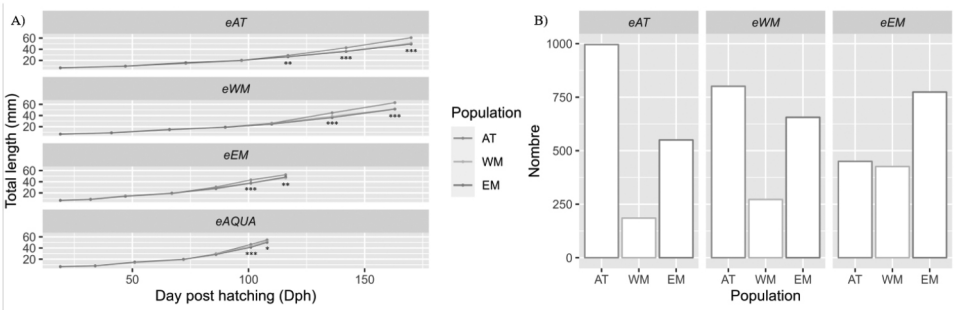

Figure 1 – A) Total length (mm) of larval European sea bass populations (AT = Atlantic, WM = Western Mediterranean and EM = Eastern Mediterranean) as a function of age in four thermal regimes (*eAT* = Atlantic regime, *eWM* = Western Mediterranean regime, *eEM* = Eastern Mediterranean regime and *eAQUA* = Aquaculture regime). *** = P < 0.001; ** = P < 0.01; * = P < 0.05; B) Number of fish per population and thermal regime at tagging (≈ 10 g mean weight).

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Results

From the fourth sampling (≈ 27.8 mm fork length), the AT fish showed a tendency to be longer than WM and EM fish. This difference became significant ($P < 0.01$, AT > WM ≈ EM) from the fifth samplings (≈ 40.2 mm fork length) in all thermal regimes. Furthermore, the size advantage of AT was larger in the coldest thermal regimes (eAT and eWM, Figure 1.A). Regarding survival, there was a strong interaction between population and thermal regime ($P < 2.2e-16$, Figure 1.B), and within each regime, there was a significant effect of population on survival ($P < 2.2e-16$ in *eAT*, *eWM* and *eEM* regimes). The AT population had the best survival rate in the *eAT* and *eWM* regimes while the EM population survived best in the *eEM* regime. The WM population had the worst survival rate in all thermal regimes. We identified three main periods of time where population-specific mortality may occur through linear regressions
of survival on temperature at different stages: between 19 dph and the first sampling (6.6-8.8 mm), between the fourth and the fifth samplings (27.8-40.2 mm), and between the fifth and sixth samplings (40.2-53.5 mm).

Discussion

The results demonstrate the growth of European sea bass is strongly influenced by thermal regimes. However, in terms of growth, we did not observe evidence of adaptation of a population to a specific regime. A local adaptation to low temperatures could explain the better growth potential of AT population than WM and EM populations under the coldest regimes (*eAT* and *eWM*). The largest size of AT population in the warmest regimes (*eEM* and *eAQUA*) could be explained by the countergradient variation phenomenon, where cold-adapted population may express better growth rates when warmer temperatures occur (Conover and Present, 1990).

The study of survival rate revealed that populations are adapted to their thermal regimes of origin. Specifically, the AT population was the better suited to the coldest regimes in terms of both growth and survival. However, for survival, there was also an adaptation of the EM population to the *eEM* regime. The WM population had the worst survival rate in all regimes, although it survived better in warm than in cold regimes. These results are consistent with previous research that suggested nonadaptive introgression and maladaptation of the WM population (Guinand et al., 2017).

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ATLANTICLAM – BRING EURO-NATIVE CLAM SPECIES FROM 'FARM TO FORK'

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Introduction

As the world population grows, demand for food increases just as much, both in quality and in quantity. Within this context, bivalves which filter-feed on phytoplankton, are the most sustainable source of animal protein. However, few gaps along the value-chain exists that needs to be overcome to increase the shellfish industry standards and to support the production, packaging, and supply of European autochthonous species such as *Ruditapes decussatus* and *Venerupis corrugata.*

The ATLANTICLAM project aims to bring Euro-native clam species from "farm to fork", in an integrated, differentiated and consumer-oriented perspective, fostering the consumption of sustainable, healthy and tasty food, contributing to tackling climate change and providing a new income source for the blue economy.

The project intends to solve the main obstacles to a wide dissemination and consumption of Euro-native clam species: availability all-year round; difficulty to match production with market demand; lack of trust on the source/origin of the clams; lack of trust on the quality and freshness of the clams; lack of solutions to increase the in-house consumption of clams.

It will be developed by Oceano Fresco and its partners: INL - International Iberian Nanotechnology Laboratory, Faculty of Biotechnology of Catholic University of Portugal,

Nofima

This project has an investment of 1.394.411 EUR and is funded under the Blue Growth Program of the Financial Mechanism of the European Economic Area (EEA) 2014-2021. The EEA (EEA Grants Portugal) financial mechanism aims to reduce social and economic disparities in Europe and strengthen bilateral relations between Iceland, Liechtenstein and Norway and the beneficiary countries.

Material and methods

At ATLANTICLAM project different tasks will be performed in order to impact the clam value chain from "farm to fork", such as (i) the development of a meticulous and highly scientific selective breeding program, in order to produce more resilient and high-value clam elite stocks ("farm"), (ii) the development of an operations model to ensure efficiency, integration and digitalization of the supply chain ("smart operations"), (iii) the development of a QR-code consumer interface allowing product traceability along the value chain, about the quality, nutritional value and environmental impact of the European clams ("traceability & source"), (iv) the development of new products (chilled clam) and sustainable packaging ("new products") and (v) the development of new recipes and commercial concepts ("fork").

Results

At the end of the EUROCLAM project, it is expected to have: 1) an improved breeding program and production optimization of clam elite stocks; 2) a smart model to predict demand and plan the harvesting and supply chain of European clams; 3) a new QR-code consumer interface, with data about all the product chain and source; 4) new chilled clam line up and new sustainable and innovative packaging; 5) new recipes and commercial concepts.

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Conclusion

With a consortium between Oceano Fresco, Nofima, INL and ESB-UCP, ATLANTICLAM will strengthen both bilateral relations and international cooperation between Portugal and Norway, sharing know-how and disseminating knowledge across borders.

Acknowledgements

ATLANTICLAM project is funded under the Blue Growth Program of the Financial Mechanism of the European Economic Area (EEA) 2014-2021 (PT-INNOVATION-0097). The EEA (EEA Grants Portugal) financial mechanism aims to reduce social and economic disparities in Europe and strengthen bilateral relations between Iceland, Liechtenstein and Norway and the beneficiary countries.

IS SAND IMPROVING *Ruditapes decussatus* BROODSTOCK CONDITIONING?

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Introduction

In Europe, in recent years, capture production of clams has decreased due to numerous factors such as overfishing, abiotic stress, diseases, and failure in recruitment. Seed production in hatcheries has become essential to ensure the sustainability of clams production. Thus, artificial broodstock conditioning allows hatcheries to extend their production season, reducing their reliance on the period of the year when wild beds become naturally mature. Also aims to achieve maximum fecundity whilst maintaining the quality and viability of the gametes. Most clam species have been demonstrated artificially conditioned without the need for substrate. However, in nature, clams tend to live buried in the substrate. This study aimed to evaluate the impact of the sand substrate on clams under artificial conditioning.

Material and methods

Adult specimens of *R. decussatus* (39.2 mm ±3.50 mm) were conditioned with and without sand at 19 ± 1°C in flow-through systems and fed with a diet of 3% meat dry weight per dry weight of algae. During conditioning, samples were collected at the beginning (T0) at after 41 days (T1) and after 73 days (T2 - end of the conditioning). To provide information regarding gonad development, condition index, histological analysis, and biochemical composition (glycogen, protein, and lipids) were performed at each sampling time.

Results

Results showed that the use of sand as a substrate did not have any effect on gonadal maturity evolution. Mortality was 56% for the sandless treatment and 59% for the sand treatment. Regarding biochemical composition parameters, it was observed an increase during the conditioning period. Between the conditions (sand/no sand) significant differences (*p- value* < 0,05) were observed in biochemical composition in the end of the conditioning period for the without sand.

Conclusion

The conditioning of *R. decussatus* broodstock did not showed to be improved when sand was used, in comparison with using only water as a substrate.

This work constitutes an important step in the improvement of broodstock conditioning in the hatchery.

Acknowledgements

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ADAPTIVE EVOLUTION FOR OPTIMIZATION OF AN INDUSTRIALLY-RELEVANT MICROALGA

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Introduction

Fish oil is currently the primary source of omega-3-polyunsaturated fatty acids (ω-3-PUFAs) for the increasingly growing aquaculture feed market, and the Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) global consumer markets [1]. The total amount of aquatic resources grown or harvested for human consumption (excluding algae) has now set a record of 157 million tonnes [2]. About 1/3 of wild fish stocks are currently overfished, and the remainder of the wild fish stocks are already exploited at maximal sustainable levels [2]. This alarming decrease in wild fish stocks caused by unregulated fishing and its deleterious effect on the marine ecosystem has recently promoted research on alternative sources of these valuable fatty acids [1] . Microalgae are thought to be the primary producers of ω-3-PUFAs in the marine food chain and could therefore serve as a direct source of these nutrients which would help to satisfy the increasing demand for sustainable seafood [3].

Marine protists used in this study can accumulate lipids in the cell as a defense mechanism against various stress factors, and can produce biomass with more than 50% of their cell dry weight as lipids, in which ω-3-PUFAs account for more than 40% [4]. What makes them advantageous over other oleaginous unicellular microorganisms is, besides accumulation of a high PUFA content, their competency for the industrial scale fermentations due to their fast heterotrophic growth and their production of toxins-free oils [5]. Considering the lack of genomic insights and detailed studies on lipid biosynthesis in thraustochytrids, as well as the complexity of stress adaptation, rational genetic engineering of these microorganisms is limited. Also, oils with high PUFA content and other valuable metabolites produced by genetically modified microalgae are still not accepted by the food and pharmaceutical industries in many countries. Therefore, Adaptive Laboratory Evolution (ALE) serves as an effective tool to study the molecular-level response of microorganisms to stress and adaptation and can aid in designing genetic engineering strategies for optimizing microbial production systems and constructing new microalgal strains [6]. In this study, a combined two-stage ALE approach is proposed to obtain a non-transgenic mutant with a potentially higher cell growth/biomass production and stable lipid production (Figure 1).

Aim

To enhance the phenotype of a thraustochytrid strain using ALE, and to identify the possible molecular mechanisms underlying such an adaptation.

Methodology

Adaptive Laboratory Evolution

A two-stage Adaptive Evolution approach was designed and first Evolution experiment was conducted using a high oxygenation stress in the culture medium. UV C mutation was applied to the starting culture at logarithmic phase to increase genotypic diversity of strain. Mutant screening was conducted by analysing cell growth and cell dry weight, as well as by analysing the fatty acid content using Gas Chromatography. Resulting mutant strain will be used in the second ALE experiment.

De novo whole Genome assembly and Transcriptome sequencing

Long-read Nanopore MinION sequencing was used for the whole genome sequencing in conjunction with Illumina NovaSeq 2x150 bp sequencing. Final de novo genome assembly was conducted using a hybrid approach combining high depth Illumina short reads with Nanopore long reads. Additional Illumina NovaSeq 2x150 bp sequencing was used for the RNA sequencing of the reference strain at the three separate growth stages.

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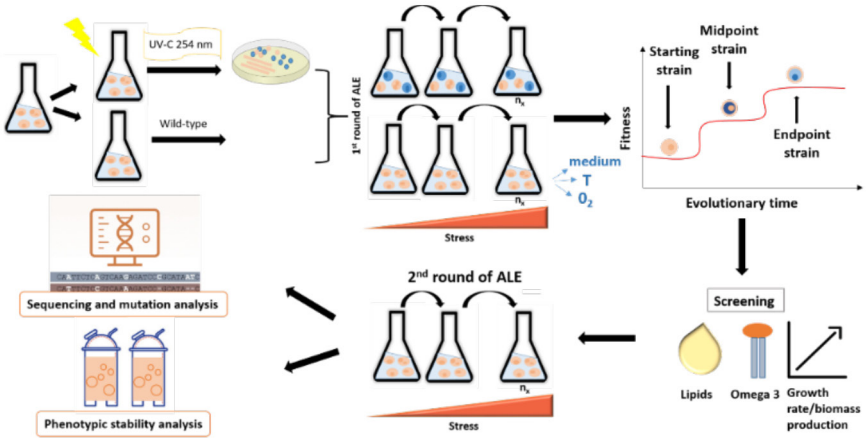
Figure 1. Schematic of a proposed two-phase ALE.



Results and Discussion

A two-stage ALE approach was designed and the first Evolution experiment using high oxygenation as a stress parameter showed significant difference in fatty acid content between final mutated cultures compared to control. Resulting mutants will be used for the second evolution experiment. The Nanopore MinION sequencing run produced around 20.3 Gb of data, altogether generating an approximate 450x coverage of the genome. Around 2.5 M reads were generated with an N50 value of up to 11.7 kb. An additional 6.8 Gb of paired-end 2x150 bp Illumina reads were generated, representing approximate genome coverage of 150x. The final Illumina-polished assembly generated with Flye showed improved quality and completeness with >95% sequence identity, 91.09% of complete BUSCO genes and greatly reduced indels to 26.10 per 100 kbp. Additional RNA sequencing of the reference strain in three separate growth stages generated around 130 Milion reads. This high-quality data will be used to generate a complete de novo Transcriptome assembly that will be used to for analysing genetic changes in final ALE mutant strains. This will also help to identify the key-genes involved and molecular mechanisms underlying evolution adaptation, and provide novel biomarkers for targeted genetic engineering for metabolic products and pathways of interest

Conclusions

A high-quality genome assembly and transcriptomic sequencing was generated that will serve as a reference for the subsequent mutant analysis. ALE experiments coupled with further omics-based analysis including transcriptomics, proteomics and metabolomics will allow for deciphering the connection between genotype and phenotype in the evolved strain compared to the original strain.

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PERPECTIVES OF AN IMMUNOBIOSENSOR FOR THE TETRODOTOXIN DETECTION IN MUSSELS

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Introduction

Intensity, frequency and geographic distribution of harmful algae blooms have increased also in areas previously unharmed (Dubois et al., 2010) like the Northern part of the Mediterranean Sea. The toxins produced by these microrganisms can contaminate seafood and represent a potential threat to human health. One of those threats is the Paralytic Shellfish Poisoning (PSP) due to non-proteinaceous paralytic neurotoxins (Tetrodotoxins TTXs), that selectively bind to voltage-gated sodium (Na+) channels interfering with or completely inhibiting the neural transmission in nerve and muscle tissues (Denac et al., 2000; Magarlamov et al., 2017). TTX is commonly present in Tetraodontidae Teleosts, anyway it has been recently reported in several shellfish species. The European Food Safety Authority (EFSA) has specified a concentrations of 44 µg of TTX or TTX analogues equivalents per kg of shellfish meat as maximum theshold for food safety (Knutsen et al., 2017) . The elective methods for TTX detections are based on chromatography separations mainly in tandem with MS like LC/MS(/MS) with hydrophilic interaction liquid chromatography (HILIC) or MS/MS and the method of extraction is optimized (Turner et al., 2023) with high sensitivity and low concentrations of TTX (from µg/ml to ng/ml). Anyway the need to expand monitoring plans are requiring analytical efforts to perform an early diagnosis, with a minimal approach also for sample preparation. To meet such requirement for a rapid preliminary screening, an immunsensor assay, based on fluorescence detection, coupled with PocOrEl (Orel d.o.o, SI) analytical devices for the detection of TTX in mussel meat is presented.

Material and Methods

A calibration curve was prepared with TTX (Creative Diagnostic Inc., USA) in buffer acetate and diluted with PBS at a ratio of 1:10 to obtain samples with 80, 60, 40, 20 and 0 µg/L of TTX; data were compared with mussels' meat spiked with the same amount of TTX to obtain the same absolute TTX concentration. The effect of the matrix in the assay that retrace an indirect ELISA test was evaluated on mussel extract after or without a filtration step through 20µm filters. A black plastic cartridge with a reaction chamber of 35µl volume has been used (Daniso et al., 2020) for immunosensor implementation. The bottom of the reaction chamber is a polyethylene (PE) transparent substrate subjected to a coating with selected organosilane (GPTES) for better adhesion of the TTX-BSA molecule (Daniso et al., 2021). The TTX-BSA (Creative Diagnostic Inc., USA) mix was deposited in the reaction chamber and let to react overnight at room temperature. Subsequently, a blocking step with defatted milk 2% was performed. Then, sample mix, composed of sample and MonoclonalAntibody-TTX (1.5 mg/ml) diluted 1:250 in PBS, was loaded in the well and was incubate at 37°C per 15 min. Afterward, the slides were washed twice to eliminate unbound MAb-TTX to the substrate and 40µl of conjugated PoliclonalAntibody with the florophore Atto 430LS (2 ml/ml) diluted 1:50 was added to the well and incubated as above. After washing twice, the cartridge was left to dry before measurements with PocOrEl (Orel d.o.o) analytical devices. Data were validated with an ELISA assay using a Sunrise (Tecan USA).

Results

The trend of the obtained results for the two sets of samples follows the expectation of a decrease in the signal with the increase of the toxin concentration of sample, and the signals in the two situations are almost equivalent. The analysis carried out with the TTX in PBS provided similar signals in comparison with the data obtained for the spiked mussels samples that contain equal amount of target toxin. The filtration step with a 20 µm filter of spiked homogenized mussels and the dilution of 1:10 of the Acetate buffer used for the extraction resulted a key step. A reduction of the Mab-TTX activity was observed when the raw homogenized mussel meat was used while the filtration step at 20µm reduce the interference of the matrix with the Mab-TTX. The cut-off value of 44 µg/kg of the analysis is marked on the graph with the red arrow; samples that exhibit a number of counts close to the corresponding cut-off figure should be considered as positive.

In conclusion, the presented implemented assay for the Poc-Orel system (OrEl d.o.o, SI) shows a good discrimination between positive and negative sample even if must be refined, at the moment is limitated at the detection of TTX and should be implemented for the analogues compound in order to meet the requirements of extensive monitoring applications.

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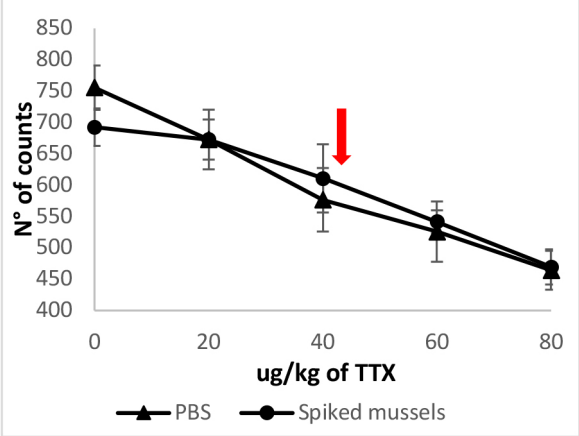

Figure 1 Mean value of the number of counts resulting from the fluorescent signal, detected using the PocOrel instrument provided from OrEl d.o.o., for 8 repetitions of spiked mussel sample compared with TTX diluted in PBS.

Acknowledgment
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ETHANOLIC EXTRACTS OF *Phaeodactylum tricornutum* CULTURED IN COPPER MANIPULATED MEDIUM INCREASE MINERALIZATION IN FISH

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Introduction

Skeletal deformities affects approximately 40% of the farmed fish produced in Europe, resulting in considerable losses for the aquaculture industry. Microalgae are rich in compounds with innovative bioactivities, in particular osteoactive molecules that hold a great potential toward bone health improvement. The manipulation of nutrients in microalgae culture medium can modulate the production of bioactives of interest. Copper plays a vital role as an essential nutrient for aquatic life, participating in various biological processes such as energy metabolism, antioxidative defense, iron metabolism, and bone growth and development (Cavalletti et al., 2022; Creaser, 1934; Mikulski et al., 2009). Manipulating the copper content in algal cultures leads to changes in the algal biomass (Levy et al., 2007), which, in turn, affects mineralization. This study aims at investigating the effect on the production of osteoactive compounds by manipulation of copper contents in the growth medium for *Phaeodactylum tricornutum*.

Materials and methods

Ethanolic extracts of *P. tricornutum* were prepared from cultures grown in increasing concentrations of copper (250-1500 µM), then lyophilized and resuspended in DMSO. The mineralogenic activity of the extracts was assessed in VSa13 cells exposed for 3 weeks to culture medium supplemented with 31.6 mg/mL of each extract and in 3-pdf zebrafish larvae exposed for 3 days to system water supplement with the same extracts. Extracellular matrix and operculum mineralization were evaluated after alizarin red S staining and quantification according to Laizé et al., 2022, Tarasco et al., 2017 and Tarasco et al., 2020.

Results

Only ethanolic extracts prepared from microalgae cultured with 1250 µM of copper showed an increase in the mineralization of the operculum (~ 20%), while all extracts increased extracellular matrix mineralization from 26% (Copper 1250) to 69% (Copper control-2,0*10^7 nmol/L), when compared to the vehicle (DMSO). This mineralization increased from the supplementation with 500 nmol to the supplementation with 1000 nmol and then decreases.

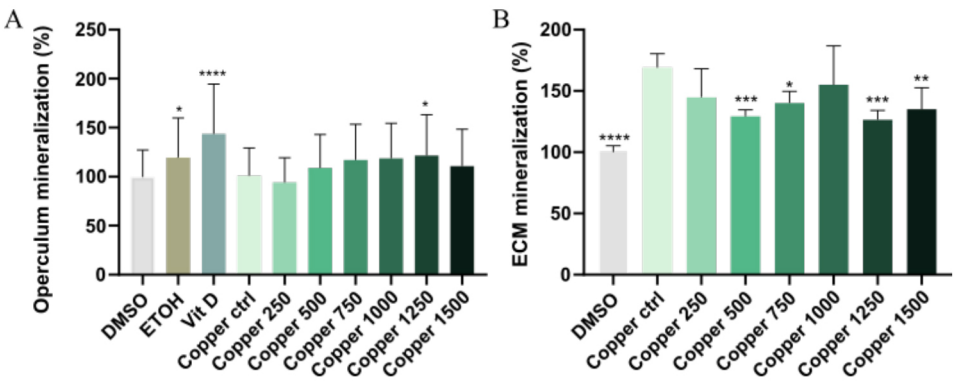

Figure 1. Effect of copper manipulated *Phaeodactylum tricornutum* extracts on (A) operculum mineralization *in vivo*, n=45 and (B) extracellular matrix mineralization *in vitro*, n=6. Statistical analysis: one-way ANOVA (**p<0.01; ***p<0.005, ****p<0.001).

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Discussion

By testing the ethanolic extracts in two different systems *in vivo* developing bone and *in vitro* cell system to evaluate ossification and ECM mineralization respectively. Both approaches have their advantages and limitations. The use of cells provides greater experimental control and the ability to explore specific molecular mechanisms involved in mineralization. However, it requires cell culture techniques and may not fully reflect the complexity of the *in vivo* environment.

On the other hand, the operculum system allows for studies that closely mimic the natural conditions of zebrafish, but the analysis is limited to this specific structure. These differences can explain the distinct effects observed. The larval operculum mineralization only increased with a copper supplementation of 1250 nmol/L. While, in the VSa13 cells, the control condition exhibited the highest mineralization. This observation suggests a potential correlation with the role of copper in biological responses in mesenchymal stem cells (Burghardt et al., 2015). Nonetheless, further studies are necessary to better comprehend the relationship between the concentration of supplementation and mineralization. In conclusion, the supplementation of 1250 nmol/L showed the most promising results for enhancing mineralization *in vivo* and potentially for addressing skeletal development and mineralization problems in fish production, such as skeletal deformities.

Acknowledgments

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THE EFFECT OF DIFFERENT DIETARY SOURCES OF SELENIUM ON IMMUNE STATUS, ANTIOXIDANT CAPACITY AND DISEASE RESILIENCE OF WHITE LEG SHRIMP (*Litopenaeus vannamei*)

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Introduction

Production of white leg shrimp (*Litopenaeus vannamei*) experienced decades of spectacular growth. Global production was estimated at around 5.2 million MT in 2021, with a forecasted volume of 6 million MT in 2023. White leg shrimp is an interesting species for the aquaculture industry, due to its high growth rate, with excellent yields. Additionally, shrimp market demand is high, making it interesting for fish farmers to produce and export globally. White leg shrimp are known to have a relatively low feed conversion ratio and environmental impact. Nevertheless, the industry is increasingly faced with challenges, such as increased pressure of diseases. One of the most severe diseases in shrimp industry is early mortality syndrome (EMS). EMS, also known as Acute Hepatopancreatic Necrosis Disease (AHPND) is characterized by high mortalities, in many cases reaching 100 percent within 30-35 days of stocking of grow-out ponds, leading to huge financial losses for farmers. EMS is caused by the toxins produced by *Vibrio parahaemolyticus*, a bacterium that colonizes the gastrointestinal tract and damages digestive organs such as the intestine and hepatopancreas. Since mortalities are high and bacteria can spread over different farms quite fast, the economic losses are huge. Shrimp-producing countries in South East Asia, such as Thailand, India and Vietnam have experienced significant drops in volumes and export. In Thailand, for example, it is estimated that in the period between 2010 and 2016, EMS has caused financial losses of just under 12 billion dollars and the associated loss of tens of thousands jobs. Recovery from this period is still ongoing, and volumes in some countries in South East Asia are still below that of before the EMS era. Since it is difficult to completely prevent bacterial presence in the water, the focus of the industry is on increasing shrimp resilience through nutrition. One possible solution is the right application of selenium (Se) in the diet. Se plays an important role in preventing oxidative stress in humans and animals, as it is part of the selenoenzyme glutathione peroxidase. Deficiencies in Se are often followed by decreased growth performance, and an increased susceptibility to diseases. In the battle against diseases like EMS, Se addition can be an important strategy to increase shrimp resilience, with decreased mortalities as result. For the addition of Se to the diet, various forms can be used. In general, it is accepted that organic Se in the form of L-selenomethionine is the only form of Se that can be stored into animal protein. All other forms of Se, such as inorganic sodium selenite or selenocysteine present as part of the organic Se in selenized yeast, cannot be stored into animal protein. Therefore, L-selenomethionine seems the preferred source to provide a safe deposit of Se inside the animal, ensuring an optimal Se status also during disease challenge. The goal of this experiment was to compare the effects of adding organic Se, in the form of L-selenomethionine to the diet and inorganic selenium, in the form of sodium selenite, on the antioxidant capacity, immune status and resistance against a *Vibrio parahaemolyticus* challenge.

Material and Methods

The animal experiment was conducted at Kasetsart University, Bangkok, Thailand. To investigate the effect of different sources of Se on white leg shrimp, three diets were created and fed in 6 replicates with 25 shrimp per repetition: 1) Control diet, a basal diet without added selenium; 2) Diet SS, control diet + 0.5 ppm Se from sodium selenite; 3) diet SM, control diet with + 0.5 ppm Se from L-selenomethionine (Excential Selenium 4000, Orffa Additives B.V.). Shrimp were fed one of the three experimental diets for 8 weeks, after which 30 shrimp were randomly selected from each treatment and immune and antioxidant parameters were measured. After the feeding trial, a challenge with *Vibrio parahaemolyticus* was conducted. Over the following period of 15 days, mortality was measured and, after the challenge period, immune and antioxidant parameters were once again measured. All generated data were analyzed using one-way ANOVAs.

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Table 1: *Vibrio* count in intestine, hepatopancreas and hemolymph white leg shrimp fed diets with different sources of selenium.

		Control	SS	SM	p-value
Before challenge	Intestine (CFU/g)	1158[a]	1119[a]	640[b]	0.018
	Hepatopancreas (CFU/g)	1905[a]	1399[ab]	1073[bc]	<0.001
Challenge	Intestine (CFU/g)	16277[a]	4524[b]	913[c]	<0.001
	Hepatopancreas (CFU/g)	13545[a]	4184[b]	1368[c]	<0.001
	Hemolymph (CFU/ml)	5	2	0	<0.001

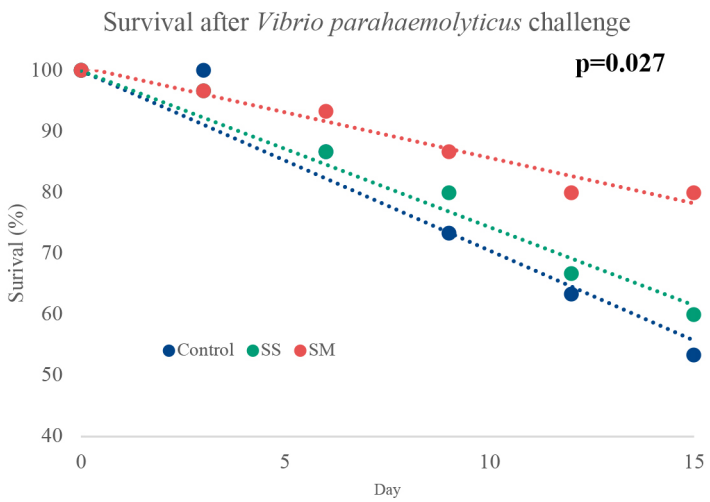

Figure 1: Survival of white leg shrimp fed different sources of selenium after a challenge with *Vibrio parahaemolyticus*.

Results and Discussion

Immunity and antioxidant capacity at the end of the 8 weeks feeding trial, showed improved health status of the shrimp for both treatments where Se was added to the diet. L-selenomethionine, compared to sodium selenite addition, showed a higher improvement in immune and antioxidant status of the shrimp. Superoxide dismutase (SOD), lysozyme activity and hemolymph protein levels were all shown to be significantly highest in animals fed the diet with L-selenomethionine ($P<0.05$). Besides, phenoloxidase activity showed a numerical improvement after addition of L-selenomethionine. On the other hand, hemocyte count and glutathione peroxidase (GPx) were unaffected by addition of Se, regardless of the source. Bacterial count before the challenge was shown to be the lowest in the shrimp fed diet SM, indicating shrimp on this diet have a better defense against the bacteria in an unchallenged environment (Table 1; $P<0.05$). Consequently, increased survival in unchallenged shrimp was observed for diet SM, showing the highest survival after 8 weeks (85.33%), followed by diet SS (83.33%) and control (77.33%) ($P<0.05$). After the *Vibrio parahaemolyticus* challenge, changes were visible in the health status of the animals, lysozyme activity and SOD were significantly improved by addition of L-selenomethionine. Other immune parameters were numerically improved by the addition of L-selenomethionine. Se addition significantly decreased the *Vibrio* count in the shrimp, with L-selenomethionine being significantly more effective compared to sodium selenite (Table 1; $P<0.05$).

After 15 days the survival of shrimp fed the diet with L-selenomethionine was significantly higher compared to the shrimp fed the diet with sodium selenite or without added Se (Figure 1; $P<0.05$). The results of survival after challenge demonstrate the increased defense capability of shrimp by addition of L-selenomethionine.

Conclusion

Addition of Se in shrimp diets seems to be of vital importance to maintain productivity in a global sector with increased disease pressure. Se is seen to improve the overall health status of animals by improving immunity, antioxidant capacity and survival of challenged and unchallenged shrimp. L-selenomethionine was shown to be highly effective in increasing shrimp health and disease resilience, compared to sodium selenite, indicating that L-selenomethionine, is a better solution for the shrimp feed sector.

MUSSEL (*Mytilus galloprovincialis*) BIOMETRIC AND BYSSUS PERFORMANCES AFFECTED BY PROLONGED HEAT WAVES

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Introduction

According to the predictive models, in the near future, the heat waves (HWs) will increase their duration up to 30 days, with negative consequences for the shellfish farming sector. Nowadays the farmers are facing the loss of mussels which come off the socks when the water temperature increases. In order to figure out the physiological mechanism behind these events and what to expect in the future, this study simulated an exposition of mussels to a prolonged HW. Morphometric indices, byssus quantity and quality through analysis of the fiber morphology were assessed.

Materials and Methods

260 specimens of farmed *M. galloprovincialis* reared into 12 aquaria were divided in two groups: the control one at temperature of 20±0,5°C and the heat wave exposed group at 28°±0,5°C. Mussels destined to byssus mechanical analysis, will be placed on square of polypropylene (PP) film in order to simulate the attach to PP socks used in farming.

At 3, 5, 10, 30 days exposure, mussels were sampled in both groups to determine the biometric indices of animal weight, wet and dry soft body and shell weight, the condition index (CI), meat yield (MY) and hepatosomatic index (HIS), clearance rate (CR), survival at air exposure. At final sampling time (30 days), byssus quantity and quality of the animals attached to PP, was evaluated by analysis under SEM observation.

Results and Discussion

About biometric indices, significant differences were found for hepatosomatic index, clearance rate and survival at air exposure where the lowest values were reported by the exposed group at the end of trial. For the HSI, the exposed group at 30 days of exposure showed a significant reduction in the digestive gland energy reserve, compared to other time points. Although, the heat wave reduced the energy reserve of the stressed group but only in long-term and did not alter the pulp yield. Moreover, the exposed group, after 5 days, started to filter at a rate significantly lower than the beginning and the control group, a sign of metabolic activity decrease. Concerning the survival at air exposure, the exposed group, at the end

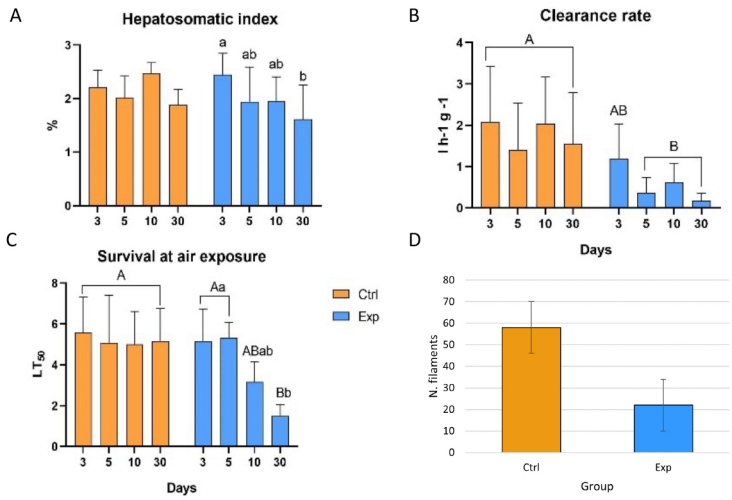

Fig. 1: Mussel HSI (A), CR (B), survival at air exposure (C), quantity of byssus filaments produced on PP plates at 30 days (D) . The uppercase letters (A,B) represent significant differences in two-way ANOVA ($P < 0.05$) between the groups. Lowercase letters (a,b) stand for significant differences between times for the same group.

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of thermic stress period, revealed a significant reduction in the capacity to survive compared to the other samplings and group. It proves that the prolonged heat wave has made these animals more susceptible to additional stress input (Fig. 1). Counting the byssus filaments, at 30 days of experiment, produced by the animals attached on the PP plate, it was found that exposed specimens made significantly fewer filaments, less than half of the other group. Probably, stressed mussels did not have enough energy to spend for the byssus formation (Fig 1D).

Conclusion

Prolonged feat waves cause a reduction in the quality of the mussel as a food product, not in terms of loss in pulp yield, but animal energy reserve and the shelflife of a product that has to be sold alive: in fact, this thermic stress reduces the survival duration outside the sea. Moreover, with the reduction in byssus filaments, stressed animals are able to detach more easily from the socks under the force of the currents, with the risk for the farmers to lose a great quantity of product.

Acknowledgements

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COMPARISON OF FARMING TECHNOLOGIES FOR *MANILA CLAM* SPAT PRE-FATTENING

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Introduction
In the Italian production systems of Manila clam there are no standard management guidelines. It is essential to define which system is more efficient and productive, especially for the pre-fattening phase of clam spat. For this purpose, different supports were tested, evaluating which allows a greater growth of the seed.

Materials and Methods
Three different pre-fattening systems for the Manila clam spat were tested at the NaturEdulis company from April to July 2023.

Spat (4.68±0,53 mm length) was placed in 1) *net lanterns* suspended in superficial sea water of Goro lagoon (FE, Italy), with a mesh size of 1.5mm; 2) *poches* suspended in less superficial sea water, with a mesh size of 1.9mm; 3) land *upwelling tanks* in the company hatchery. During four sampling points, one per month, growth/performance parameters were monitored. 30 clams were sacrificed, in the aquaculture laboratory of the University of Bologna, to evaluate the length, width, height, individual total weight, wet and dry flesh and shell weight, condition index (CI), the specific growth rate (SGR) (ln weight 1-ln weight 0/interval time*100) and the daily shell increment (DSI) (shell length1 –shell length0 / duration of the experiment*100). At the beginning and the end of the trial, clearance rate also was assessed for each support (CR).

Moreover 3L of sea water from the different pre-fattening sites were collected in bottles in order to analyze the concentration of the A chlorophyll (Chl).

Results and Discussion
The most interesting results can be found in the length and total weight. In fact the clams increased the length significantly during the time in each support, following the chlorophyll trend (Fig. 1A). During May and June (2-3 time sampling) the A chlorophyll concentration do not show significant difference between the supports. However, a significant increase can be found during all the months for each support collocation. Finally the high Chl values in lantern can have contributed to the best performance of clam grew in this support, on the contrary for the upwelling tank system. Despite the clearance rate capacity by clams was no different between the support (Fig. 1B), it is possible to notice the difference in the animal growth. Making a comparison between the supports at the same time, in the second sampling clams from poche support had the shorter length and at the third time this situation appeared for the clams from upwelling. At the end, poche and upwelling supports showed the same length, different from the longer clams from lanterns (Fig. 1C). There is not the same significant trend for the weight values. It is possible observe that the weight in the poche. Comparing the supports, in the end, the clams in the lanterns had the greatest weight (different from upwelling and the smallest poche) (Fig. 1D). The condition index results indicate that the clams in the poche did not increase the CI during the months. At the end, this support maintained the same initial CI value, that is the smallest between the supports (no significance for lantern and upwelling) (Fig. 1E).

Conclusion
Observing the different growth parameters, the lantern seems to be the best support that can provide a faster growth and good performance of the Manila spat. On the contrary, the poche was not an efficient system, maybe due to the massive biofilm that was forming around the mesh, avoiding a sufficient water flow.

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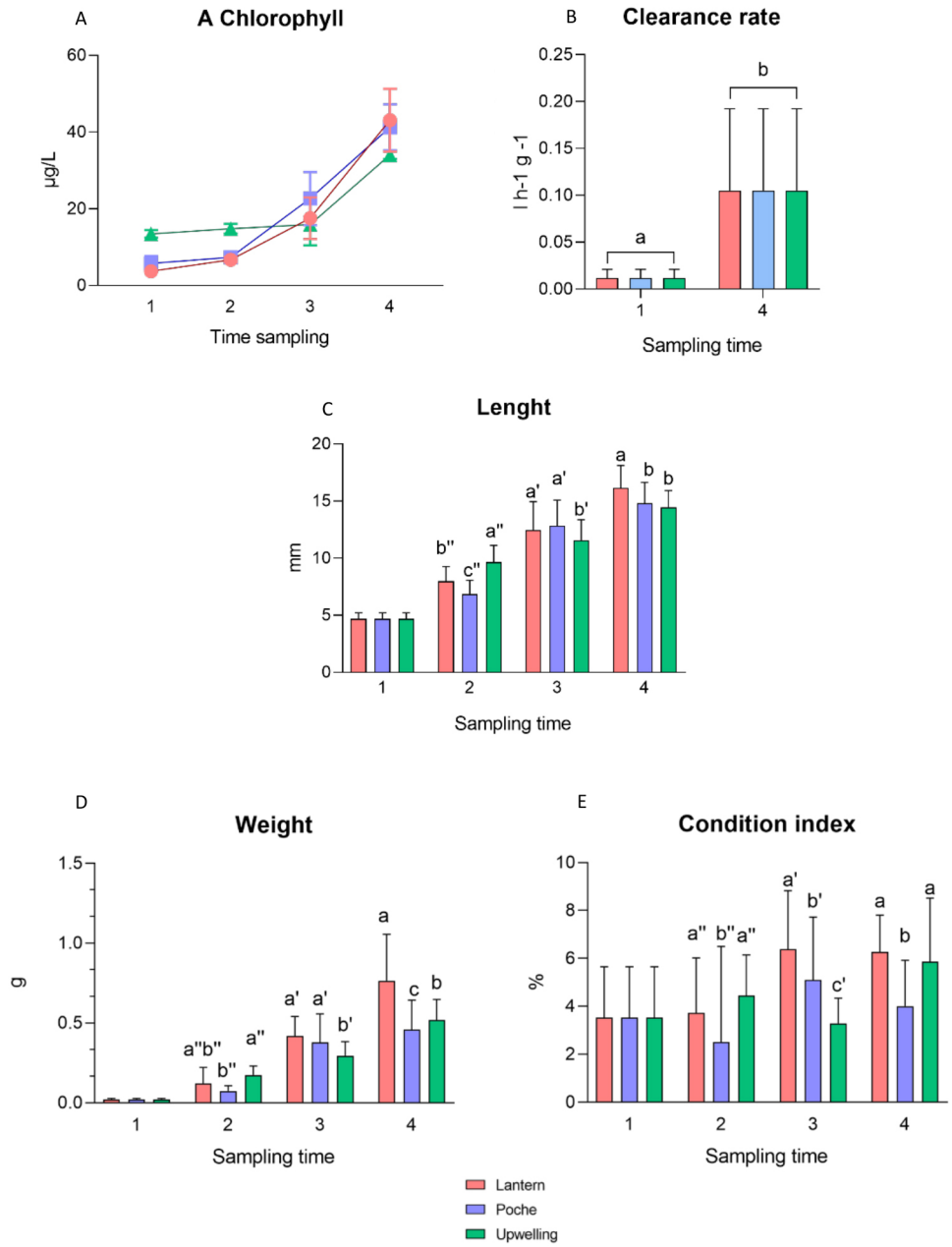

Fig. 1: A Chl (A), CR (B) Clam length (C), weight (D), CI (E). The lower letters (a,b,c; a',b',c';a'',b'',c'') represent significant differences in two-way ANOVA ($P < 0.05$).

Funding

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UNDERSTANDING THE FUNDAMENTAL CONTRIBUTION OF LIVE FOOD IN THE FEEDING REGIMES FOR MARINE FISH LARVAE. A CASE STUDY OF THE GILTHEAD SEABREAM *Sparus aurata* L

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Introduction

Live food consumptions in the feeding regimes of marine fish larvae have been decreased considerably during the last decades, making the producers less reliant on the success of rotifer cultures and reducing the amounts of *Artemia* that are being used. A lot of progress has been made in diet formulations and production techniques, proving that early starter feeds are accepted well by marine larvae as soon as mouth opening occurs. In the meantime, quantities of live feed consumed during larval rearing have been reduced considerably. Despite these improvements, excluding completely the live preys from the menu does not result in successful larval production for any of the commercially most important marine species.

As nutrition has a large impact on fish gut mucosal health status, larval mucosal architecture and microstructure, larvae were examined through histology to evaluate diet-induced alterations. Additionally, transcriptome analysis by 3' end RNA-sequencing of pools of larvae was conducted to perform an in-depth analysis of the impact of live food deprivation on the fish larval health status. Fish growth and survival were evaluated as ultimate outcomes.

Materials and methods

Test set-up

Nearly hatched seabream larvae, originating from the same pool of eggs, were stocked at a density of 100 larvae per liter in 390L tanks. Seawater was provided through a semi-closed water renewal system at a temperature of 18±1°C. Dissolved oxygen levels were kept around 100% saturation. Larvae were reared under green water conditions. A standard live food regime (LFC) was compared to a treatment where no live food (No LF) was introduced, providing a special early start-feeding diet from the onset of exogenous feeding.

Weekly biometrics

20 Larvae per tank were sampled every week and controlled for Standard Length (SL).

Histology

6 Larvae per group were sampled at 20 and 35dph for histological analyses of the posterior intestine (PI). Whole larvae were fixed in Bouin's fixative and stained with May-Grünwald/Giemsa. PI folds height, width and enterocyte height were measured.

3' end RNA-sequencing

270 Larvae were sampled from the 2 experimental groups (LFC and No LF) at 20dph. Larvae were washed with PBS, proceeding with RNA extraction with Genezol. RNA concentration, purity and integrity were checked before proceeding with the RNA sequencing. A bioinformatic pipeline was applied to statistically analyze differentially expressed genes (DEGs) and assess enrichment of Gene Ontology (GO).

Results

The weekly length measurements showed very early significant differences between the No LF and LFC groups and the last survivor in the No LF group died at 36dph, indicating the poor biological performance of the latter.

Histomorphometric analyses of distal intestine from larvae at 20 and 35 dph revealed significantly shorter and narrower villi, and shorter enterocytes in the No LF compared to LFC treatment. Nevertheless, no coarse histological damages were visible in the gut mucosa in the No LF group.

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RNA sequencing performed on pools of larvae at 20 dph highlighted 486 Differentially Expressed Genes (DEGs) between both groups: 260 and 226 were down- and up-regulated, respectively, in the No LF treatment compared to LFC. Based on statistical significance and extent of gene expression change, the absence of live food resulted in the disturbance of many Gene Ontology biological processes such as lipid transport, proteolysis, immune response, glycolysis and cartilage development.

Conclusions

This study indicates the types of biological processes that are highly influenced when the live food is removed from the standard larval feeding protocol. Live food remains fundamental, ensuring proper development and growth of gilthead seabream larvae and assesses its efficacy as a naturally derived functional feed.

AN INTEGRATION OF ZOOTECHNICS AND TRANSCRIPTOMICS AS A JOURNEY TO UNDERSTAND FACTORS AFFECTING LARVAE QUALITY IN FISH

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Introduction

The life cycle of most teleost fishes embraces a crucial phase known as larval period, during which larvae undergo many evolutionary events (e.g., start of the exogenous feeding, the yolk-sac reduction and the inflation of the swim bladder) that chiefly affect their morphological, behavioral and physiological status (Urho, 2002). Those changes appear to have a different impact on the performance (often referred to as quality) of fish larvae (Kjørsvik et al., 2003). Among others, growth-related traits have been commonly used as parameters to assess larval quality and were reported to significantly vary between the specimens even of the same cohort (Mun et al., 2019; Wang et al., 2021). Despite that, mechanisms and factors leading to different performances during the early life history of fish are largely unknown. Therefore, using a family-based experimental approach, we intended to thoroughly investigate the relation between zootechnical and transcriptomic profile of Eurasian perch (*Perca fluviatilis*) larvae in order to gain deeper insight into the developmental processes influencing larval quality.

Material and methods

Sixteen unique families of Eurasian perch were created by controlled reproduction. Eggs were fertilized *in vitro* using cryopreserved semen (Judycka et al., 2022), and then incubated in a recirculating aquaculture system (RAS). The hatched larvae were reared for 30 days following the standardized procedure described by Palińska-Żarska et al. (2020) and during the larvicultural period several zootechnical data were collected (e.i., length, weight, swim bladder inflation rate, cannibalism rate, mortality). In addition, larvae at mouth opening stage (right after hatching) were sampled for transcriptomic analysis. Obtained RNA-sequencing data were mapped to the reference genome *P. fluviatilis* and later processed with Weighted Gene Coexpression Network Analysis (WGCNA) using a R package (Langfelder & Horvath, 2008). Gene ontology (GO) enrichment analysis was carried out using ShinyGO (Ge et al., 2020).

Results

The analysis revealed statistically significant differences ($p < 0.05$) between families for length and weight at hatching and throughout the rearing period (first feeding, oil droplet reduction, weaning and at the end of the larval period), cannibalism rate, mortality and specific growth rate (SGR). There was no significant difference for the swim bladder inflation effectiveness (SBIE).

WGCNA of RNA-seq data showed interactions between genes (grouped in different modules according to their Pearson correlation) and the zootechnical data collected. There were significant modules related to weight at mouth opening, cannibalism and mortality but mostly to the length at mouth opening. Specifically 5 modules were positively correlated to length at mouth opening and 3 modules were negatively associated to it. We found that in positively length-related modules, ribosome biogenesis, translation processes, rRNA processing were most enriched biological processes. On the contrary, in modules negatively correlated to length, the most enriched processes included neurogenesis, neuron projection development, cell-cell signalling, cell projection and cytoskeleton organization.

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Discussion

Here, for the first time, we attempted to explore differences in larval performances by comparing 16 different families in Eurasian perch. WGCNA highlighted the importance of length data at hatching and the results indicate candidate genes and biological processes providing ground basis for understanding mechanisms leading to length heterogeneity in fish.
Ribosomal proteins (RP), such as *rps19* and *rps6*, being positively correlated with the length of newly hatched larvae, are suggested as potential biomarkers in controlling larval development. Furthermore, the analysis outlines a negative link between genes related to cytoskeleton, nervous system and the length trait. During the early life stages, larvae are still evolving and genes involved in the formation of the cytoskeleton and axon growth could play a crucial role in larval performance. In this context, *IQ motif containing GTPase activating protein 2 (iqgap 2)* gene could largely influence the early developmental stages of larvae (Fang et al., 2015).

The outcomes of our study provide new insights into the processes involved in the determination of larval quality and propose WGCNA as a robust approach to investigate the transcriptomic profile of Eurasian perch larvae.

Acknowledgment

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WHAT IS THE DIETARY ZINC REQUIREMENT OF *Litopenaeus vannamei*? - A META-ANALYTIC APPROACH

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Introduction

The mineral zinc (Zn) plays an important role in several physiological functions of shrimp and therefore it is important to know the minimal dietary Zn requirement for shrimp. The bioavailability of Zn, and therefore the requirement, may depend on the Zn source used in feed supplementation (i.e. organic Zn vs inorganic Zn). To date, more than 17 studies reported Zn requirements of 4 shrimp species, of which more than 10 focussed on *Litopenaeus vannamei*. Despite, an overall consensus is lacking on dietary Zn requirement of *L. vannamei* as these study results were highly variable and recommendations range from 15 to 261 mg Zn/kg diet (Bharadwaj et al., 2016; NRC, 2011; Pan et al., 2022; Shi et al., 2020). This study thus attempt to determine the minimal dietary Zn requirement of *L. vannamei* using a meta-analytical approach.

Methodology

Data from published and unpublished studies including a Zn dose-response experiment with *L. vannamei* and at least three dietary Zn levels were collected. Ten studies were selected based on these criteria. Two data sets were created depending on the Zn source (organic vs inorganic). Selected data were standardized prior to the meta-analysis to eliminate inter-study differences due to initial shrimp sizes, magnitudes of growth, and trial duration. For example, weight gain (WG) data were normalized using the following equation; relative weight gain (%) = actual weight gain of the group/maximum weight gain in the given study × 100. Meta-analysis was done using the linear plateau (LP) model (Prabhu et al., 2013). In case the LP model did not fit, a linear regression model was applied. The analyses were done on different response criteria; WG, whole body and hepatopancreas Zn, and Zn deposition (%).

Results

The meta-analysis showed that minimal dietary organic- and inorganic-Zn requirements were 72 and 104 mg/kg diet, respectively, to attain maximum WG (Fig. 1).

Among 10 studies, 5 studies reported whole body Zn (wet weight) and there was a positive linear correlation with dietary Zn levels ($R^2 = 0.49$, $P < 0.001$). Only 3 studies reported Zn deposition (%) and a negative correlation was observed ($R^2 = 0.89$, $P < 0.001$). Three studies reported hepatopancreas Zn, but no specific relation was observed ($R^2 = 0.02$, $P > 0.1$).

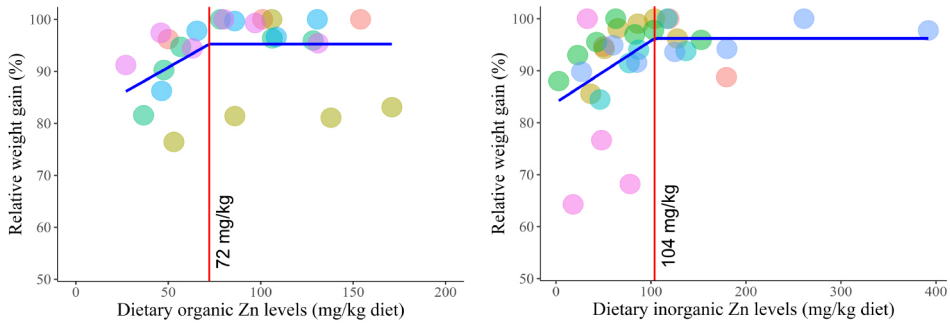

Fig 1. Minimal dietary Zn for *Litopenaeus vannamei* (organic Zn source left graph and inorganic Zn source right graph) estimated using a linear plateau model based on weight gain. Different colors in the graphs indicate different studies.

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Discussion

All studies used WG as a response criteria to estimate minimal dietary Zn requirement of shrimp. The meta-analysis showed that the estimated minimal dietary organic- and inorganic-Zn were respectively 32% and 60% lower than the maximum values reported in literature. The large differences in WG as well as Zn requirements among studies could be a results of different factors like differences in environmental and dietary factors. Diet composition may have an impact on Zn requirement; for example, presence of phytate may increase Zn requirement of shrimp (Davis et al., 1993). Due to a low number of studies it was however not possible to test this in the current meta-analysis.

Apart from WG as response criteria, some studies also looked at whole body and hepatopancreas Zn, or Zn deposition (%). However, estimation of minimal dietary Zn using these criteria was not possible in the current meta-analysis due to the low number of studies.

This meta-analysis showed dietary organic- and inorganic-Zn requirements of 72 and 104 mg/kg respectively for *L. vannamei*. The number of studies used in this meta-analysis was less to draw an absolute conclusions. Further studies determining the minimal dietary Zn requirements of *L. vannamei* are therefore suggested.

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RECORDING DISEASES AND BIODIVERSITY OF EUROPEAN FLAT OYSTER (*Ostrea edulis*) IN THE BELGIAN PART OF THE NORTH SEA: OPPORTUNITIES AND CHALLENGES FOR RESTORATION AND SUSTAINABLE AQUACULTURE

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Introduction

The European flat oyster *Ostrea edulis* is one of the most appreciated mollusks due to its gastronomic, cultural and environmental value, and plays an important role in the marine ecosystem through, e.g., its reef-forming capacity. Despite its historical and ecological importance, flat oyster reefs have completely disappeared from the Belgian part of the North Sea (BPNS). Next to overexploitation and habitat destruction by bottom fisheries, diseases caused by *Bonamia* and *Marteilia* parasites contributed to the demise of oyster populations. To evaluate the feasibility of restoring and cultivating flat oysters, within the H2020 UNITED project we started a demonstration project inside a Belgian offshore wind farm. The goal of this study was two-fold: On the one hand to determine the status of flat oysters introduced in the BPNS and their offspring regarding bonamiosis and marteiliosis, and on the other hand to characterize the fouling biodiversity associated with flat oysters.

Methodology

Flat oysters implemented were initially certified *Bonamia* and *Marteilia* free and originated from Norway (adult oysters) or from England (oyster spat). After six months to two years, these oysters, and their offspring, were sampled (N = 356) during late spring (May – June) or autumn (September – October) from both nearshore and offshore sites within the BPNS. The associated fouling biodiversity was also sampled and taxonomically identified *in situ* and in the lab.

Each oyster sample was split in two, one for histology analysis and the other for Real-Time PCR analysis. Only in case oysters tested positive for the detection of *Bonamia* sp. or *Marteilia refringens* parasites through qPCR, the histologic slides were further analyzed.

Results & Discussion

In none of the tested flat oyster samples, *Bonamia* or *Marteilia* parasites were detected, while the control samples were positive. This is the first time these oyster diseases have been monitored in the BPNS, allowing to demonstrate disease-free status, which is a promising result for oyster restoration and cultivation projects envisaged in the BPNS. Nonetheless, other diseases might be present or emerge in this new oyster population. Thus, it is crucial to continue monitoring by checking health status via histology and qPCR, especially if mortality occurs.

A high species diversity was associated with flat oyster habitat, including other reef-forming species such as the Ross worm *Sabellaria spinulosa*. However, also high densities of tube-dwelling amphipods such as *Jassa* spp. and *Monocorophium* spp. were recorded, posing challenges to offshore aquaculture.

Conclusion

This research is a first, but necessary, step to pave the way for the restoration and sustainable aquaculture of the once abundant flat oyster in Belgium. When situated inside an offshore wind farm, the combination of restoration and aquaculture can be a prime example of sustainable marine multi-use. Furthermore, the restoration of flat oyster reefs brings back a lost habitat with its associated biodiversity and ecosystem services and helps to appreciate again the cultural, ecological and economic value of this once so-important species.

INDOLE SIGNALING, A PROMISING TARGET TO CONTROL VIBRIOSIS IN AQUACULTURE

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Introduction

Diseases caused by pathogenic vibrios cause major losses in aquaculture. The use of antibiotics in order to control these infections has led to the development and spread of antibiotic-resistant pathogens, rendering antibiotic treatments ineffective and causing problems for food safety (Defoirdt et al., 2011). Therefore, novel methods to control vibriosis are needed. A promising alternative is the interference with quorum sensing, bacterial cell-to-cell communication (Defoirdt, 2018). Recent research has shown that indole controls virulence-related phenotypes in pathogenic bacteria and indole is one of the cell-to-cell signaling molecules produced by vibrios. Consequently, we aimed at investigating the interference with indole signaling in order to control vibriosis in aquaculture.

Results and Discussion

We found that indole controls several virulence-related phenotypes in vibrios, most notably biofilm formation and motility. Further, we found that the addition of 100-200 µM indole decreases the virulence to aquatic organisms in all marine vibrios studied thus far, including *V. anguillarum* (in sea bass), *V. campbellii* (in brine shrimp and giant river prawn), *V. crassostreae* (in blue mussel), *V. parahaemolyticus* (in brine shrimp) and *V. tasmaniensis* (in blue mussel) (Li et al., 2014; Yang et al., 2017; Zhang et al., 2022c; Zhang et al., 2022d). At these concentrations, indole did not affect the growth of the vibrios. This is important because it indicates that there will be a lower risk for the spread of resistance against the virulence-decreasing effect of indole signaling when compared to antibiotics.

Given the fact that indole signaling controls the virulence of *Vibrio* species, we have been searching for more potent indole analogues to control vibriosis. Indole analogues are widely present in nature (Lee et al., 2015). Most notably are the auxin plant hormone indole-3-acetic acid and its precursors such as indole-3-acetamide and indole-3-acetonitrile (Zhao, 2010). Indole-3-acetic acid has been shown to decrease biofilm formation and motility of *V. campbellii*, *V. harveyi* and *V. parahaemolyticus* strains at 200 µM and to protect brine shrimp larvae from these pathogens when added at 400 µM to the rearing water (Zhang et al., 2023). The auxin precursors indole-3-acetamide and indole-3-acetonitrile were shown to have a similar effect as indole-3-acetic acid, with indole-3-acetonitrile being active at a relatively low concentration of 10 µM (Yang et al., 2017; Zhang et al., 2022b). Interestingly, auxins are not only produced by terrestrial plants, but also by algae and seaweeds (Stirk et al., 2004; Lin et al., 2022). Hence, micro-algae and seaweeds could be interesting sources of auxins to control vibriosis in aquaculture.

In addition to natural indole analogues, we have also studied synthetic derivatives. A first group of derivatives are indene, 2,3-benzofuran and thianaphthene, in which the N atom of indole is replaced by C, O and S, respectively. All three of these compounds were found to increase the survival of brine shrimp larvae challenged with *V. campbellii* to around 80% or more when added to the rearing water at 200 µM. Further, they significantly decreased swimming motility, but had no effect on biofilm formation. A second interesting group of indole analogues are halogenated indoles. We investigated the impact of 31 halogenated indoles on *V. campbellii* (Zhang et al., 2022a). None of the compounds affected growth of *V. campbellii* for concentrations up to 200 µM, whereas 10 compounds increased the survival of brine shrimp challenged with *V. campbellii* to over 80% when added to the rearing water at 20 µM, and 5 compounds (6-bromoindole, 7-bromoindole, 4-fluoroindole, 5-iodoindole and 7-iodoindole) did so when added at 10 µM. All of these compounds decreased swimming motility of *V. campbellii* at 10 µM and most of them inhibited biofilm formation at 100 µM.

In conclusion, we found that indole signaling controls the virulence of all vibrios we have tested thus far in different host organisms (fish, bivalves and crustaceans) and we identified several indole analogues that are highly effective in blocking the virulence of vibrios. These results indicate that indole signaling is an interesting target for the control of vibriosis in aquaculture.

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TRANS-GENERATIONAL VALIDATION OF CANDIDATE DNA VARIANTS FOR RESISTANCE TO VIRAL NERVOUS NECROSIS AND *Vibrio harveyi* IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

The sustainable development of European seabass aquaculture strongly depends on the ability to control the epidemics that can impact farms. For open ocean farms, one of the main threats is viral nervous necrosis (VNN) caused by the NNV virus which can cause up to 90% mortality. A bacterial pathogen, *Vibrio harveyi* is also considered a highly problematic pathogen across species, including seabass. The mortality caused by *Vibrio harveyi* can reach 50% in case of an outbreak. The French seabass breeding and hatchery industry is now leading the application of new selection methods, notably by being the first in Europe to have implemented genomic selection in this species.

In this study, we obtained whole-genome sequences (WGS; NovaSeq sequencer, Illumina) of sires and/or dams from several selection cohorts which enabled us to perform fine-mapping of QTL regions for VNN and *Vibrio* resistance. GWAS was performed for each population and each resistance phenotype separately, using a Bayesian sparse linear mixed model (BSLMM) with GEMMA software. The most interesting SNPs from each QTL identified by these models were integrated into a panel of SNPs genotyped with the Agriseq technology, in order to evaluate to identify to the effect of these candidate variants in the next generation of fish, for the two diseases studied.

Materials and methods

A total of 5799 European sea bass (*Dicentrarchus labrax*) from two French breeding companies, EMGi and FMDS, were used in a first study (S1), to analyze VNN resistance. In a second study (S2), 3498 European sea bass (*Dicentrarchus labrax*) from the French breeding companies EMGi was used to analyse resistance to *Vibrio harveyi*. To study survival, all offspring were experimentally challenged to VNN in S1 and to *Vibrio harveyi* in S2. Both challenges were performed at the SYSAAF-ANSES Fortior Genetics platform (ANSES, Plouzané, France) to assess resistance by enumerating dead or surviving individuals. All challenged individuals were genotyped on the ThermoFisher 57K DlabCHIP SNP chip (Griot et al., 2021). The whole-genome of the sires and/or dams used in the crosses of the commercial populations were sequenced, on a NovaSeq sequencer (Illumina), in order to identify all genetic variants characterizing both populations. Variant calling was processed according to the DeepVariant best practice and after classical filtering steps we identified nearly 1 million SNPs shared by the two commercial populations for S1, and 2.5 million SNPs for S2. Second, FImpute v2.2 software was used to obtain an imputed genotype for these million SNPs for all S1 and S2 offspring, using their known 57K genotype.

GWAS was performed for each population separately, using a Bayesian sparse linear mixed model (BSLMM) on a dataset of these imputed SNPs for all challenged individuals, using GEMMA software. Several QTL regions were identified on the European sea bass genome, 18 QTL in the S1 analysis (Delpuech et al., 2023) and 14 QTL in the S2 analysis with a Bayesian factor higher than 10. From these QTL, 282 candidate variants for NNV resistance were selected, as well as 82 candidate variants for *Vibrio harveyi* resistance.

A validation approach for these candidate variants was performed. For this purpose, two groups of European sea bass offspring were created: the first group consisted of 2,772 individuals challenged for resistance to *Vibrio harveyi*, and the second group consisted of 3,401 individuals challenged for resistance to NNV. Genetic data from the tested individuals were obtained by targeted genotyping using AgriSeq sequencing (Thermofisher). A total of 563 markers were genotyped and these markers can be divided into three groups, 199 markers to perform parentage assignment, 282 markers to detect NNV resistance, and 82 markers to detect *Vibrio harveyi* resistance. These disease resistance specific markers were genotyped in order to validate in the offspring generation a single nucleotide polymorphism identified by GWAS in the parental generation.

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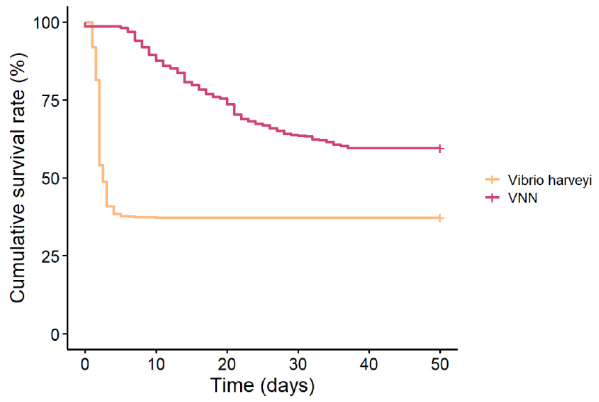

Figure 1: Kaplan-Meyer curve for VNN challenges in red, and Vibrio harveyi challenges in orange. On the one hand, the NNV virus was integrated in the tank by balneation, while for the VIbrio harveyi analyses the bacteria was directly injected into the fish.

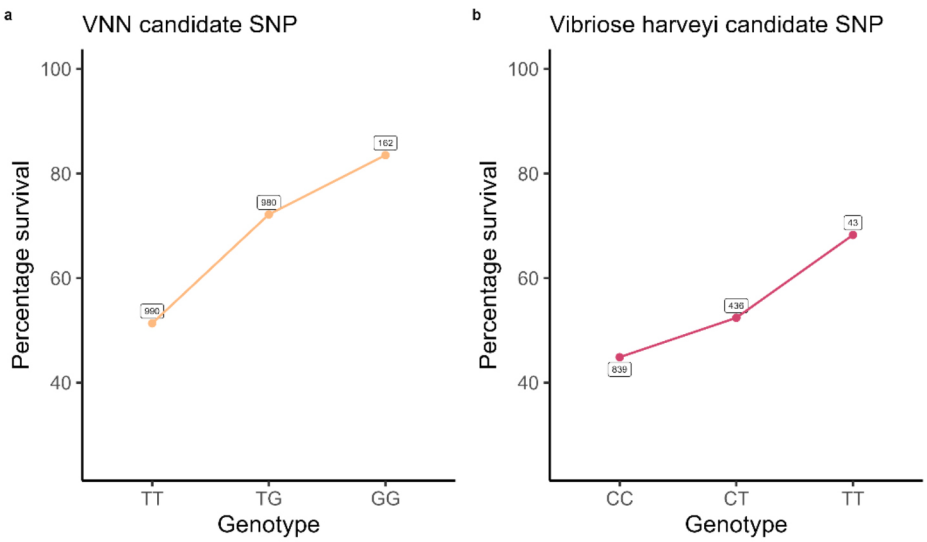

Figure 2: Effect of the main VNN QTL on the survival of individuals challenged with VNN (a) and of the main Vibrio QTL on the survival of offspring challenged with Vibrio (b) for each genotype at these markers. For both graphs the number of individuals is indicated in the box for each genotype.

Results

Challenges for individuals of the offspring generation were conducted over approximately 40 days. Survival rates were 59% for VNN challenges and 37% for Vibrio challenges. Peak mortality was at 17 days post-infection for VNN challenges and at 2 days post-infection for Vibrio challenges (Figure 1).

Survival rates were close to 50% as expected in both challenges. We studied the effect on survival of the highest-effect QTLs identified in the parental generation, on LG12 for VNN (Figure 2a), and on LG22-25 for *Vibrio harveyi* (Figure2b). For each of the candidate markers presented, better survival was confirmed for resistant genotypes compared to susceptible genotypes, identified from previous generations via GWAS analyses.

Further analyses on validation of models for estimation of breeding values will be performed on the same data and presented at the conference.

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COMPARATIVE ANALYSIS OF THERMAL STRESS EFFECT ON BRAIN AND GONADS OF TWO *Genypterus* SPECIES

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The *Genypterus* genus include relevant native species with economic relevance and high potential to Chilean aquaculture diversification. Has been observed that increase in temperature could generate stress in these species. However, still the knowledge on how thermal stress affect at brain and reproductive level is limited. The objective of this work was compare the effect of heat stress in brain and gonads in two *Genypterus* species, the red cusk-eel (*G. chilensis*) and black cusk-eel (*G. maculatus*).

Red and black cusk-eel juveniles were collected and separated into control and stress groups. The stress group was subjected to high-temperature stress (19°C) for five days and then fish were euthanized, sampling brain and gonads for RNA purification and reverse transcription for posterior qPCR. Relative gene expression evaluation through RT-qPCR in each species was performed, normalizing with the geometric mean between two reference genes (actb and taf12). Multiple t-test was performed to identify significant differences.

High temperature produces a significant increase in heat shock protein on brain on the stress group on red cusk-eel (hsp70, hsp60 and hsp90, Fig. 1A, B and C) and black cusk-eel (hsp70 and 90, Fig. 1D and E), join with an oxidative stress response in red cusk-eel and black cusk-eel (sod1 and gpx1, Fig.1F, G, H and I). In the case of gonads thermal stress increases hsp60 on black cusk-eel, but no in red cusk-eel (Fig. 1J), with an oxidative stress response in red cusk-eel (gpx1 and cat, Fig. 1K and L) and black cusk-eel (cat, Fig. 1M). This study showed how thermal stress can affect *Genypterus* species, determining the differences and similitudes in brain and gonad response to thermal stress, information is relevant to the understanding of species response to thermal stress. Funding: ANID FONDECYT Inicio 11230153, Programa de Inserción Académica PIA 82510015.

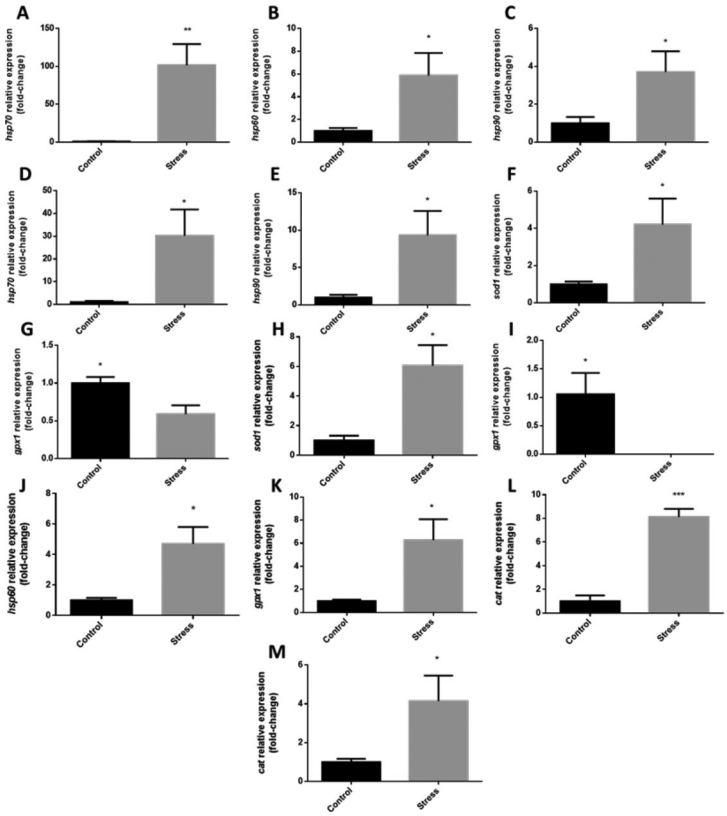

Figure 1. Comparisson of gene expresión on brain and gonads of red cusk-eel and black cusck-eel. Gene expresión on brain of red cusk-eel (A = hsp70, B = hsp60, C = hsp90, F = sod1, G = gpx1) and black cusck-eel (D = hsp70, E = hsp90, H = sod1, I = gpx1); and gonads on red cusck-eel (K = gpx1, L = cat) and black cusck-eel (J = hsp60, M = cat).

DETERMINATION OF MICROPLASTIC POLLUTION IN FISH OF *Genypterus* AND *Merluccius* GENUS IN CHILEAN COAST

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The Chilean teleost fish includes several economically important relevant species for Chilean marine industry. Several of these species have aquaculture potential as red cusk-eel (*Genypterus chilensis*), pink cusk-eel (*Genypterus blacodes*) and black cusk-eel (*Genypterus maculatus*) or are relevant for Chilean fisheries as the Chilean hake (*Merluccius gayi*). However, these species remain in marine ecosystems, therefore, are exposed to different environmental stressors and pollutants. One of these pollutants is microplastics, which are abundant in marine environments. These fish species could be exposed to microplastic presence; however, little is known about the microplastic pollution in the population of these species. This study aimed to evaluate the prevalence of microplastic in *Genypterus* and *Merluccius* species across the Chilean coast.

These species of teleost fish were sampled in different locations of the Chilean coast registering length, weight, and the tissue of the animals. The tissue was digested in KOH and then filtered using Whatman glass microfiber and analyzed using a high-resolution optical microscope. We found microplastic presence in all the *Genypterus* species, showing that this genus is affected by microplastic pollution. In the case of *Merluccius gayi*, we found important contamination with microplastic in the intestine, with more than 20% of prevalence, which presents higher levels compared to previous reports for this species in other locations. In this sense, the condition factor K in this population of Chilean hake was not influenced by the microplastic presence, presenting a low R^2 between microplastic number and K. This information is evidence that microplastic pollution level is a relevant issue for species of *Genypterus* and *Merliccius* genus, evidencing that microplastic pollution is dependent of the species and geographical location, requiring more studies to determine its effect at the physiological level on these species. Funding: ANID FONDECYT Inicio 11230153; Programa de Inserción Académica PIA 82510015; Concurso Interno de Investigación, Creación e Innovación Tecnologica UST 11310013.

EPIGENETIC CHANGES IN PYLORIC INTESTINE OF ATLANTIC SALMON FED DIETS WITH INCREASING LEVELS OF LIPIDS AND CHOLINE

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Introduction

Substantial replacement of fishmeal and fish oil with plant ingredients in the diets has caused certain challenges in health and welfare of the Atlantic salmon. Excessive lipid accumulation or steatosis in the pyloric caeca and mid intestine is such a gut health challenge predominant in farmed salmon. Severe steatosis can cause lipid malabsorption resulting floating faeces in surrounding waters also called lipid malabsorption syndrome (LMS). Through a series of studies, it has been recently established that supplementation of choline, which is deficient in plant-based diets, can prevent the steatosis in Atlantic salmon (Hansen et al., 2020a; Hansen et al., 2020b; Krogdahl et al., 2020). Choline is an essential nutrient and a precursor to produce phosphatidylcholine, the major phospholipid in the lipoproteins that facilitates the transport of lipids from the enterocytes. Moreover, choline can influence epigenetic regulation by being the key methyl donor for DNA or histone methylation.

Epigenetic mechanisms, such as histone modifications, DNA methylation, noncoding RNA and chromatin structure rearrangements can produce heritable changes in gene expression without changing actual genomic sequence. Alterations in DNA methylation in different genomic regions differentially influence gene activities producing diverse physiological and phenotypic changes. Considering the role of choline as methyl donor in DNA methylation, this study was conducted to evaluate the epigenetic changes in pyloric intestine of Atlantic salmon fed diets containing increasing levels of lipid and choline.

Materials and Methods

Four diets were formulated to have lipid levels of 16% (L16), 21% (L21), 26% (L26) and 31% (L31). The first 3 diets were choline deficient and had levels of 1542-1619 mg/kg while the L31 diet had somewhat higher, but still suboptimal choline level of 2310 mg/kg. A feeding trial was conducted with Atlantic salmon parr with initial weight of 25g in duplicate tanks per diet group, at 8□, for 8 weeks.

A genome-wide analysis of DNA methylation patterns was performed by reduced representation bisulfite sequencing (RRBS) to examine the global epigenetic alterations in the pyloric intestine. Library preparation for RRBS was performed with the NuGen ovation RRBS methyl-seq system (Tecan Genomics). Briefly, DNA from 6 replicate samples per diet group were digested with MspI followed by adapter ligation, final repair, bisulfite conversion, amplification and subsequently sequenced on the NextSeq 500. Bisulfite-specific DNA mapping to Atlantic salmon genome and methylation information was extracted using Bismark aligner. Differentially methylated cytosines (DMCs, q value ≤ 0.1 and methylation differences $\geq 10\%$) in CpG context between the genomes of Atlantic salmon fed different diets were analyzed using methylKit package. Genomic feature annotation of DMCs was performed using the HOMER package. Functional annotation of the genes associated with DMCs was performed using g:Profiler online tool and manually inspecting the Ensembl and NCBI data bases.

Results and Discussion

This study revealed that increasing levels of lipid and choline in the diet induced genome-wide epigenetic changes in the pyloric intestine of Atlantic salmon. The highest number of differential methylations were observed between the fish fed diets with largest choline differences (L31 vs L21) and the lowest number of differential methylations were observed between the fish fed diets with similarly low choline levels and highest lipid differences (L26 vs L16). The observations indicated that choline and/or high lipid levels epigenetically modulated several genes of membrane components and transporters, adipose lipolysis and lipogenesis, and microRNAs important for lipid homeostasis. Our observations support the vital role of choline in epigenetic regulation also in Atlantic salmon similar to that reported in higher vertebrates (Zeisel, 2017; Korsmo et al., 2019). The knowledge could be valuable for further optimization of the feed formulations for Atlantic salmon.

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SERUM REDUCTION DOES NOT AFFECT PROLIFERATION IN ATLANTIC STURGEON STEM CELLS

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Introduction

Stem cells are characterized by the unique abilities of self-renewal and differentiation, making them a powerful field of research for many applications (Hong et al., 2011). Fish stem cells are gaining popularity in the last few years thanks to the increasing interest in cellular aquaculture as an alternative and potentially more sustainable protein source to fishing. Currently, two of the main challenges, in developing cell-based seafood, are the lack of available fish cell lines and the poor knowledge about fetal bovine serum (FBS) substitutes for cell cultivation (Goswami et al., 2022). Here, we evaluate the effects of serum reduction on the proliferation of an Atlantic sturgeon cell line (AOXlar7y) derived from larval tissue (Grunow et al., 2011).

Materials and methods

Cell culture

For these experiments, Atlantic sturgeon stem cells from passages 43 to 47 and 78 to 83 were cultured in L-15 medium (Gibco) with 10% of fetal bovine serum (PAN Biotech) at a temperature of 25 and 28°C for proliferation analysis. All experiments were performed within six replicates using different passages.

Real-time cell electronic sensing

To evaluate the effect of lower serum concentration on cell growth, the impedance of the sturgeon stem cells was determined at 2%, 5%, and 10% serum concentration for 7 days. Cells were incubated at 25° C and 28°C in a 96-well plate and cell behaviour was measured via Cell impedance (CI) using the xCELLigence RTCA SP instrument (Roche Diagnostics GmbH). The CI is influenced by three cell parameters: cell number, cell adhesion, and cell size/morphology and can be seen as an indicator of proliferation.

Cell confluence

Cells were seeded in two 12-well plates (TPP) at a concentration of 70,000 cells per well and with three different serum concentrations (2%, 5%, 10%) and incubated for 2 days at 25 and 28°C. Cell confluence was evaluated using the Cell and Gene Therapy Catapult Cell Confluency Tool (https://ct.catapult.org.uk/resources/confluency-tool). Data were obtained from six microscope pictures per well which were taken on the third day of cultivation. Mean ± SD was analysed from each experimental trial.

Actin Filament staining

The morphology of AOXlar7y cells was evaluated by immunofluorescence labelling staining against β-actin. Cells were seeded on 3-chamber slides (Ibidi) at a concentration of 70,000 cells per chamber and cultured for 2 days at 25 and 28°C at 2%, 5% and 10% FBS concentration. For staining, fixed and permeabilised cells were stained with Phalloidin-iFluor 594 (Abcam) and DAPI (Carl Roth GmbH + Co. KG).

Results and Discussion

In this study, we evaluated the effect of serum reduction from 10% to 5% and 2% via real-time cell impedance measurement. The results show that a concentration of 5% FBS does not affect AOXlar7y cell proliferation. The curve from 5% is comparable to that of cells cultured with 10% of FBS, which is the standard amount of serum commonly added in most cell cultures. The proliferation curve of cells cultivated with 2% of FBS is instead markedly lower, confirming the direct correlation between serum quantity and cell proliferation capacity. The effect of the three FBS concentrations was further confirmed by comparing the cell confluence of each condition, which indicated an increase in proliferation for cells cultured at the higher temperature (28°C). The actin filament staining has been used to visualize the cytoskeleton to detect signs of stress or differentiation due to temperature increase and/or serum reduction. In particular, an abrupt serum starvation can be a stimulus for the stem cells differentiation (Messmer et al., 2022), making it more difficult to reduce serum during common stem cell maintenance. The results showed no evidence of differentiation, and cytoskeleton structures remained largely homogeneous between cells under different conditions. However, the cytoskeleton labelling occurred only after two days of cultivation, so further analysis is required after long-term culture.

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Conclusions

The results show the possibility to lower the serum concentration for culturing the Atlantic sturgeon cell line AOXlar7y to 5%, without compromising their self-renewal capacity and without showing signs of stress induction or differentiation. By further investigating the properties of this cell line and the mechanisms by which it responds to certain culture conditions, it may allow us to determine the characteristics that make certain cells more proliferative even in the presence of less nutrition-rich, consequently cheaper, and more sustainable cultivation media. This principle is important not only for in vitro meat production, but also for general laboratory research, as it would make these powerful in vitro models more accessible worldwide.

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AN IMPROVED PROTOCOL FOR TESTING ESSENTIAL OIL ACTIVITY ON FISH PATHOGEN BIOFILM

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Introduction

Aquaculture is recognized as the fastest-growing food-producing industry globally for human consumption (Naylor et al., 2022). The European aquaculture sector alone yields more than 3 million tons of fish yearly, as reported by the Food and Agriculture Organization (FAO, 2020). Numerous marine and freshwater aquatic organisms are vulnerable to bacterial diseases such as vibriosis and photobacteriosis, yersiniosis and aeromonosis respectively, just to mention a few very common ones. The origin of fish pathogens can be correlated to various factors, but their ability to withstand and cause disease outbreaks in aquaculture systems suggests that biofilms are a possible source of persistence, especially for multidrug-resistant bacterial strains that have adapted to the facility settings and may serve as the main source of infection for farmed fish. Therefore, the capacity to develop a biofilm represents a significant concern with possible implications in disease outbreaks as a pathogen reservoir as already demonstrated for some bacterial agents of importance in aquaculture such as Yersinia ruckeri (Coquet et al., 2002). Microbial cells can adhere to each other and to host cells and abiotic surfaces, such as glass, polystyrene plastic, and seashells which promotes colonization and the formation of biofilms. The biofilm comprises densely populated bacteria shielded by a robust exopolymer matrix that firmly attaches to a surface. Forming biofilms leads to the failure of antimicrobial agents, with 1000-fold greater resistance to them (Uruen et al., 2020). The main objective in counteracting fish pathogen infections is the identification of substances (possibly of natural origin, e.g., essential oils), which can effectively target pathogen biofilms. The first step of this approach is the study of *in vitro* systems, which help to demonstrate the efficacy of the selected antibiofilm agents. The present work aimed to improve the currently available techniques to obtain a formed biofilm by fish pathogens as the first step in studying antibiofilm substances.

Materials and Methods

The following strains were tested: *Yersinia ruckeri, Vibrio harveyi, Photobacterium damselae* subsp. *piscicida, Pseudomonas aeruginosa, Aeromonas salmonicida* subsp. *salmonicida, Pseudomonas anguilliseptica, Tenacibaculum maritimum.* Each strain was grown on a specific agar medium and subsequently sub-cultured in Mueller Hinton broth. The density of the inoculum was adjusted to 0.5 McFarland standard, corresponding to approximately 1x10□ CFU/mL. To determine the optimal protocol for studying the efficacy of essential oils (EOs) on biofilm formation, a comparison was made between the growth of bacteria with and without sterilized carapace (obtained from shrimps, 1x1 cm). This assessment was carried out in 24-well plates, where the bacteria were inoculated with a 1:100 dilution of a 0.5 McFarland suspension. The plates were incubated for five days at 28 ºC for biofilm development. After washing samples, the biofilm was detached from the surfaces by water bath sonication for 30 minutes, and the resulting suspension was diluted to 50% in Mueller-Hinton broth. 100 µL of each suspension were inoculated into 96-well plates to monitor their growth curves (24h to OD= 630 nanometers) by Cytation 5 Imaging multi-mode Readers (Agilent biotek). Further, 150 µl of the microbial suspension from the biofilm was resuspended with an equal volume of Mueller-Hinton and treated by adding a commercial mixture based on EOs (MIX-GL) at the concentration of 2xMIC. The treatment was left for 20, 40, 80, and 160 minutes or for 24h. Subsequently, the samples were centrifuged at 2000 rpm for 10 minutes, and the pellet was resuspended in culture broth and inoculated in a 96-well plate to monitor the growth curves (for 24h at OD= 630 nanometers). The cells grown with and without carapace were tested for the sensibility to MIX-GL.

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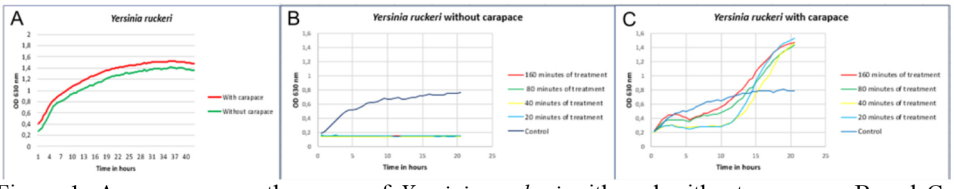

Figure 1. A, average growth curves of *Yersinia ruckeri* with and without carapace; B and C, treatment at different times with MIX-GL at 2 MIC concentration on cells grown without (B) and with carapace (C).

Results

Wide differences in biofilm formation have been shown between strains with the best ability to form biofilm by *Yersinia ruckeri*. All the strains tested could attach to polystyrene plates and shrimp carapaces to some degree, but there are differences in the developed biofilm in relation to the tested surfaces. Using shrimp carapaces as attachment surfaces, it has to be noted that crystal violet can not be used for measuring directly biofilm formation because the absorbance of crystal violet has been shown to be very high, altering the evaluation of formed biofilm: this is probably due to the affinity of carapace to cristal violet also in the absence of biofilm. Therefore, biofilm formation values were calculated by cell growth measurement after detaching them from the different surfaces (polystyrene plates and shrimp carapaces). The formed biofilm on the carapace was higher than that grown-on polystyrene. This result is very important to test the efficacy of antibiofilm compound activity. In fact, it is important that biofilm is allowed to grow in optimal conditions to evaluate the antibacterial efficacy of EOs properly. As shown in Fig. 1 (A), at starting point OD value of *Yersinia ruckeri* was higher in the sample where cells were grown with carapace, and this difference was maintained for all the growth cycle curves. The better performance of cells grown with carapace is accompanied by a higher resistance to the antibacterial effect of EO (Figura 1, B and C).

Conclusion

The choice of the experimental model is crucial to evaluate the antibiofilm activity better and to accurately identify the effective dose of antimicrobial natural substances, such as EOs, avoiding the use of ineffective doses that could select resistant strains.

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INVESTIGATING THE EFFECTS OF INCREASING LEVELS OF EPA AND DHA ON HEALTH, LIPID METABOLISM AND STRESS RESPONSE IN JUVENILES LUMPFISH (*Cyclopterus lumpus*)

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Introduction

Lumpfish (*Cyclopterus lumpus*) is used as a cleaner fish against sea lice infestations in salmon aquaculture. It is widely used in Canada, Norway, Scotland, and Faroe Islands due to its better tolerance to cold water. However, their welfare is still poor as high mortalities occur during the deployment in sea cages. A tailored diet, that covers the nutritional requirements, is essential to provide good welfare, improve survival and maintain their delousing efficacy. Long chain polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid EPA (20:5n-3) are required for growth as well as development and reproduction (Sargent et al., 1999).This study aims to evaluate the effect of five experimental feeds with diverging EPA+DHA levels on juvenile lumpfish to investigate the effects on fish performance, lipid and fatty acid metabolism as well as stress response.

Materials and Methods

Lumpfish, approximately 20 ± 2 g (mean ±SD), were sourced from Nesvík Marine Centre (Faroe Islands) and transferred into an experimental flow through system. Each tank was stocked with 24 lumpfish and was randomly assigned to one out of six diets, in quadruplicate. A basal extruded diet was coated with either rapeseed oil (RO), krill oil (KO) (Aker Biomarine, Norway) or a blend of both oils, generating five experimental feeds with decreasing EPA+DHA levels (22.8-5.6 % total fatty acid; 0KO, 25KO, 50KO, 75KO, 100KO, respectively). A commercial diet was also used as a control (COM). All feeds were produced by Havsbrun (Faroe Islands). Feed was manually delivered at a feeding rate of 2.5% of their body weight. Uneaten pellets were daily syphoned and weighed out in order to record daily feed intake. Four sampling points were carried out throughout the trial which lasted for 52 days: a basal sampling (S0), two nutritional samplings (S1 and S2; 21 and 47 days, respectively) and a stress challenge (S3).

During S1and S2, fish were measured for morphometric data, as well as liver and viscera weight. Three whole fish were stored at -20° for proximate analysis, while ; whole intestine, brain, gills, eyes and liver were dissected from two fish and stored at -20° for lipid analyses. At S3, fish were exposed to an acute stressor (chasing and confinement), after which, fish were left to recover and sampled 1 hour and 6 hours after the stress for morphometric data and plasma cortisol. Linear models were used to investigate the effects of the diets, while a post hoc Tukey HSD test was performed to identify differences between dietary treatments. Principal component analysis (PCA) was performed on fatty acid profile of tissues.

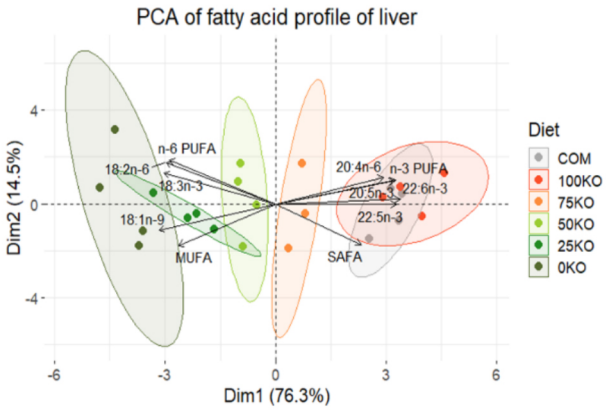

Fig.1. Principal Component Analysis (PCA) biplot of lumpfish liver showing separation of tanks depending on dietary treatments and relative influence of fatty acid profile. Lumpfish were fed one commercial control diet (COM) and five experimental diets with decreasing levels of krill oil (100KO, 75KO, 50KO, 25KO, 0KO).

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Results and Discussion

Preliminary data analysis showed no significant differences in growth parameters, survival and condition indices, in both S1 and S2, between dietary treatments. Despite this, significant differences were found in the mean cumulative feed intake, with fish fed 25 – 100KO inclusion had a higher feed intake throughout the trial (on average 18.1 g), while fish fed the commercial diet had a lower feed intake (10.1 g ± 3.7). There was an interaction in terms of whole body lipid content between diet and time, indicating that some feeds (COM and 75KO) increased the lipid content of the fish faster than the others. Fish fed 25KO had the highest liver lipid content, while those fed 100KO had the lowest. There was also an effect of diet on the whole intestine lipid content (, with fish fed COM showing the highest lipid content in this tissue, while those fed 100KO and 0KO had the lowest. Significant differences were observed in liver between the dietary treatments in terms of fatty acid composition. Liver fatty acid profile reflected dietary input (Betancor et al., 2014), as can be observed in the PCA (Fig.1). The increasing dietary inclusion of EPA and DHA lead to an increase of n-3 fatty acids in the liver, while fish fed diets with high rapeseed oil inclusion (50KO, 25KO, 0KO), displayed higher levels of oleic acid (18:1n-9) and n-6 fatty acids such as 18:2n-6. Significant differences were found between dietary treatments in plasma cortisol 6 hours after the stress, where fish fed a higher inclusion of krill oil (100KO, 75KO, 50KO), had significant lower levels of cortisol. In agreement, fish fed vegetable oils showed higher cortisol plasma levels than fish fed a fish oil rich diet (Perez-Sanchez et al., 2013).

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BEHAVIORAL COMPARISON AMONG WILD AND DOMESTICATED ZEBRAFISH STRAINS: EFFECTS OF DOMESTICATION

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Introduction

Domestication is the process by which a species bred in a captive environment, and modified across successive generations from its wild ancestors, becomes more useful to humans who control increasingly its reproduction and food supply. Domestication has been described in five levels, as the acclimatization of wild fish to human facilities (level 1), the completion of the life cycle in captivity partially (level 2), then completely with wild outputs (level 3) or without wild outputs (level 4), and, finally, to selection programs to meet specific breeding goals (level 5) (Teletchea and Fontaine, 2012). Among traits that change under a domestication process, behaviors as phenotypes are the most directly influenced (Pasquet, 2018). However, few studies have investigated the behavioural differences between wild fish at early domestication (i.e., levels 1 & 2) and fish bred for several generations in captivity (levels 3 and above), despite the fact that such changes can occur very rapidly in the domestication process (Huntingford, 2004). Using the mirror-biting test, we compared behavioral traits involved in activity, aggression and stress, between wild caught zebrafish acclimatized to captivity (F0), their offspring (F1) reared in captivity, and three laboratory wild-type strains (AB, TU, and WIK).

Materials and methods

We tested 174 zebrafish in the mirror biting test under 5 different groups considering the different levels of their domestication: F0 (level 1), F1 (level 4), AB, TU, and WIK (all laboratory strains were considered at level 5). The F0 were wild fish collected from Bangladesh and acclimatized to captive conditions from June 2022. The F1 was the first generation produced by the F0 in captive condition in March 2023. The individuals were housed in 3.5 L tanks part of a recirculated water standalone rack (Tecniplast) under conditions that met the physico-chemical requirements of the species.

An individual 18h acclimatization in the experimental tank with all sides covered occurred before the beginning of each test. All tracking was performed with the R- package trackR (Garnier 2022) in RStudio (R Core Team 2022; version 4.2.1). The tracking was conducted for each individual, when the mirror cover was removed and lasted one hour generating 90,000 frames. Behavioural analysis was done using RStudio (R Core Team 2022; version 4.2.1), by coding an optimized script to obtain behavioural results from the data table produced by trackR after tracking. The most relevant behaviors for the mirror-biting test were based on definitions used in the literature (*see* (Audira et al., 2018; Kalueff et al., 2013)). All statistical analyses and formatting of results were carried out using RStudio. First, correlation between traits were assessed (i.e. Pearson's correlation) and for highly correlated traits, one of the traits was randomly chosen. Second, a principal component analysis (PCA) was done to assess divergences between the groups tested. Third, the potential divergences between the five groups of zebrafish were further assessed by a global multi-response permutation procedures (MRPP) using R-package vegan (Oksanen et al., 2020) and 10000 permutations. In parallel, we also tested a potential divergence between sexes and the two AB age classes with a MRPP. As these results were not statistically significant (p-value = 0.56 and 0.62, respectively), we no longer distinguished between the sexes and ages in the analyses that followed. Fourth, when the global MRPP was significant (e.g. p-value < 0.05), we performed a pairwise MRPP between each pair of groups and applied a Bonferroni correction. Fifth, an indicator value (IndVal) (Dufrêne and Legendre, 1997) R-package labdsv (Roberts, 2023) was performed to determine which behaviors were most expressed by each of the five groups of fish. Finally, we investigated the intragroup variability by calculating the distance between individuals and the centroid of each group in PCA space. These distances were then compared between groups using a global Kruskal-Wallis test and followed, if the test result was statistically significant, i.e. p-value <0.05) by a Dunn's test using the R-package rstatix (Kassambara, 2023).

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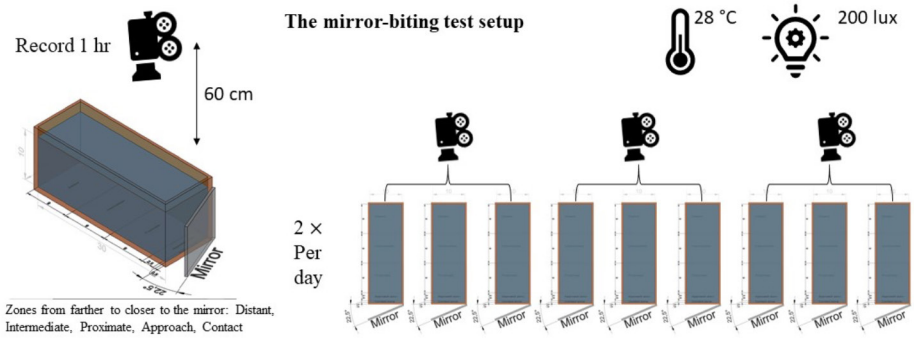

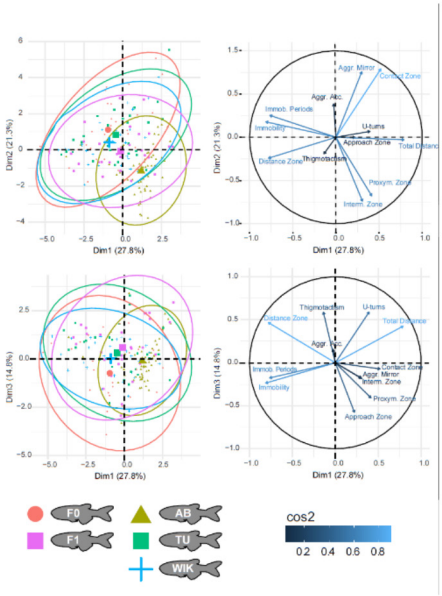

Results

The three first axes of the PCA were taken into consideration and they represented 27.8% of the variance (axis 1), 21.3% (axis 2) and 14.8% (axis 3) (Figure). The behaviors contributing to the maximum of variance on the three axes differed. The first axis represented activity, opposing immobility to travelled distance. The second axis represented aggressiveness opposing presence in the contact zone, and aggression to the mirror and presence in zones farther from the mirror. The third axis was more linked to stress opposing thigmotaxis and acceleration to the presence in zones near the mirror. The different strains and generation differed relatively to the PCA analysis. The overall MRPP was significant (A=0.0963, p<0.01). AB and ABw differed from all the other strains and F1. Wild (F0) differed from F1. According to IndVal, the F0 were characterized by periods of immobility and aggressiveness to the mirror. The F1 displayed more erratic behaviors to the mirror alternating aggressiveness (U-turns, aggressive acceleration) and stress (thigmotaxis). AB and ABw were more characterized by activity, and exploration of the different zones. The Dunn's test showed intra-strains variance with AB showing less variability than Wild and F1 (Dunn test: z=4.81 p<0.0005, z=6.2, p=0.0006 respectively).

Discussion

The results showed behavioural differences between the two generations of wild zebrafish (F0 and F1), while AB differed from TU and WIK, although they are supposed to be at the same level of domestication (i.e., 5). Lower aggressiveness in F1 agrees with the domestication increasing docility scenario (Wilkins et al., 2014). Activity, stress and aggressiveness have been linked to the domestication of organisms as modifications from their wild counterparts (Milla et al., 2021). This divergence from the wild during the domestication process is due to the increasing control of humans over a species life cycle, along with the decreasing gene flow from the wild in successive generations (Teletchea and Fontaine, 2012). Behavioral traits have been described as the first to be affected by this process in fish (Huntingford, 2004; Pasquet, 2018) as well as in mammals (Sánchez-Villagra et al., 2016) with main changes involving increased docility (Lu et al., 2022), reduction in avoidance behavior (Álvarez and Nicieza, 2003), changes in risk-taking behavior and in general decreased stress response in the rearing environment (Milla et al., 2021; Pasquet, 2018).

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HOW AQUACULTURE POTENTIAL CHANGES DURING DOMESTICATION: SEEKING THE BEST WAY TO MAXIMIZE IT

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Introduction

Aquaculture's contribution to the total fisheries and aquaculture production is growing, projected to be 53% by 2030 (FAO, 2022). However, to ensure food security and sustainability, it is required to increase the diversity of species that dominate global aquaculture (FAO, 2022). Thus, new domestication programs will be required. Domestication is the process by which a species bred in a captive environment, and modified across successive generations from its wild ancestors, becomes more useful to humans who control increasingly its reproduction and food supply. This process has been described through the five 'levels of domestication', from the acclimatization of a wild species in a captive environment (level 1) to the partial completion of the life cycle in captivity (level 2), with (level 3) or without gene flow from the wild (level 4), up to the selection for traits of socio-economic importance (level 5) (Teletchea and Fontaine, 2012). Historically, domestication has led to many success stories, with however numerous unfruitful attempts due to increased complexity in terms of time and species specificities that hindered the process (Teletchea, 2019). Success requires certain prerequisites with regards to the species biology and phenotypic plasticity making it able to adapt to the farmed environment (Braithwaite and Salvanes, 2010; Driscoll et al., 2009; Mignon-Grasteau et al., 2005). In captivity growth and survival, behavior, reproduction and nutrition shape among others, the so called 'aquaculture potential' being the quantified amount of expression of all traits/biological-functions favorable for domestication and subsequent production (Toomey et al., 2021). However, the aquaculture potential could be modified during the domestication process, according to the management of base populations. Different scenarios of stock management such as no selection, single function (i.e., usually growth-related traits on) selective breeding program (SBP), or multi-function (i.e., selecting on several traits simultaneously) SBP exist. Thus, the right decision for stock management could improve the aquaculture potential and eventually the resilience of stock performance.

Objective of the study

In 2022, we started a domestication program using zebrafish (*Danio rerio*) collected from the wild (Bangladesh) to monitor the evolution of the aquaculture potential over successive generations. The zebrafish has been chosen due to its short generation intervals, the extended information available on its breeding and biology, and since it has been proposed as a good model for aquaculture research (Piferrer and Ribas, 2020). The goal is to apply and monitor 3 stock management approaches: 1) no selection = random SBP, a 2) single-function SBP, and a 3) multi-function SBP from the onset of domestication, and assess their consequences on the aquaculture potential. Our hypothesis is that the multi-function SBP could maintain a more genetically diverse population to be exploited in selective breeding, compared with the traditionally applied in aquaculture single-function SBP, that may promote deleterious alleles, loss of genetic diversity due to increased selection pressures, and genetic correlations with undesirable traits. It is a major challenge to maintain animals' ability to adapt to an increasingly unstable environment (climate, economic context, societal demands), and we question whether a multi-functional SBP approach could provide that higher level of adaptability even with a compromised growth potential. Thus, to validate our hypothesis a comparison from the onset of domestication is needed, with a single-function SBP, and a random selection SBP as a point of reference.

Methods

We reared separately 11 families from the base (F0) population, under 3 SBPs (Multi-function, Single-function, Random) from the onset of domestication. For each family were assigned 3 tanks, each corresponding to an SBP, in a recirculated Tecniplast™ rack (5 fish per L). A main prerequisite was to prevent inbreeding, and thus a pedigree was constructed up to the 4th generation that would not allow the breeding of candidates with same ancestry. At tagging size (70 dpf) breeding candidates were tagged with a visible implant elastomer (VIE, Northwest Marine Technology, USA) to allow the recording of a set of easily measured, least invasive, phenotypic traits. Phenotypic traits were categorized in 1) 'Active traits' used for the ranking and selection of the best breeding candidates (male and female) from each SBP (Multi- & Single- function, not Random) within family, and 2) 'Consequence traits', a broader set of traits (including all Active traits) involving behavioral

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experiments, a thermal challenge, and disease resistance challenge test, used to monitor the evolution of the aquaculture potential. For the Multi-function SBP *active traits* were related to growth, reproduction, and welfare, while for the Single-function *active traits* were only growth-related. Once the best breeding candidates were selected, inter familial mating was performed and a new generation was produced.

Expected Results

This study, currently in progress, require the phenotyping information from more than one generation in captivity in order to be able to assess how the different selective breeding programs shape the evolution of the aquaculture potential. Here is presented the workflow to describe the hypothesis and propose an alternative way that could be beneficial for future domestication efforts in aquaculture, considering animal welfare and sustainable production. However, apparent behavioural differences have been already observed in the mirror test experiment between F0 and F1 (publication in progress), as it has been described that behavioural phenotypes are the first to be altered under domestication and more specifically in one generation in zebrafish (Pasquet, 2018).

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WILL MARINE BIOTOXINS BE A GREATER THREAT TO EXTENSIVE BIVALVE FARMING IN A CLIMATE CHANGE CONTEXT? – THE CASE STUDY OF *Mytilus* spp. EXPOSED TO *Prorocentrum lima* FOLLOWING THE OCCURRENCE OF AN EXTREME WEATHER EVENT

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Introduction

Every year, approximately 15 million tonnes of marine bivalves are produced for human consumption, out of which 89% come from extensive aquaculture production (Wijsman et al., 2019). The demand for these much appreciated seafood products has substantially increased over the last years, catapulting the expansion of this food production sector. Yet, the sector has been facing consistently higher mass animal mortality events worldwide (Soon and Ransangan, 2019; Soon and Zheng, 2020). The occurrence of harmful algae blooms (HABs) is among the most common factors that can lead to the drastic animal losses (Griffith and Gobler, 2020; Soon and Zheng, 2020). *Prorocentrum lima* is a toxic benthic dinoflagellate that has a cosmopolitan distribution from temperate to tropical oceans. *P. lima* is capable of producing diarrhetic shellfish toxins (DSTs), such as okadaic acid (OA) and dinophysistoxin (DTX) to which bivalves are highly exposed upon active suspension feeding during harmful algal blooms (HABs) (Corriere et al., 2021). When the occurrence of an HAB leads to the detection of these toxins in bivalves' flesh at levels above permissible levels, extensive bivalve farming areas are obliged to close, sometimes during long periods of time, as to avoid potential human hazards related with the exposure to these contaminants. Recently, HABs outbreaks have been increasing in frequency, duration and intensity in coastal areas throughout the world, as a result of climate change effects, such as warmer average seawater temperatures, alteration of typical seasonal patterns and, especially, increased occurrence of marine heatwaves which abruptly rise temperatures during a very short period of time. These extreme weather events can be particularly critical to the aquaculture sector in two ways: on one hand, farmed bivalves are acutely forced to live in conditions outside their physiological threshold without any previous thermal acclimation, potentially leading to substantial animal losses; on the other hand, acute temperature shifts often trigger the occurrence of HABs (Maulu et al., 2021), which further defy bivalves' resilience and hamper their harvest for human consumption. Hence, it is utmost important to acquire deeper insights on toxins' bioaccumulation mechanisms and the deleterious ecotoxicological responses they elicit, at both optimal and altered abiotic conditions, as such data will be crucial to develop early warning tools (e.g. modelling) to mitigate the devastating consequences that HABs can have in the aquaculture sector, at ecological, economic and public health levels.

Material and Methods

Mytilus spp. collected from Porto Brandão (Portugal) were transplanted to laboratory facilities at the Portuguese Institute for the Sea and Atmosphere (IPMA I.P.). Here, they were distributed into 12 tanks within recirculation aquaculture systems, comprising 4 treatments (NoToxin+20ºC, NoToxin+24ºC, Toxin+20ºC, Toxin+24ºC), each carried out in triplicate. Bivalves were kept at 20 ºC (the same temperature registered in their natural habitat), while being fed with a non-toxic commercial dried microalgae (*Tetraselmis* spp.) solution. After 20 days of acclimation, seawater temperature was raised to 24 ºC in treatments simulating a marine heatwave (i.e. NoToxin+24ºC and Toxin+24ºC). The occurrence of an HAB was then simulated in half of the tanks (i.e. Toxin+20ºC and Toxin+24ºC treatments) by replacing the non-toxic commercial dried microalgae (*Tetraselmis* spp.) solution for a toxic *P. lima* solution. Upon 5 days of exposure to these conditions, the

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simulated HAB was stopped, i.e. all animals were fed again with the non-toxic microalgae solution for another 5 days of trial. Mussels were collected at the beginning of the trial (i.e. T20 – before exposure), after 5 days of exposure (i.e. T25 – maximum time of exposure) and at the end of the trial (i.e. T30 - recovery), in order to assess DSTs (OA, DTX1, DTX2) bioaccumulation/detoxification in *Mytilus* spp., as well as the ecotoxicological responses [total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), lipid peroxidation (LPO), heat shock proteins (HSP70), lactate dehydrogenase (LDH) and citrate synthase (CS)] in different mussel tissues (digestive gland, muscle and gills).

Results

Preliminary results indicate that DSTs uptake was higher at 20 ºC than at 24 ºC (T25), most likely, due to bivalves' ability to remain inside their shells when subjected to stressful environmental conditions, which might have prevented them from being in direct contact with toxic microalgae. Yet, during the recovery period (T30) toxins' concentrations were not significantly different in the two tested temperatures, therefore, suggesting that the exposure to a marine heatwave did not compromise mussels' ability to detoxify these contaminants. Regarding ecotoxicological responses, even though results are still being analysed, the preliminary data already evidenced remarkable alterations of bivalves' antioxidant (CAT, SOD and LPO) and metabolic (LDH and CS) enzymes activity upon exposure to *P. lima*, especially at warmer temperature. After 5 days of recovery period, most of the analysed biomarkers did not return to basal levels.

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Aurantiochytrium sp. AS FEED ADDITIVES IMPROVED THE RESISTANCE OF PACIFIC WHITE SHRIMP TO VIRAL CHALLENGE ASSOCIATED TO THERMAL STRESS

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Introduction

The microalgae *Aurantiochytrium* sp. is a rich source long-chain polyunsaturated fatty acids (LC-PUFA), containing a high lipid content ranging between 55 and 75% in dry matter, with a docosahexaenoic acid (DHA) concentration of up to 48% of the total fatty acids. Furthermore, it has a cell wall composed of β-1-3 glycans, which have been related to the increase of shrimp immune response (Gupta et al., 2012; Marchan et al., 2018, Rojo-Cebreros et al., 2017). The dietary addition of *Aurantiochytrium* sp. in shrimp under optimal cultivation temperature showed an improvement in the specific growth rate (Wang et al., 2017), with an increase in the level of DHA in the muscle (Guimarães et al., 2019). This work aimed to assess the effect of different addition levels of *Aurantiochytrium* sp. on immune parameters and resistance of Pacific white shrimp reared under suboptimal temperature (22°C) to viral infection in association with thermal stress.

Materials and methods

The rearing trial was performed at the Marine Shrimp Laboratory of Universidade Federal de Santa Catarina, Brazil (LCM/UFSC) using 400 L tanks stocked with 100 *Litopenaeus vannamei* shrimp m^{-3}. They were reared for nine weeks in a clear water system under suboptimal temperature (22°C), and fed diets with 0 (control diet), 1, 2, 3 and 4% of *Aurantiochytrium* sp., all in triplicate. At the end, shrimps were transferred to Aquaculture Laboratory of Instituto Federal Catarinense – Araquari campus, Brazil (LAq/IFC – Araquari), where they were acclimated for 48 h, then infected with white spot syndrome virus (WSSV). The challenge trial lasted seven days, the first four under 22°C and the last three days under 28 °C (thermal stress). A negative control was made with animals inoculated with a WSSV-free inoculum. The mortality was monitored every 3 h throughout the challenge and hemolymph samples were collected before the infection, and at day 4 and 7 of the challenge. The hemato-immunological parameters evaluated were total hemocyte count, phenoloxidase activity, agglutinin titre and total protein concentration. Kaplan-Meyer test was applied to mortality data and multivariate analysis was used to analyze hemato-immunological and mortality data.

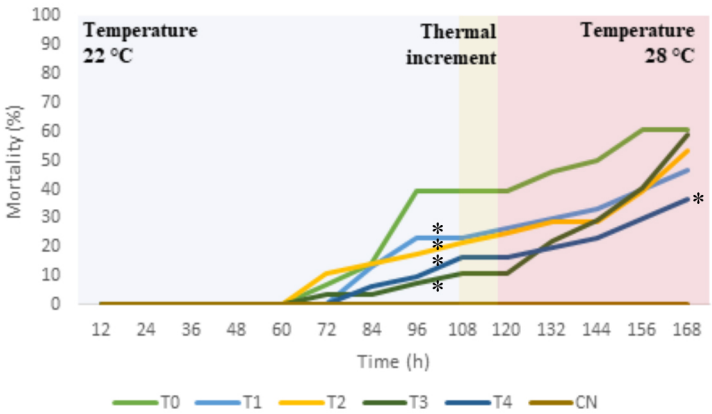

Figure1. Cumulative mortality of *L. vannamei* fed the microalgae *Aurantiochytrium* sp. (0, 1, 2, 3 and 4%) after infection with WSSV associated with heat stress. The negative control (CN) was comprised by uninfected animals submitted to the thermal stress. *ANOVA one-way, followed by Tukey test (p=0.0001)

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Results

All *Aurantiochytrium* sp. fed treatments showed lower mortality after four days of viral challenge under 22 °C when compared to the control diet. However, after thermal increase only the 4% treatment showed lower mortality (Figure 1). Viral infection significantly impacted shrimp immune response. Similarly, thermal stress also affected shrimp immune response, infected with WSSV or not (negative control). Multivariate analyses (MANOVA, Mahalanobis distance and Tocher clustering) showed that animals fed control diet and infected with WSSV, in addition to animals from the 3% treatment before thermal stress, the 4% treatment before and after thermal stress, and, to a lesser extent, the 1% level before and after thermal stress, were significantly different from the remaining groups.

Discussion and conclusion

Both, viral infection and thermal stress, caused significant impact on shrimp immune response. This has been reported in previous studies (Fan et al., 2016, Immanuel et al., 2012). However, this study also revealed that *Aurantiochytrium* sp. dietary addition might help shrimp to cope with these stressors, and consequently showing great potential to be used in the shrimp farming industry. In short, the 4% supplementation of the microalgae *Aurantichytrium* sp. resulted in better immune response and lower shrimp mortality during the viral challenge associated with thermal stress. At low temperature (22°C), microalgae supplementation, in particular the 3% and 4% levels, increased the shrimp resistance to viral infection, demonstrating great potential for use in periods of mild temperatures in subtropical regions.

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UNRAVELING THE IMPACT OF KOI HERPESVIRUS (KHV) AND CARP EDEMA VIRUS (CEV) INFECTIONS ON COMMON CARP (*Cyprinus carpio*) SEMINAL PLASMA PROTEOME

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Introduction

Common carp, *Cyprinus carpio*, is an economically significant fish species in Europe and Asia for inland aquaculture [1]. However, koi herpesvirus (KHV) and carp edema virus (CEV) infections have emerged as substantial threats to carp populations. KHV, an *Alloherpesviridae* family member, causes koi herpesvirus disease marked by gill necrosis and internal bleeding, while CEV, belonging to the *Chordopoxvirinae* subfamily of *Poxviridae*, results in "koi sleepy disease" with clinical signs including lethargy, congested gills, enophthalmos, and skin alterations [2,3]. However, the mechanisms of immunity to these viruses in common carp are still not well understood, especially the immune regulation in the gonad to viral infection. This study examines and compares the seminal plasma proteome of KHV and CEV-infected carp males in relation to non-infected males (CTR).

Material and Methods

In the infection experiments 4 year old common carp males were used, eight carp males were bath-exposed to KHV (320 $TCID_{50}$/ml), while other native carp males were exposed to clinically affected, virus-shedding donor fish carrying CEV, as CEV cannot be propagated *in vitro* [2], uninfected specific pathogen free males served as control. Semen and tissues (gill and gonad) were collected seven days post-infection and stored at -80°C until further analysis. Viral load and gene expression were quantified using qPCR and tissue histology and changes in spermatocyte morphology were examined using hematoxylin-eosin and eosin-nigrosin staining. The differentially abundant proteins (DAPs) in seminal plasma were identified using data-independent acquisition mass spectrometry (DIA-MS) between the control and virus-infected groups (n=8 for each group). DAPs were considered significant if their fold change (FC) was ≥1.5 and the p-value was less than 0.05. Functional annotation was performed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses.

Results

The highest levels of both viruses were detected in the gills, confirming the development of infection. In this tissue gene expression analysis indicated that, CEV induced strong antiviral, proinflammatory complement responses with suppression of T and B cell responses. KHV, on the other hand, induced less drastic inflammation with no adverse effects on the adaptive immunity. The changes in gene expression in the testes had a similar pattern but were less pronounced, despite the fact that both KHV and CEV were found in the testes and semen, with KHV being more abundant than CEV in the semen (Figure 1A). Histological evaluation showed evidence of necrotic changes in the testicular lobules caused by both viral infections, resulting in an increased number of damaged spermatocytes as indicated by the eosin-nigrosin staining technique.

Using DIA-MS, a total of 2,248 seminal plasma proteins were identified, with 2,143 proteins in non-infected males, 2,493 in KHV-infected males, and 2,106 in CEV-infected males. Principal component analysis (PCA) revealed a significant impact of the infections on the seminal plasma proteome (Figure 2B). In KHV-infected males compared to the control group, 660 DAPs were identified, comprising 229 up-regulated and 431 down-regulated proteins. Similarly, CEV-infected males exhibited 1,079 differentially abundant proteins, with 123 up-regulated and 956 down-regulated proteins. A comparison between CEV and KHV-infected males identified 883 up-regulated and 149 down-regulated proteins. Venn diagram

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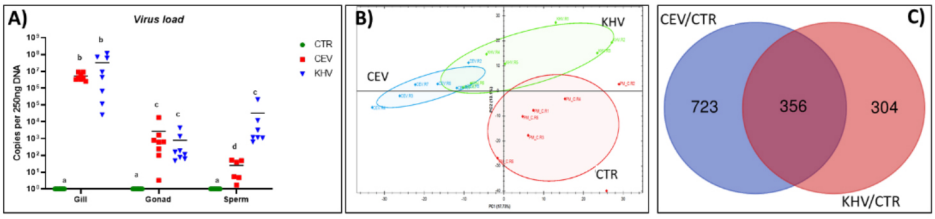

Fig. 1. Virus load in the carp gill, gonad and semen collected 7 dpi with KHV, CEV and control (CTR, A); Principal component analysis of seminal plasma proteome (B); Venn diagram of DAPs between infected and control males (C).

displayed that a large number of DAPs (356 proteins) were commonly changed by both the KHV and CEV infections (Figure 1C). Among these common proteins, 54 were up-regulated, 287 were down-regulated, and 15 showed opposite direction changes. Specific proteins were identified as highly induced (FC>10) under KHV infection, including PYCARD, UBE2E1, HBA1, TTN, PLAUR, LXN, SCG3, and ARSA while CEV infection led to increase of CFB, hatching enzyme, and HBA. GO and KEGG analysis revealed that proteins associated to innate immune response, triglyceride metabolic process, complement and coagulation pathway, response to reactive oxygen species were up regulated whereas proteins related to leucocyte and neutrophil mediated immunity, carbohydrate derivative, viral process and protein folding were down regulated following CEV infection. KHV led to up-regulation of proteins related to RNA catabolic process, protein translation and localization, protein folding and viral process whereas proteins related to leucocyte and neutrophil mediated immunity, complement activation and cell adhesion were down regulated.

Conclusion

The presence of viruses in the semen and gonads indicates that both KHV and CEV can breach the blood-testis barrier, leading to the development of reproductive system infections. These infections of the testes, in combination with systemic immune responses, could affect the functioning of the male reproductive system of carp. The findings provide valuable insights into the differential abundance of seminal plasma proteins in virus-infected males, contributing to our understanding of the molecular mechanisms underlying reproductive responses in aquaculture species.

Acknowledgements

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COMPARATIVE PROTEOMICS OF EGGS AND OVARIAN FLUID IN SIBERIAN STURGEON (*Acipenser baerii*)

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Introduction

Sturgeon species, considered living fossils, exhibit unique reproductive system, including gametes, compared to modern teleost fish [1]. Sturgeons are not only important from an evolutionary perspective but also highly valued for their black caviar and high-quality meat. Unfortunately, overfishing for meat and caviar production has led to a severe decline in the sturgeon population, with 27 sturgeon species currently listed as endangered on the Red List. Therefore, gaining a deeper understanding of the molecular processes underlying sturgeon egg formation and quality is of great scientific and practical importance. However, there is a notable lack of comprehensive data concerning the protein composition of sturgeon eggs and ovarian fluid (OF) and their functional significance. To address this knowledge gap, this study aimed to conduct a comprehensive comparative proteomic analysis of Siberian sturgeon eggs and OF.

Material and Methods

The experiments were conducted on five Siberian sturgeons females (age 9-14, body weight 16.5 ± 2.7 kg) maintained at the Department of Sturgeon Fish Breeding Inland Fisheries Institute in Pieczarki, Poland. Eggs and OF from the same female (n=5) were collected using a catheter [2]. OF was collected from the surface of the egg with a pipette, taking care to avoid any contamination with blood. Proteins from eggs (100 mg) were extracted by homogenization (4 x 30 s) in 4 volumes of lysis buffer (8 M urea, 2% CHAPS, 30 mM Tris, pH 8.5) and then sonicated (6 x 5 s) on ice. Samples were analyzed using liquid chromatography–mass spectrometry (LC–MS/MS) system composed of an Evosep One HPLC System (Evosep Biosystems) coupled to an Orbitrap Exploris 480 mass spectrometer (Thermo Scientific, FDR < 1%). To estimate the protein abundance, emPAI was calculated using unique precursors and normalized by total abundance. To estimate differences in composition between two sample types, median emPAI values were divided and then transformed into fold change values. Functional annotation was performed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. Western blotting was used to validate the mass spectrometry results.

Results

The eggs collected for analysis were characterized by high quality with a fertilization rate at the second cleavage (4 h postfertilization) of 97.5 ± 0.3 and hatching rate of 78.4 ± 6.5%. A total of 565 proteins were identified in eggs, while 617 proteins were identified in OF, with 772 proteins showing differential abundance. Among the differentially abundant proteins, 407 were more abundant in eggs, while 365 showed higher abundance in OF. Furthermore, we identified 287 proteins specific to eggs and 339 proteins unique to OF, along with the top most abundant proteins (emPAI >10) in each; in eggs, the top most abundant proteins were vitellogenins, nucleoside diphosphate kinase A2, cofilin-2, cystatin-B, ubiquitin carboxyl-terminal hydrolase isozyme L1 and zona pellucida sperm-binding protein 3 whereas in ovarian fluid abundant proteins included albumin, apolipoprotein A1, transferrin, hemoglobin, hemopexin, nucleoside diphosphate kinase A and fish-egg lectin.

In eggs, pathways related to mRNA translation (eIF signaling), protein degradation (ubiquitin–proteasome pathway) and metabolic pathways (oxidative phosphorylation, glycolysis, fatty acid β-oxidation, and sirtuin signaling pathway) were found to be the most significant canonical pathways. Conversely, OF proteins were primarily associated with immune system processes, including the complement and coagulation cascade, neutrophil and leukocyte-mediated immunity, cholesterol metabolism, cytoskeleton signaling, clathrin-mediated endocytosis signaling, and integrin and epithelial adherens junction signaling. Eggs were enriched in proteins localized to mitochondria and ribosome components, whereas

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OF-specific proteins highlighted extracellular matrix and secretory vesicles. 117 egg proteins and 102 OF proteins were involved in the reproduction processes including ovulation cycle, oocyte maturation, prevention of polyspermy, ovarian aging, fertilization, sperm-egg interaction and embryonic development.

The changes in the abundance of four selected proteins (ALB, FGB, FN1, VTG2) analyzed using 1D Western blotting were found to be consistent with those obtained from the LC–MS/MS analysis. Western blot analysis confirmed the absence of FGB and FN1 proteins in eggs while demonstrating their exclusive presence in OF. A strong signal of ALB was also detected in OF; however, due to its low concentration in eggs (140 times lower than in OF), it was not visualized under the current method conditions (below the limit of detection). Additionally, Western blot analysis revealed a six-fold higher abundance of VTG2 in eggs compared to OF.

Conclusion

This study presents the first comprehensive characterization of the protein composition of sturgeon OF and eggs, shedding light on their distinct functional roles. The findings not only advance our understanding of sturgeon reproduction but also shed light on egg-OF signaling and the origin of the OF proteins. Moreover, the identified proteins offer potential biomarkers for predicting egg quality, contributing to the development of effective breeding strategies for sturgeon species.

Acknowledgements

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ALGAE VERTICAL PROJECT: SUSTAINABLE MICROALGAE PRODUCTION FOR NEW FUNCTIONAL AQUAFEED

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Microalgae are advantageous ingredients for aquafeeds due to their nutritional quality (such as content in omega-3 fatty acids and protein), increasing resistance to pathologies, reducing nitrogenous excretions, and may even improve growth, physiological activity and, ultimately, fish quality[1]. Microalgae are good alternatives to conventional fish feed ingredients with low sustainability (fish oil and meal) or nutritionally unbalanced with low levels of omega-3 fatty acids (plants)[2]. Despite the fact that microalgae-derived products have been widely used in several industries besides aquaculture, such as food, feed, cosmetics, and agriculture[3], they present several technical and scientific challenges that still require optimizations during their production. One of the main hurdles in the application of industrially produced microalgae products are their high costs, which are influenced by factors such as contamination incidence and the usage of expensive and less sustainable production inputs[4,5]. ALGAE vertical project will seek to work in the current technical bottlenecks of the field, from sustainable production and processing evolving throughout the value chain to placing value-added products on global markets, such as the aquaculture sector. Novel strategies to tackle microalgae production challenges will be studied and evaluated in three Demonstration Units at Necton, Hubel Campina and GreenCoLab. This project will also investigate the optimization of microalgae production sustainability concerning the four main inputs required for their growth: water, nutrients, CO_2, and energy. New prevention or mitigation strategies to avoid culture collapses due to contaminants will also be investigated, towards higher control and stability of the cultures. Additionally, a thorough characterization of the microalgae biomasses regarding their biochemical components, functionality and presence of toxic compounds will be conducted to ensure high-quality microalgae biomass applications, in particular for aquaculture. This will increase the overall sustainability of microalgae production facilities, significantly reducing their production costs, and improving the quality of the products. This work will be part of the Algae Vertical, a consortium of 19 companies and 19 research entities led by Necton. The ultimate goal of the Algae Vertical is to improve the whole value chain of microalgae production and commercialization to give rise to new microalgae-based products, patents and services. The Algae Vertical is part of the Blue Bioeconomy Pact, a project led by Inovamar and funded by the European Union through the Portuguese Recovery and Resilience Plan (PRR), to foster the growth and development of the Blue Bioeconomy in Portugal.

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NEW LIVE FEED ENRICHMENT COMBINING MICROALGAE AND PROBIOTICS APPLIED IN GUILTHEAD SEABREAM (*Sparus aurata*) LARVICULTURE

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Introduction

Development of live feed enrichment products for marine fish larvae is crucial to improve species survival and development. Marine fish larvae have typically low survival at weaning due to the challenges in their nutrition and health management (e.g. pathologies). Altricial larvae present immature systems (e.g. nervous, digestive, immunologic) requiring highly digestible live feed in early nutrition. Live feed used in hatcheries (rotifers and artemia) are zooplanktonic species that do not present the adequate nutritional profile for marine fish larvae development and require bioencapsulation of nutrients through enrichment processes with adequate products. Currently, most efficient enrichment products still use fisheries derived ingredients such as fish meal and fish oil in their formulations, thus it is essential to develop functional and sustainable enrichment solutions to support nurseries. The development of new sustainable products based on the combination of microalgae and probiotics is a valuable formulation strategy to support fish nutrition and health in early life stages. Different microalgae species contain a variety of nutrients essential for marine fish larvae and can be used in formulations to meet specific nutritional requirements and as substitutes for the fish-derived ingredients. Additionally, the inclusion of probiotics in the enrichment formulations may support the improvement of larvae resilience to pathologies and other stressful factors (e.g. transport)[1]. ALLARVAE project focused on the development of microalgae products in powder with probiotics for hatcheries, generating the VITABLOOM gama. The objective of this study was to develop a live feed enrichment product based on the combination of microalgae and probiotics able to promote high gilthead seabream (*Sparus aurata*) larvae survival at weaning and improve their resilience to stressful events.

Material and methods

Two Allarvae pilot enrichments ALLarvae and ALLarvae POC (ALL and ALL POC) were formulated with microalgae and probiotics. ALL POC included a red microalgae *Porphyridium cruentum*. The enrichments were manufactured and tested according to characteristics such as decantation, microalgae agglomerates abundance and dimensions and biochemical profile. Live feed was enriched, analysed and used in *S. aurata* larviculture. The control treatment was a high-quality commercial enrichment product (CP). Larvae were cultured until weaning with commercial products or pilot ALLarvae formulation as enrichment in rotifers and artemia in triplicate (200L tanks), with an initial density of 76 larvae/L. At 38 days after hatching (DAH) a stress challenge of transport was performed (220Km). Larvae oxidative stress (glutathione peroxidase and catalase activities) and survival were evaluated.

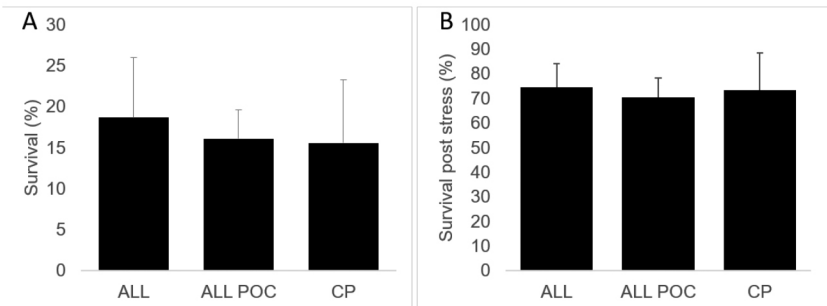

Fig 1 – Gilthead seabream (*Sparus aurata*) larvae survival fed with live feed enriched in Allarvae treatments (ALL, ALL POC) or with the control (CP): A) survival (38 DAH), and B) survival post stress challenge of transport (38 DAH). Data is expressed as means and standard deviation.

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Results and Discussion

The design of the pilot enrichment products was performed according to *S. aurata* nutritional requirements, the biochemical analysis showed that both formulas were nutritionally balanced. *S. aurata* requires 46% of proteins and 22% of lipids[2], ALL presented 34.5% of proteins and ALL POC 34.1%, both formulas revealed 24.2% of lipids, while control showed 19.3% of proteins and 38% of lipids. ALLarvae enrichment products (ALL and ALL POC) promoted similar larvae survival and growth compared to the commercial product (Fig 1), this result suggests that ALLarvae successfully met *S. aurata* nutritional requirements. During stress conditions ALLarvae pilot formulas promoted good biological efficiency resulting in similar survival to the control treatment (Fig 1). There were no significant differences between pre and post stress on the activity of oxidative stress enzymes, suggesting no deleterious effect provoked by the use of the new formulations.

ALLarvae POC enrichment product contained *Porphyridium cruentum*, a red microalga with bactericide properties and immunostimulant polysaccharides. The formula promoted successful larval weaning. The selected formula for final product development was ALLarvae (ALL). ALLarvae is a sustainable enrichment product in powder based in the combination of microalgae and probiotics that promoted good larvae biological performance in *S. aurata*. The availability of this sustainable and effective enrichment in powder is highly advantageous for aquaculture industry due to the logistics simplification, displaying a balanced nutrition for fish and supporting their health.

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LIVER TRANSCRIPTOME DURING SEAWATER TRANSFER IN TWO DIFFERENT ATLANTIC SALMON FAMILIES FED FUNCTIONAL DIETS CONTAINING *Debaryomyces hansenii* YEAST-BASED PRODUCTS

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Introduction

The liver, an important immunological organ in the gut-liver axis, has also recently been linked to smoltification during seawater transfer (SWT) (1). Previous studies have shown liver plasticity when fish were exposed to a different functional ingredient (2) and variation in genotype-specific transcriptomic profiles in liver in responses to dietary interventions (3). The main objective was to evaluate the effect of functional diets containing *D. hansenii* yeast-based products (LAN4 and LAN6) on hepatic transcriptomic profiles in two genetic groups of Atlantic salmon during SWT. The study also investigated whether these functional diets could facilitate the parr-smolt transformation and SWT and whether genetic background affects the hepatic transcriptome and growth performance in Atlantic salmon.

Materials and methods

Fish from two genetic lines (Family A and Family B, AquaGen, Norway) were fed standard diets prior the experiment, vaccinated and transferred to 18 experimental tanks (60 fish per tank) for a total of 7 weeks in fresh water (FW) and then moved to SW for 6 weeks (20 fish per tank). During the whole experimental period, fish were fed a control diet (CD) or two experimental diets containing 0.1% of either component LAN4 or LAN6 (*D. hansenii* yeast-based products). Dead fish with skin ulcers were detected during a natural outbreak in the SW phase, and samples were collected for pathogen detection. At the end of each phase, fish were sampled for various tissues including liver samples for RNA-seq analysis, and comparison between groups (Figure 1) was performed using KEGG enrichment analysis in ShinyGO v.077.

Results and discussion

Fish were healthy during the 7-week FW phase with no mortalities. Seven fish died during the 5th week of the SW phase due to skin ulcers caused by *Moritella viscosa.* All groups tested positive for the bacterium in gill samples, but none of the fish had clinical signs of winter ulcers at the end of the SW phase. No significant differences were found in growth parameters between dietary groups within the same family, but significant differences were observed between families in both FW and SW phases, with Family A exhibiting better growth than Family B, in both water phases.

Figure 2. Top significantly upregulated KEGG pathways in Family A and Family B in LAN4 and LAN6 dietary group compared between FW and SW phase.

KEGG pathway analyses revealed unique pathways linked to distinct effects of water phase, diet, and family in SWT.

In the comparison between FW and SW, Family A showed unique significantly upregulated pathways related to lipid family changes and anabolic processes when fed LAN4 diet during SWT, while Family B had unique downregulated pathways related to immune signaling and activation when fed the same diet. (Table 1, Figure 2).

In the LAN6 group, Family A had unique upregulated pathways related to protein production and cellular growth proliferation. Family B had no unique upregulated pathways in the LAN6 group, but unique downregulated pathways related to primary bile acid biosynthesis, retinol metabolism, lysosome, fatty acid, and sphingolipid metabolism. These results suggest that LAN4 and LAN6 (*D. hansenii* yeast-based products) may have distinct modes of action in salmon liver, resulting in differences in their transcriptomes. These differences may be influenced by the fish's genetic background and environmental conditions.

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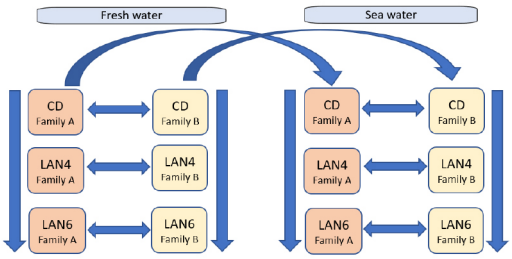

Figure 1. Experimental approach for the characterization of the hepatic transcriptomic profiling of Atlantic salmon fed novel aquafeed with *D. hansenii*-based products.

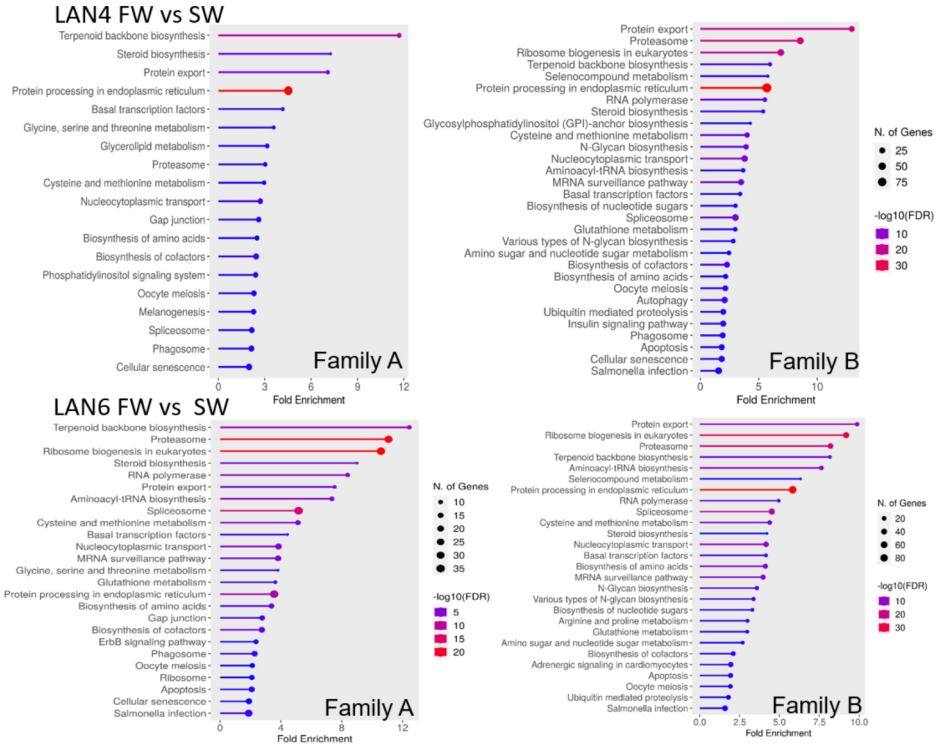

Figure 2. Top significantly upregulated KEGG pathways in Family A and Family B in LAN4 and LAN6 dietary group compared between FW and SW phase.

Table 1. Unique up-and down-regulated pathways in Family A and B in LAN 4 group (FW vs SW).

Fold Enrichment	Pathways	Percentage
Unique significantly upregulated pathways Family A (LAN4) FW vs SW comparison		
3.2	Glycerolipid metabolism	9
2.4	Phosphatidylinositol signaling system	7
2.3	Melanogenesis	6
Unique significantly downregulated pathways Family B (LAN4) FW vs SW comparison		
3.8	Porphyrin and chlorophyll metabolism	13
3.5	ABC transporters	12
2.7	Notch signaling pathway	9
2.7	ECM-receptor interaction	9
2.4	TGF-beta signaling pathway	8
2.2	Biosynthesis of cofactors	8
2.1	Ubiquitin mediated proteolysis	7
2	Carbon metabolism	7
1.9	Focal adhesion	7
3.8	Porphyrin and chlorophyll metabolism	13
3.5	ABC transporters	12

GILTHEAD SEA BREAM MICROBIOTA SHIFTS ASSOCIATED WITH THERMAL STRESS AND DIETARY INTERVENTION DURING A RECORD HEAT SUMMER

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Introduction

Gut microbiota can contribute to regulate growth, nutrient utilisation, disease resistance and physiological stress responses to cope with dietary changes and heat stress, among other environmental stressors. Hence, the composition and function of gut microbiota varies in farmed gilthead sea bream not only with diet, sex, age and season, but also with host genetics that shapes a more plastic microbiota that co-selects with fast growth (Naya-Català et al., 2022; Piazzon et al., 2020). Experimental evidence also indicates that natural and synthetic fat emulsifiers are able to improve growth in gilthead sea bream through changes, at least in part, in gut microbiota composition (Ruiz et al., 2023a; 2023b). The potential of emulsifiers to mitigate the effects of heat stress is proven in broilers (Yin et al., 2021). However, it is not yet known whether similar microbiome shifts are observable with changes in dietary fat levels and the extreme temperature rises associated to global warming. To bridge this gap, we investigated the combined effect of fat level and emulsifier supplementation (Volamel Aqua, Nukamel) in gut microbiota and conventional blood stressor markers during the extremely hot summer of 2022 at the Spanish Mediterranean coast.

Methods

Four isoproteic plant-based diets with 6% FM and two different dietary lipid levels (14%, 16%) with/without Volamel Aqua (0.1%) were formulated and produced by Research Diet Services (RDS, the Netherlands), resulting in four experimental diets: High fat diet (HFD), high fat + emulsifier (HFD-EMS), low fat diet (LFD) and low fat + emulsifier (LFD-EMS). Juveniles of gilthead sea bream (*Sparus aurata*; 12 g initial body weight) were allocated in triplicate 500 L tanks under natural photoperiod and temperature conditions at IATS latitude (40°5'N;0°10'E) from May to August and hand-fed daily until visual satiety. During the first half of the trial (46 days), emulsifier supplementation supported a 10% improvement of feed conversion ratio (FCR) with the increase of water temperature from 20 ºC to 25 ºC. Such improvement was masked during the second half of the trial with the achievement of the historical record of water temperature at our latitude (30.49 ºC, August 9th, 2022). At this time, 12 fish per diet were anaesthetized with MS-222 and sampled for blood (circulating glucose and cortisol) and for intestinal mucus (adherent intestinal microbiota) analyses. Previous samples from fish with the same genetic background not exposed to extreme heat temperatures and fed a commercial standard formulation were used as reference values (REF). Microbial DNA was extracted (High Pure PCR Template Preparation Kit, Roche) and V3-V4 region of the 16S rRNA was amplified and sequenced with Illumina platform. Sequences were quality filtered and taxonomically assigned following a custom pipeline using SILVA database.

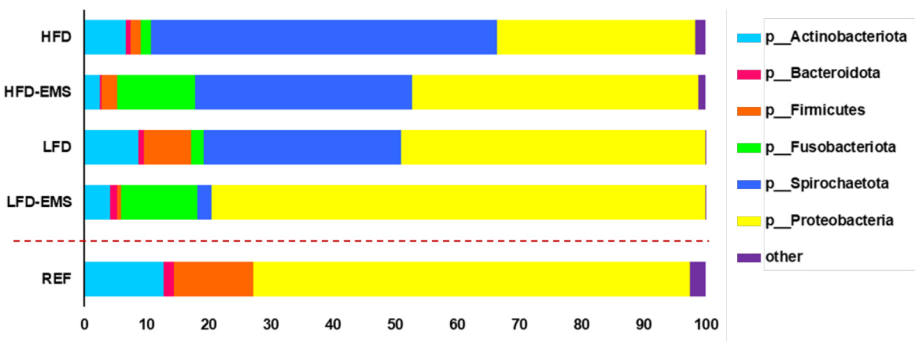

Figure 1: Stacked bar chart representing the relative abundance of bacterial phyla for each of the experimental diets (HFD, HFD-EMS, LFD, LFD-EMS) compared with the reference group (REF) of fish with the same genetic background not exposed to extreme heat temperatures.

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Results and discussion

As shown in previous studies, *Proteobacteria* followed by *Firmicutes*, *Actinobacteria* and *Bacteroidota* were the most abundant phyla in the gut microbiota of REF fish (Figure 1). Conversely, with the increase of temperature, we found that *Spirochaetota* increased dramatically in HFD and secondly in LFD fish. This trend was partially reversed with the addition of the emulsifier, which shaped a microbiota profile in LFD-EMS fish closer to that of fish not exposed to extreme temperatures. At a closer look, partial least-squares discriminant analysis (PLS-DA) highlighted up to 11 genera with a high discriminant value after filtering by VIP>1 and 0.5% abundance. *Brevinema (*representing almost the total contribution to the *Spirochaetota* phylum) was the most abundant genus with a significant discriminant score, and its decrease with the emulsifier addition was concurrent with an increase in relative abundance of *Photobacterium*, *Vibrio*, *Cetobacterium,* and *Bacillus*. Other genera exclusively lowered by the emulsifier or synergistically with the decrease of dietary fat content were *Thauera,* and *Streptomyces* and *Staphylococcus*, respectively. Additionally, both dietary fat level and emulsifier supplementation altered glucose and cortisol levels, being achieved the lowest values of these blood stress markers in LFD-EMS fish.

Concluding remarks

The increase in *Brevinema* genus appeared associated to extreme summer temperature in our experimental model, confirming and extending the possible use of *Spirochaetota* phylum as a marker of heat stress in both fish (Steiner et al., 2022) and pigs (Le Sciellour et al., 2019). Intriguingly, the mitigation of microbiota dysbiosis was favoured by a low dietary fat level, which would depict that dietary intervention can contribute to alleviate in a large extent the negative impact of global warming in farmed fish, although this was not accompanied herein by the improvement of growth performance during episodes of extreme high temperatures. However, the above microbiota shifts were associated to a decrease in plasma glucose and cortisol levels, especially in LFD-EMS fish, which would be indicative of a low energy cost of growth in fish fed low fat diets with the emulsifier supplementation during extreme warming conditions.

Funding

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ELEVATED WATER CO_2 CAN PREVENT LOW-MINERALISED BONE IN POST-SMOLT ATLANTIC SALMON (*Salmo salar*, L.)

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Introduction

Land-based aquaculture systems can have an elevated content of metabolically-derived carbon dioxide (CO_2) in the water. Interestingly elevated CO_2 levels have been suggested to induce nephrocalcinosis and to increase the bone mineral content [1,2]. This raises the question if dietary phosphorus (P), one of the main components of bone minerals, can be reduced in systems with elevated CO_2 levels. The optimisation of dietary P levels is desirable, as excess of dietary P can cause water pollution [3]. Using mono-ammonium phosphate (MAP) as a dietary P supplement, this study aims to analyse if the requirements of dietary P are reduced with an elevated water CO_2 level.

Materials and Methods

Atlantic salmon post-smolt (start weight 200 g) were fed MAP-supplemented diets with a low (0.28%) (0.5P), regular (0.56%) (1P), or very high (2.34%) (3P) estimated available P for 13 weeks. Animals from all diet groups were either subjected to water with an increased CO_2 level (20 mg/L) ($20CO_2$) or to standard control levels of CO_2 level (5 mg/L) ($5CO_2$). Animals from all experimental groups were reared in duplicates. Analytics included radiography, whole mount Alizarin red S staining, mineralised and non-demineralised histology, gene expression analysis, and plasma, and mineral content analysis.

Results and Discussion

The growth of animals fed the 3P diet, $5CO_2$ and $20CO_2$, was reduced compared to $5CO_2$ animals fed either 0.5P or 1P [3]. $5CO_2$ animals fed 0.5P diet showed a known low-mineralised vertebrae phenotype characterised by extended areas of non-mineralised bone; osteomalacia. Plasma P levels in 0.5P animals were reduced. Animals fed 1P and 3P diet animals had fully mineralised vertebrae irrespective of the CO_2 level. The 3P diet had no further effect on bone mineralisation. Unexpectedly, 50% of the $20CO_2$/0.5P animals showed a moderate increase of vertebral centra mineralisation, on x-ray images, whole mount Alizarin red S stained specimens, and histological sections (Fig. 1B,E,E'). This indicates that animals were able to use the dietary P more efficiently when reared in water with high CO_2 levels. Fibroblasts growth factor (*fgf23*) expression, a hormone synthetised by osteoblasts and osteocytes which increases renal phosphate release [4] was downregulated in 0.5P animals. This suggests that more phosphate is retained under levels of increased water CO2 and reduced dietary P intake.

Figure 1. Representative vertebral centra of Atlantic salmon fed a low P diet and reared under low CO_2 (5 mg/L) (A,D,D') or high CO_2 (20 mg/L) conditions visualised on x-ray images (A-C, scale bar = 1.5 cm), whole mount stained with Alizarin red S (D-F), and on non-demineralised histological sections stained by von Kossa/Van Gieson (D'-F') (scale bar D-F' = 1 mm). (A,D,D') Characteristic reduced mineralisation is observed in animals fed a low P diet, characterised by the increased size of radiolucent spaces between the vertebral bodies (A) and by extended areas of non-mineralised bone at zygapophyses (arrowhead in D) and vertebral body endplates (arrowhead in D'). (B,E,E') A moderate increase of mineralisation is observed in animals fed a low P diet and reared at increased water CO_2 levels, characterised by the reduction of radiolucent spaces (B) and reduced areas of non-mineralised bone (E-E'). (C,F,F') A regularly mineralised vertebral bodies in low P animals reared in high CO_2 characterised by a further reduction of the radiolucent spaces (C) and areas of non-mineralised bone (F-F').

The current study indicates that the requirements for dietary P in seawater stages of Atlantic salmon can be reduced when animals are reared in water with elevated CO_2 levels. This offers a possibility to lower the P content in salmon feeds for animals reared in systems prone to accumulate CO_2.

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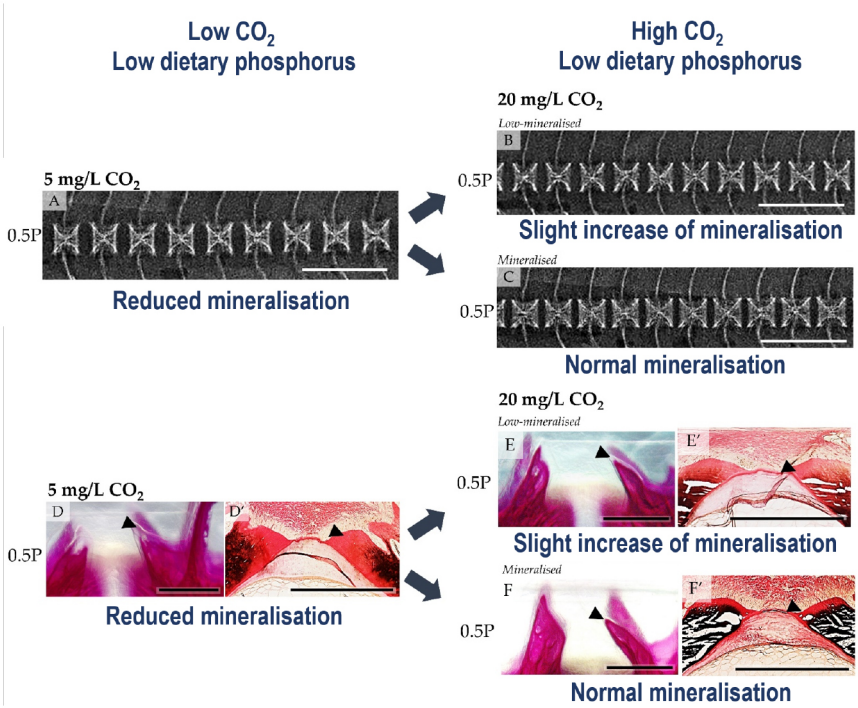

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POTENTIAL OF LOCAL FISH MEAL PRODUCTION FROM AQUACULTURAL SLAUGHTER REMAINS FOR FISH FARMING IN AUSTRIA

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Introduction

Fish meal is a highly demanded resource when it comes to the production of formulated feed for fin fish aquaculture and furthermore in general it is an important source of protein. But in times of a biodiversity-, climate- and energy crises new paths for its provision have to be taken.

The implementation of the Austrian "Strategic Plan 2020 for Aquaculture" caused an increase in domestic production volumes of edible fish. At the same time the amount of fish slaughter by-products has increased. Most of the remains are processed away from the production site. Considering that the slaughter yield is around 50% per individual, a high percentage is eliminated from the on-farm production cycle for human consumption.

In the frame of the project "Sustainable utilization of fish carcasses for the circular economy in Austrian aquaculture" (No. 101742) financed by the Federal Ministry for Agriculture, Forestry, Regions and Water Management of the Republic of Austria and DaFNE, the processing of fish slaughter by-products is one of the main tasks besides a sociological study to clarify the potential of local aquaculture development scenarios and the interest in local fish meal production.

Materials and Methods

To process fish slaughter by-products of the African catfish (*Clarias Gariepinus*, Burchell 1822) a prototype of the Swiss company "Value Recovery Solutions AG" was tested – the further named "VRS Jumbo". This machine can process 250kg of wet by-products by comminution, water evaporation and sterilization within a time span of 7 to 11 hours. After a cool down period the final products are a liquid ("fish oil") and a solid component (fish meal). Several parameters of the default settings were adapted in the testing phase for the following experiments: In a first run 7 x 200kg fish slaughter by-products (carcasses, skins, fish-offals, heads – randomly collected) of the African catfish were processed. From the received fishmeal, samples have been put into high density polyethylene cans and these have been stored at -20°C until further analysis. The wet chemical analysis was conducted at the Feed Laboratory Rosenau – Regional Chamber of Agriculture, Lower Austria - while the amino acids were analyzed at the Institute of Animal Nutrition, Livestock Products, and Nutrition Physiology at BOKU- University, Vienna.

For the second part of the study – the sociological investigation for pin-pointing clearer directions of local aquaculture developments – consultations and opinions of stakeholders have been analyzed. For the narrative interviews three clusters of persons were targeted: (1) administration and legislation, (2) education and consulting and (3) fish producers. For each cluster a specific interview guideline was developed whereas the first question was in all three clusters covering the potential of Austrian aquaculture. The other question-blocks were specific for each cluster and its operations. Several dates were offered for persons from each cluster to participate in an online discussion. The meetings were recorded and further transcribed, analyzed and evaluated.

Results

Analysis of the first runs of the production of African catfish meal revealed that (all values are mean values of the 7 analyzed procedures) the dry matter content (DM) is 92.1%, the crude protein content (XP) is 43%, the convertible energy is 18.62 MJ ME, the crude fat content (XL) is 30%, the crude fibre (XF) is 0.4% and the crude ash content (XA) is 17%. In comparison to other commercial fish meal (60-65% XP) the African catfish meal has a lower protein content, but it has more energy and fat, while crude fibre and crude ash are reduced.

The evaluation of amino acids shows that the ratios are similar to other commercial fishmeal. Especially lysine and methionine as the first limiting amino acids in fish meal present a mean of 2,57 g/100g (lysine) and 0,79 g/100g (methionine).

Within the sociological study limited or missing research results are often the main reasons for consultants and teachers (cluster 2) not being able to provide education and counseling services for fish farmers in terms of fish slaughter by-products processing. Fish producers (cluster 3) actually prefer rendering plants as optimal locations for the disposal of fish slaughter by-products. Furthermore, most of them don't have any preferences in terms of processing fish slaughter by-products at their own companies or at a central location. From cluster 1 no feedback was received.

EFFECT OF SHORT AND LONG TERM ANTIOXIDANT SUPPLEMENTATION IN *Sparus aurata* SPERM QUALITY

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Introduction

The negative impact of oxidative stress on spermatozoa is one of the main damages suffered by these cells, as described by some authors (Guthrie & Welch 2012; Cabrita *et al*., 2014)). In the specific case of fish sperm, there is an increased vulnerability to this type of damage due to the high content of polyunsaturated fatty acids in their membranes. Quality parameters such as: sperm motility, cell viability, apoptosis induction and DNA integrity, are affected by oxidative stress (Cabrita *et al*., 2014). There are many external factors that contribute to this situation, but nutrition is crucial in the regulation of oxidative events, since it allows the incorporation of compounds to support the action of the antioxidant system present in the spermatozoa and seminal plasma (Félix *et al*., 2020). The main goal of this study is to assess the impact of antioxidant diet derived supplementation in fish sperm quality and compare a short and long-term administration to understand if putative effects persist over time or if the feeds lose their effectiveness.

Material and methods

Adult gilthead sea bream (*Sparus aurata*) were fed with 3 different diets (n=21 each): A) control diet, B) vitamins C and E supplemented diet (Vit C+E) and C) selenium and zinc supplemented diet (Se+Zn), along two years. During each reproductive season, males were sampled for sperm collection 4 times, and an average of the results from the first and second year was done in order to evaluate the short (ST) and long-term (LT) effects of the supplementation on sperm quality. From the collected sperm, different quality parameters were evaluated: spermatozoa motility using the CASA system, lipid peroxidation by MDA quantification, cell viability and reactive oxygen species (ROS) through flow cytometry, and DNA fragmentation using Comet assay. Methods have been described elsewhere (Cabrita *et al*., 2011, 2014).

Results and discussion

The motility analysis performed in this study showed a significantly higher spermatozoa progressive motility at long-term (LT) for both supplemented diets. At ST (short term), fish fed with Vit C+E presented significantly higher curvilinear velocity (VCL) than the control and at LT it was higher than the other two treatments. Like in this study, Sarmento *et al*. (2017) also reported better progressive motility (PM) in Nile tilapia (*Oreochromis niloticus*) fed with supplemented vitamin C. This was also observed in *Solea senegalensis*, once fish fed with vitamin E presented higher PM and VCL (Beirão *et al*., 2015). However, the effect of a single vitamin, E or C, or even other antioxidant compounds may be species-specific (Cabrita *et al*., 2011). Vitamin C and E deficiency produces reproductive impairments in seabream such as immature gonads and low hatching rates, demonstrating to play an important role in gamete maturation (Izquierdo *et al*., 2001). In terms of cell viability, there were no significant differences, neither between treatments nor between periods of supplementation. In the case of ROS production, no significant differences were found among the 3 groups at ST, but there was a significant increase in the Se+Zn supplemented group at LT, suggesting a decreasing effect of this diet over time. All 3 groups presented a significant increase in lipid peroxidation at LT, with Se+Zn showing the highest MDA levels. Regarding DNA fragmentation, at ST, Vit C+E displayed higher DNA degradation than the control. A general increase in DNA fragmentation was detected at LT, without significant differences between the control and Vit C+E group, which may indicate that more time is needed to detect the positive effects of this supplement.

Conclusion

According to these results a short-term beneficial effect of vit C+E diet in terms of oxidative stress was observed, although there were no evidence of a reduced effect at long term. On the other hand, Se+Zn diet presented a depleted action over time. Further studies concerning a longer period of supplementation should help to better understand these mechanisms.

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UTILIZING YOLOv5 TO AUTOMATE COUNTING OF ERYTHROCYTE CELLS IN FISH SPECIES RAISED AT FISHFARM KREUZSTEIN AT THE FEDERAL AGENCY FOR WATER MANAGEMENT (BAW, IGF)

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Introduction

Haematological and biochemical parameters of blood are widely used to determine fish health status and the effect of environmental factors (Fazio 2019; Esmaeili 2021; Witeska et al. 2022). One commonly used parameter is the erythrocyte count (RBC, red blood cell count) in a set volume of blood (e.g. Huffman et al. 1997). Traditionally, erythrocytes for RBC are analyzed via manual inspection which is time-consuming and can also lead to interobserver differences (Fazio 2019). Alternative options for blood analysis, such as automatic haematology analyzers and flow cytometric methods, have been proposed (Fazio 2019), but the necessary instruments for such analyses are quite costly and may not be easily available for small labs. Nowadays automatic image recognition is well established in various areas and has become more straightforward to use in custom-tailored applications. YOLO, which stands for 'you only look once' is such an architecture based on a convolutional neural network (CNN) (Redmon et al. 2016; Nakahara et al. 2018). The underlying algorithm divides each picture into a grid system, in which each cell of the grid is responsible for object identification. This method is characterized by high speed in identification and rapid training results. The aim of this case study was to utilize open-source image recognition software to automate blood cell counting for salmonid fish raised at Fish Farm Kreuzstein.

Material and Methods

Blood was collected from young of the year brown trout (*Salmo trutta*). Due to their small size fish were anaesthetised with an overdose of MS222 and 10μl blood was collected with a pipette from the cut heart ventricle. The blood was instantly fixed in 1ml 0.1mol^{-1} cacodylate buffered 4% glutaraldehyde solution (pH 7.4). Blood samples were put on a Neubauer counting chamber and pictures of blood were taken at a 40-fold magnification with a light microscope (Motic BA2010 LED). Identical sections of the picture were cropped using Preview (version 11.0). For image recognition, we used YOLOv5 from Ultralytics (Jocher et al. 2022). Transfer training was conducted with pre-trained model weights yolov5s (COCO128 dataset) and 78 labelled images (5487 instances) and one background image. The epoch size was 300 (default) and for image size 640 (default). The validation dataset included 17 and the test dataset nine images. We did no fine-tuning or comparisons of different models. The trained model weights were exported to TensorFlow.js format. The frontend interface for the end-user was created with VueJs and the meta-framework Quasar. Deployment was done via GitHub Pages. The annotated files, current trained weights and single-page application code is available under GNU Affero General Public License v3.0 on GitHub: https://github.com/HannesOberreiter/baw-fish.

Results and Discussion

These results exhibit the potential of object detection algorithms for research purposes and rapid prototyping. As our first MVP (minimal viable product) was already running after three days of work. While licensed software is often not accessible to small labs, object detection algorithms like YOLOv5 (now also available as YOLOv8) allow for relatively straightforward use for different recognition applications. After training with only 79 labelled images, the resulting best model weights showed a mAP50 of 97% (Precision 96%, Recall 94%) on the test dataset. The use of modern JavasScript frameworks, in our case VueJS, allowed us a rapid development of a user-friendly interface for lab personnel to upload their images and see the results. The visualization of the results was very important to allow human-based decisions in edge cases, for example, problems of overlapping cells when blood cells aggregate. The exported final model weights in TensorFlow.js format were quite small (around 20MB) and allowed to outsource the prediction to the device of the user. This means we only need static asset hosting and no expensive server hosting to do the calculations. Our goal was to find a quick solution for a previously tedious task, which was achieved with tools that have good and relatively easy-to-understand documentation and are well-established. Of course, team members with programming skills are needed to make use of these technologies, but if available, programmes like YOLOv5 grant interesting opportunities for workflow improvement and automatization. This is not only limited to blood cell counts but could also be extended to other applications like fish egg counting, the measurement of cells or cell type identification, limited only by programming skills or time constraints.

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AQUEOUS-PROCESSED RAPESEED/CANOLA PROTEIN CONCENTRATE: TOWARD RECYCLING NON-NUTRIENT DENSE MEAL AND CONTRIBUTING TO A MORE SUSTAINABLE SALMON FEED PRODUCTION SYSTEM

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Introduction

Rapeseed/canola (*Brassica napus* L.) stands as an underdeveloped reservoir of beneficial nutrients for aquaculture. Aqueous extraction of soluble proteins from defatted rapeseed/canola meal represents an innovative processing method that is safe, environmentally friendly and with minimal impact on protein denaturation (Aider and Barbana, 2011; Campbell et al., 2016). This processing method can create a rapeseed/canola protein concentrate (~78% crude protein) with low levels of phytic acid and insoluble fibers, and that has potential to contribute to circular economy by increasing the use of crop by-products. In this research, the process was applied to produce an aqueous-extracted protein concentrate (APC) destined to serve as ingredient in aquaculture feeds. A series of studies were conducted to (1) evaluate the long-term effect of graded inclusion levels of APC on growth, feed and nutrient utilization, body composition, fillet color and gut histology of post-smolt Atlantic salmon, and (2) estimate the nutrient digestibility of APC in juvenile Atlantic salmon in freshwater and in post-smolt Atlantic salmon.

Materials and methods

Rapeseed/canola processing: Defatted meals obtained from rapeseed/canola crushing plants were processed at a pilot-scale facility through different steps involving protein extraction, precipitation and separation using water as solvent and no chemical such as hexane.

Long-term efficacy: Post-smolt salmon (228.0±4.9g) were fed graded inclusion levels of APC over a six-month study. Eight isoproteic and isolipidic experimental diets containing 0 (Diets A, E), 10% (Diets B, F), 15% (Diets C, G) and 20% (Diets D, H) APC were randomly allocated to 24 750-liter tanks at 33 fish per tank. Diets A through D were formulated to mimic commercial salmon feeds in geographies where processed animal proteins (PAPs) are used (e.g. Americas), whereas diets E through H included no PAP (European-type diets). The proximate (protein, lipid, dry matter, ash) and amino acid composition of the test ingredient, diets and whole-body of salmon were analysed according to AOAC methods. Effect of APC inclusion levels on growth performance, nutrient utilization, fillet color, gut histology and pellet physical characteristics were measured and analysed statistically.

Nutrient digestibility studies: Juvenile salmon (57.3±6.7g) were fed with a reference diet and three test diets combining the reference diet with APC in 90:10, 80:20 and 70:30 ratios in freshwater (14.9±0.4°C). Feces were collected using the settling column passive method. Another digestibility study was conducted with post-smolt Atlantic salmon (227.7±4.1g) also fed a reference diet and three test diets combining the reference diet with APC in 90:10, 80:20 and 70:30 ratios in saltwater (25ppt; 13.7±0.5°C). Feces were collected using the manual stripping method. For both studies, the proximate (protein, lipid, dry matter, ash) and amino acid composition of the test ingredient, diets and feces were analysed according to AOAC methods. The apparent digestibility coefficients were estimated using equations from NRC (2011) and results were analysed statistically.

Table 1: Mean (standard error) apparent digestibility coefficients (ADCs) of APC in Atlantic salmon at juvenile and post-smolt life stages.

Nutrient	ADCs	
	Juvenile	Post-smolt
Protein	91.6 (2.0)	95.7 (0.8)
Lipid	89.9 (3.2)	90.9 (3.1)
Essential amino acids	84.7 (2.9) – 97.5 (2.8)	93.9 (0.6) – 98.0 (0.6)

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Results

Rapeseed/canola processing: The crude protein content of APC was consistent across the three lots processed during this project at 77-79% (as-fed). The amino acid profile was comparable to that of fishmeal.

Long-term efficacy: Growth performances, measured using the thermal-unit growth coefficient (TGC), varied between 0.143 and 0.160, and were not significantly different among treatments ($P>0.05$). Feed conversion ratios (FCR) were ≤ 1.08 and, although there was a significant APC effect ($P=0.003$), differences were marginal and did not correlate with APC inclusion. Over the 168-day study, the best TGC and FCR were obtained with salmon fed 10% CPC, regardless of formula type. Proximate composition, nutrient deposition rates and retention efficiencies were similar across treatments. Although not significant, fillet redness improved with APC inclusion. APC inclusion level correlated positively and significantly with distal intestine villi length ($P=0.031$). Villi length of salmon fed 20% CPC (1053.3 ± 28.0 µm) was significantly superior to that of fish fed the control diet (931.7 ± 21.4 µm) ($P=0.006$). The proportion of distal intestine with no inflammation of the lamina propria and submucosa increased with APC inclusion.

Digestibility studies: The results of both digestibility studies are summarized at Table 1.

Discussion and concluding remarks

Rapeseed/canola processing: The environmentally friendly aqueous processing method is suitable for producing protein concentrates from oilseeds.

Long-term efficacy: APC stood as a promising sustainable and safe protein alternative for salmon feeds in this long-term study. With a high protein content and an amino acid profile comparable to fishmeal, dietary inclusion of APC at all levels tested in this study supported growth and optimal nutrient utilization while improving fillet redness and gut health of post-smolt Atlantic salmon.

Digestibility studies: Digestibility of protein, lipid and essential amino acids of APC was high at all life stages and unaffected by APC inclusion level.

To conclude, the next steps will consist in describing further the positive effects of APC on fish welfare/health in challenged fish and assessing the environmental impact/performance of this innovative ingredient.

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QUALITY AND QUANTITY: THE EFFECTS OF LIGHT CONDITIONING ON *Palmaria palmata* BIOMASS PRODUCTION FROM VEGETATIVE CULTURE

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Palmaria palmata is a valuable Atlantic seaweed; Ireland is a major producer. Current retail value is €80-€120 kg^{-1} (dry), with 80-100 tonnes (wet weight) wild-harvested annually. Cultivation is also possible; at sea (longlines) and on shore (tanks). In a series of vegetative growth trials, frond tissue ('tips') were grown to assess 1) effect of photoperiod (12:12 and 16:8 light:dark; L:D) with nutrient addition (seawater control, 50 % or 100% F/2 culture medium twice wk^{-1}, 100 % F/2 once wk^{-1}); 2) effect of light quality on cultures in 16:8 L:D red, green, blue and white light LEDs compared to older cool white LEDs 3) effect of 12 days in green light, then 12 days in blue light (12:12 L:D in 20 (25%), 40 (50%) and 80 (100%) µmol m^{-2} s^{-1} blue light, 100% green light control, 100% blue light control). Specific Growth Rates (SGRs) of tips were highest (13.89 % day^{-1}) in 16:8 (L:D) and 100 % F/2 twice wk^{-1}. However, 12:12 L:D cultures had significantly higher Total Nitrogen (4%) than 16:8 cultures. Light quality affected biomass of cultures; greatest biomass achieved jointly under Green LEDs and old cool white LEDs, followed by red light, with blue light the least effective. SGRs ranged from 12.2-8.9 % day^{-1}. In the green/blue light experiment, green light alone produced significantly the best biomass results, with overall increases of 220%, compared to green-then-blue light cultures, with 50% and 100% blue cultures not significantly different with 105% and 130%, respectively). Blue light controls increased the least (50%), indicating that initial green light enhanced biomass of blue cultures. Protein results of all trials will be discussed.

EFFECTS OF FISHMEAL REPLACEMENT BY DEFATTED *Hermetia Illucens* ON METABOLISM AND IMMUNE RESPONSES OF EUROPEAN SEABASS (*Dicentrarchus Labrax*)

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Introduction

Fish meal is currently the major protein source for use in aquafeeds, however, due to its dependence on finite fish stocks, a vast amount of research effort is focused on finding alternative protein sources. The use of insect protein as sustainable alternative to animal- and plant-based feeds has been proposed for human and animal consumption. Insects have advantages as feed components, since they reproduce easily, grow fast, have low feed conversion ratio and small need of arable land and water. Moreover, insects are rich protein sources, with well-balanced essential amino acid profiles, rich sources of fat, vitamins, minerals and healthy compounds (i.e. chitin, antioxidants, antimicrobial peptides). It has been found that insect-based diets modulate fish microbiota and improve their immune system, reducing the use of antibiotics in aquaculture. Recently, the European Union (EU) authorized the use of insect meal in aquafeeds (Basto A, 2021), (Kotzamanis Y, 2020). In that frame, the present study aims to explore the impact of partial replacement of fish meal by defatted *Hermetia illucens* (HI) focusing on the underlying mechanisms involved in immune responses, in European sea bass, an economically important fish species for European aquaculture.

Materials and methods

Five experimental diets containing different percentage of fish meal substitution by defatted *H. illucens* (HI6 20%, HI6 30%, HI6 40%, HI6 50% and HI6 60%) and a positive control diet (commercial diet without HI) were used. European seabass juveniles were assigned to experimental tanks and each diet was allocated in triplicate groups. The feeding trial was continued over a period of 73 days. At the end of the feeding trial, fish were weighed and analyzed for whole body composition. Blood was drawn by the caudal vein of fish and serum was isolated by centrifugation. Head kidney and spleen tissues were removed and either used immediately for leucocyte cell suspension or stored at – 80 °C. All animal handling and sampling procedures were conducted in accordance with Greek and EU laws and regulations.

The serum samples were used for serum biochemistry and immunological parameters assessment, according to well established protocols. The factors which were studied include total protein amount, glucose, hemoglobin, nitrite ions (NO_2^-), lysozyme, myeloperoxidase, proteases, proteases inhibitors, immunoglobulin (IgM) and complement C3. Total RNA was extracted from fish head-kidney and spleen and real-time PCR assays were carried out to analyze the expression pattern of different immune relevant genes (IRF7, ISG12, MxA, IL-1b, IL-10, STAT3, IgHM, CD4, CD8a and hepcidin). Leucocyte cell suspensions were prepared for evaluation of macrophage activation by phagocytosis, reactive oxygen intermediates (ROI) and nitric oxide assays. The proliferative ability of the leukocytes was also assessed.

Results and Discussion

The total protein amounts weren't noticeably modulated by any of the experimental diets similarly to serum hemoglobin levels. On contrary, the glucose levels were significantly altered by the HI6 30% experimental diet compared to control diet while all other diets didn't show significant differences on the glucose levels. Non-specific immune responses were assessed in fish serum by measuring the levels of nitrite ions, lysozyme, complement C3, myeloperoxidase, as well as protease and anti-protease activities. A significant decrease in nitric oxide levels of fish fed HI6 40 – 60% diets was observed when compared to the control diet. A more intense decrease was evident for lysozyme levels, since all experimental diets had less serum lysozyme than the control diet. The myeloperoxidase levels were also altered by the experimental diets: The HI6 60% MPO levels was significantly lower compared to the control diet, while the MPO levels in the other experimental diets were slightly decreased. The remaining parameters (C3 levels, proteolytic and anti-proteolytic activities) were not affected by any of the experimental diets. The immunoglobulin M (heavy chain) expression was determined both in the molecular

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and protein level. In serum, the IgM levels were slightly increased with experimental diets compared to the control diet, however no significant modulation was observed. Accordingly, the IgHM gene expression levels were slightly down-regulated. Gene expression profile was analyzed to evaluate the modulation of immune-related genes in sea bass fed with two experimental diets: HI6 30% and HI6 60%. The studied genes in sea bass head kidney were related to cytokines (IL-1b, IL10) and interferon pathway (ISG12, IRF7, MxA, and STAT3) whereas those analyzed in spleen were T-cell markers (CD8a and CD4) and an antiviral peptide (hepcidin). All analyzed genes were differentially expressed to some extend but gene expression differences were not statistically significant for any of the tested groups.

The possible immunostimulant effect of fish diet replacement was assessed by means of the phagocytic, respiratory burst, and nitrite production activities as well as clonal expansion of HK cells obtained from fish fed with increasing diet replacements after incubating them with various microbial stimuli *in vitro*. The phagocytosis ability of HK macrophages exhibited a concentration dependent increasing pattern despite the fact that the phagocytic capacity of the HK cells was the same among the groups tested. The respiratory burst activity was significantly increased in 60% substitution diet while nitrite production unveiled that diet substitution did not further enhanced cells capacity to respond. Accordingly, diet substitution did not affect mitogen-induced proliferation of HK cells after simultaneous triggering with ConA and PMA in comparison with control cells.
Concluding, the experimental diets containing different percentage of fish meal substitution by defatted *Hermetia illucens* didn't induce any immune response, which would indicative harmful effects of insect meals on the fish physiology.

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INVESTIGATING THE PHOSPHATE REMOVAL CAPACITY AND ANTIBACTERIAL ACTIVITY OF MOLLUSC SHELL WASTE

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Introduction

Water is a vital resource for the sustenance of life and it forms the basis of the blue economy. Water quality is often threatened by pollution such as nutrient pollution (eutrophication) and microbial contamination. This causes a deterioration of water quality, infections and in worse cases, death of both humans and animals. Various methods have been used in the removal of microbiological contaminants in water such as physical treatments (filtration), biological processes, chlorination, ultra violet (UV) disinfection and ozonation. However, these methods are not effective in removing nutrients such as phosphates from the same wastewater. Adsorption is one of the methods used in the removal of phosphate from wastewater. The use of low-cost materials makes adsorption a cheap and easy method to use. Mollusc shells are by-products generated during shell processing and the sustainable use of these by-products is key to the actualization of a blue economy within the shellfish processing industry. Can these 'waste shells' and low-cost material, be used in the removal of nutrients (specifically phosphate) and water-borne bacteria? This study aims to investigate the phosphate removal capacity and antimicrobial activity of mollusc shell waste powder in both its non-heat treated and heat-treated forms, against four bacterial strains which affects both humans and fish.

Materials and methods

Waste shells were cleaned and oven dried at 105□ for 2 h, sieved to their respective particle size and calcined for 2 h. Two Gram-negative bacteria, *Escherichia* coli *Pseudomonas stutzeri,* and two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis,* which are widely distributed in the environment (such as in water and soil), were selected as the indicator organisms. The indicator bacteria were prepared following the National Committee for Clinical Laboratory Standards (NCCLS) protocol. The antibacterial activity of the non-heat treated and heat treated mollusc shell waste powder were determined using the well diffusion method at a concentration of 25 mg. Batch experiments were conducted to determine the phosphate removal efficiency of the shells. The shell samples were characterized using physico-chemical methods such as Scanning Electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR) and X-ray powder diffraction (XRD) analysis.

Results

Non-heat treated mollusc shells showed no antibacterial activity against all the bacteria strains. However, the heat treated mollusc shells had good antibacterial activity with activity highest for *B. subtilis* and *P. stutzeri,* followed by *E. coli* and *S. aureus*. Furthermore, the results of the batch experimental study showed that the non-heat treated shells had a lower phosphate removal efficiency when compared with the heat-treated shells. The result of the characterization study showed that heat treatment of the shell powder resulted in a change of structure, morphology and elemental composition all of which contributed to its increased phosphate removal capacity and antibacterial activity.

Conclusion

Heat treated mollusc shells used in this study exhibited antibacterial activity against all the bacteria strains, thus demonstrating a broad spectrum of activity for both gram-negative and gram-positive bacteria. Furthermore, the heat-treated shells show a higher phosphate removal capacity when compared with the non-heat treated shells. Research is currently ongoing on the use of the shells for the simultaneous removal of phosphate and water-borne bacteria from both synthetic and industrial wastewater.

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INSECT OIL AS A DIETARY INGREDIENT IN AQUAFEEDS FOR SILURIFORM FISH

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Introduction

Cheaper alternatives to fish oil are necessary due to the growth of aquaculture and the unfavourable costs of fish oil. Although several alternatives to fish oil have been tested and potential benefits demonstrated, the effects of insect oils on growth and health of siluriform fish are less well defined. To narrow this gap, we highlight the importance of black soldier fly (*Hermetia illucens*) larvae oil as an ingredient in the diets of siluriform fish. The insect oil, which was produced by a defatting procedure was used as a test ingredient in the diets of African catfish (*Clarias gariepinus*) and European catfish (*Silurus glanis*). Here, we present first results on growth, nutrient utilization, plasma biochemistry, enzyme activities, and antioxidation capacity in both species.

Materials and methods

Three isonitrogenous (430 g/kg) and isolipidic (110 g/kg) diets (reference and two experimental) were formulated for the two species, the main difference being the oil source. The reference diet contained a mixture of fish oil and rapeseed oil (1:1 ratio) while the two experimental diets contained either a 1:1 mixture of insect oil and rapeseed oil (H50) or insect oil only (H100). Two feeding trials, one for European catfish and another for African catfish were conducted, lasting for eight weeks. The first trial involved a total of 630 European catfish juveniles (average body weight of 28.1 ± 0.17 g) originating from the institutional hatchery facility at the Research Centre for Aquaculture and Fisheries (HAKI). Fish were distributed into three dietary groups (REF, H50 and H100), each replicated three times (70 fish per tank) and reared in a recirculation aquaculture system equipped with nine 1 m^3 fiberglass tanks. A second trial involving 900 juvenile African catfish (average weight: 29.1 ± 1.69 g) were treated similarly to the first trial. During the eight weeks period, fish were fed daily until apparent satiation using automatic feeders. Water quality parameters (temperature, dissolved oxygen, and pH) were monitored regularly, while nutrients (ammonia, nitrites and nitrates) were monitored on a weekly basis.

Results

All the diets (REF, H50, and H100) promoted adequate growth and feed utilization in both species. Fish growth was more than five-fold of the initial body weight, with no significant differences in growth performance, nutrient utilization, or body indices between fish fed the reference and experimental diets. The plasma cholesterol, glucose, phosphorus, calcium, and total protein contents were not significantly affected; only the globulin (for *S. glanis*) and albumin, and the albumin to globulin ratio, significantly differed between the dietary fish groups in each species. This may indicate upregulated nutritional or metabolic activities in insect-based diets since albumins are involved in transport functions. Plasma alanine transaminase (ALT) activity did not differ between dietary groups of fish for both species, while for the alkaline phosphatase (ALP), significant differences were observed only for *S. galnis* but not in *C. gariepinus*. The activity of plasma lipase significantly differed between dietary groups of *S. glanis* and not in *C. gariepinus*, but overall was highest in fish fed H50 diet and lowest in fish fed H100 diet for both species. No significan differences were observed in lysozyme activity, immunoglobulin and myeloperoxidase content of plasma from the different dietary fish groups in both trials. The activities of intestinal digestive enzymes (amylase, lipase and trypsin) were insignificant in *S. glanis* while in *C. gariepinus*, only amylase activity was significantly higher in H50 than REF and H100 fish groups. The liver antioxidant indices of both *S. glanis* and *C. gariepinus* were nonsignificant between the different dietary groups, but indicated better performance of insect-based diets.

Conclusion

Overall, these results indicated that complete substitution of fish oil or vegetable oils with black soldier fly larvae oil is possible in the practical diets of siluriform fish, without causing deleterious effects on the growth, nutrient utilisation, plasma biochemical parameters, and antioxidation capacity.

FEEDING DURING RAS OFF-FLAVOURS DEPURATION: DOES FEEDING HELP REDUCING DOMINANT OFF-FLAVOURS IN RAS-REARED FISH?

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Introduction
The presence of water-borne off-odour compounds in fish, such as geosmin and 2-methylisoborneol (Abd El-Hack et al., 2022), or less commonly reported feed-borne compounds, such as beta-ionone, butanoic acid, or indole (Podduturi et al, 2017; Mahmoud et al., 2018), can drastically reduce its palatability, leading to consumer rejection and lower market demand of recirculating aquaculture systems (RAS) products. A common off-flavour mitigation strategy is to purge the fish in flow-through systems prior to harvesting while ceasing feeding. However, this procedure results in the loss of fat content and precious market weight, as well as in a far greater consumption of water. Other technological strategies utilise intense ozone and ultraviolet (UV) light, which further increase costs in RAS farms (Abd El-Hack et al., 2022). In one recent study, feeding was observed to increase excretion rate of geosmin in Nile Tilapia (Schram et al., 2021), with important implication on reducing the negative impacts of standard off-flavour purging methods. The aim of this study was to isolate the effect of water born vs feed born off-odour odours in juvenile male Russian sturgeon (*A. gueldensteadtii*) reared in RAS as well as to understand the effect of feeding during standard purging practices.

Methods
A commercial scale experiment was designed to trial four groups of test fish: two groups reared in clean flow through (FT) water and two in RAS water. In each water type, one group of animals was starved for 3 weeks and one fed at standard feeding regime with a commercial feed previously in use. After 3 weeks in experimental conditions, three animals were sampled per test group, euthanised, filleted and frozen at -20°C. Subsequently, the dominant off-odour compounds in the two water sources, in the commercial feed and in the fish fillets of each test group were determined via olfactometric detection of single odour-active volatile compounds coupled with instrumental analytical methods. Aroma distillates were obtained from fish fillets by using solvent assisted flavour evaporation (SAFE), the dominant off-odorants − i.e., exhibiting higher sensory detection thresholds and concentrations − were determined by aroma extraction dilution analysis (AEDA) and finally identified by gas chromatography-mass spectrometry/olfactometry (GC-MS/O) as described by Mahmoud et al., (2016).

Results
Dominant off-odour compounds present in the fish fillets were sourced from both commercial feeds and water types. RAS water was found to be more diverse in off-odour compared to FT water, and ubiquitous dominant off-odours to be detectable with higher AEDA dilution factors (FD) in the RAS water. Geosmin and 2-MIB were present in both water types yet reaching higher FD in RAS water. The dominant off odours present in the commercial feed comprised feed-borne compounds previously published (see Podduturi et al, 2017; Mahmoud et al., 2018), being dominated by butanoic acid (“cheesy, sweaty)”, methyl butanoic acid (“sweaty”), thymol (“thyme-like”), heptanoic acid (“pungent, sour, wax-like”) and 4-hydroxyphenyl acetaldehyde (“horse stable-like) notes. Juvenile male Russian sturgeons reared in RAS water were more diverse in dominant off-odour compounds compared to those reared in FT water. Geosmin and 2-MIB were observed in all four test groups with different FD factors, being in general higher in RAS water. Within each water type, feeding was observed to reduce the FD levels of highly dominant odour compounds, being a strong indication of reduced concentrations, while increasing on the other hand the overall diversity of the odorants, albeit all with relatively low FD factors. Accordingly, feed-borne off-odour compounds were absorbed into fish fillets either by ingestion or by excretion-reabsorption via respiration from RAS water, yet these substances were not detectable in the group starving in FT water. Of the four test groups, the animals fed in FT water yielded the least concentrated dominant off-odours and the highest organoleptic palatability.

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Points of Discussion

In line with the results published by Schram et al. (2021), this study confirms that feeding during standard RAS off-odours purging practices in FT water helps reducing the levels of highly dominant off-odour compounds, such as geosmin. Therefore, feeding during purging should be taken into consideration by aquaculture farmers to reduce the economic loss of standard purging practices. In general, we are convinced that the lack of understanding of the biochemical mechanisms improving off-flavour depuration during feeding should receive greater attention in future research. At the same time, the uptake of highly unpalatable feed-borne odorants in fish fillets is confirmed in this study, in accordance with findings of previous studies. As feed-born volatile organic compounds are obviously absorbed into fish fillets, this source of off-odours should be addressed by feed manufacturer in the future, and the potential for flavour modulation should be considered. Finally, the possibility to create "purging" fish feed formulas resulting in highly palatable fish products from RAS is a promising strategy as demonstrated by this study.

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DURATION OF INCUBATION & LARVAL DEVELOPMENT OF STERLETS (*A. ruthenus*) IN RIVER WATER UNDER NEAR_NATURAL CONDITIONS

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Day-degrees, also known as cumulative temperature units (CTUs) have been used to predict the duration of early development in fish. For most sturgeon species, the available data in literature was conducted under constant conditions. However, there is a lack of information on CTUs of early development in sturgeons under natural or near-natural conditions. The aim of this study was to observe the duration of incubation and the duration from hatch until feeding of sterlets (*Acipenser ruthenus*) under near-natural. This study was embedded within the LIFE Sterlet project and data was gathered from the year 2018 to 2022. The rearing of fish larvae took place in the LIFE Sterlet hatchery container with Danube water without biological, chemical or thermal water treatment to simulate natural conditions. Temperatures were monitored on a daily basis and day-degrees were calculated by summing the temperature over time. Preliminary results indicated slower larval development of the sterlet than described in literature. Hatching started at about 106 day-degrees earliest and took maximal 213 day-degrees. Feeding started between 171 and 2012 day-degrees. The findings of this study provide valuable insights into the temperature-dependent development of sterlet larvae under near-natural conditions and can assist in the design of optimal rearing of sturgeons for conservation efforts.

BLACK SOLDIER FLY LARVAE *(Hermetia illucens)* MEAL IN DIETS FOR ATLANTIC SALMON SUSTAINS GROWTH AND GUT HEALTH DURING FIELD CONDITIONS

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Introduction

To support the rapid growth in the aquaculture industry, there is a need to develop sustainable feed resources as alternatives to fish meal and plant ingredients. Insect meals such as black soldier fly larvae (BSFL) meal are promising alternatives with a low environmental footprint. Previous controlled short-term experiments suggests that BSFL meal is a suitable protein source for Atlantic salmon (1). The aim of this experiment was to investigate how insect meal inclusion impacts growth performance and gut health of Atlantic salmon during the grow-out period in seawater under production field conditions.

Methods

A total of 320,000 Atlantic salmon (*Salmo salar*) with an initial and final weight of 1,200 and 4,500 g, respectively, were fed diets containing either 0, 4, and 8 % defatted BSFL meal (IM0, IM4, IM8) for 5 months in a field experiment on a commercial salmon farm (Nordfjord, Norway). The experimental diets were produced in a twin-screw extruder and the technical quality was assessed for expansion, hardness, durability and sinking velocity. Samples from intestinal content were taken from distal intestine (DI) for microbiota analysis with 16s ribosomal RNA sequencing and DI tissue was histologically analysed with haemotoxylin and eosin, periodic acid-Schiff and Alcian blue staining. In addition, samples were taken from DI for RNA sequencing.

Results and discussion

The physical pellet quality of all diets was high, but expansion, durability and sinking velocity decreased significantly with 8 % BSFL inclusion, indicating that high inclusions of BSFL may reduce pellet quality. No significant differences in growth rate, feed conversion ratio or mortality were observed between the dietary groups. Histological findings were not significantly attributable to diet, but there was a tendency of increased numbers of ectopic goblet cells in the IM4 diet (p = 0.058). For gut microbiota, alpha diversity was not significantly affected by insect meal inclusion, however, beta diversity significantly increased (Fig. 1A and 1B). These findings suggest that insect meal inclusion changed the microbial composition without increasing microbial richness. *Bacillaceae and Lactobaciallaceae* were significantly more abundant in groups fed BSFL. This is in line with previous studies on salmon smolts [1] and post-smolts [2] fed BSFL. Many bacterial species of *Bacillaceae* can produce chitinase [3] and lactic acid bacteria are considered beneficial in the fish gut [4]. RNA-seq of the DI revealed that IM4 compared to IM0 showed only down-regulated terms related to lipid metabolism and biosynthesis (Fig. 2A). When comparing IM8 to IM0 (Fig. 2B), two terms were up-regulated (response to estrogen and positive regulation of gene expression) and one was down-regulated (fatty acid biosynthesis). Moreover, the comparison between IM4 and IM8 (Fig. 2C) showed twenty-one up-regulated terms related to immune signaling, cell proliferation and extracellular components, possibly corresponding to the tendency of increased prevalence of ectopic goblet cells in the DI in the IM4 group.

Conclusion

Replacing conventional protein sources with moderate levels of defatted BSFL meal did not compromise growth performance or health in Atlantic salmon under field conditions. These findings suggest that black soldier fly larvae meal at moderate inclusion levels is suitable as a sustainable, alternative protein source for Atlantic salmon in seawater.

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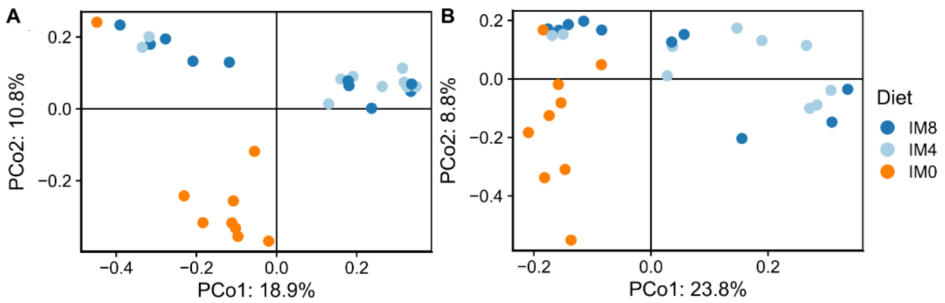

Figure 1: *Principal coordinate analysis (PCo) plots of beta diversity of gut microbiota in salmon fed experimental diets. The indices are* ***A****: Jaccard distance* ***B****: Aitchison distance. The samples are grouped by diet: IM0: 0 % insect meal; IM4: 4 %; IM8: 8 %.*

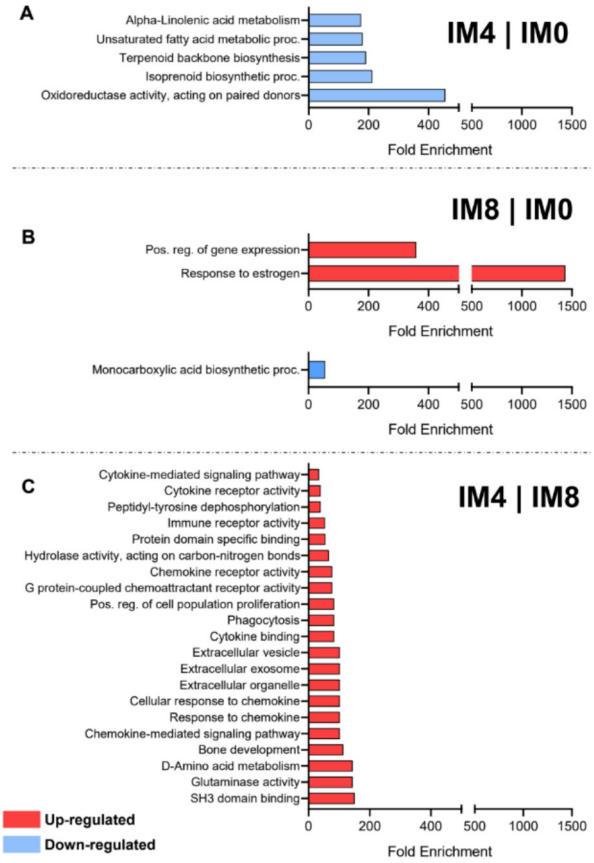

Figure 2: *RNA-sequencing of the distal intestine: Significantly enriched gene ontology (GO) terms of fish fed 0 % insect meal (IM0), 4 % (IM4) and 8 % (IM8).* ***A****: IM4 vs. IM0.* ***B****: IM8 vs. IM0.* ***C****: IM4 vs. IM8.*

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PRICKLY PEAR FRUIT (*Opuntia ficus-indica*) PEEL IMPROVES STRESS TOLERANCE, IMMUNE RESPONSE, AND ANTIOXIDANT ACTIVITY IN NILE TILAPIA (*Oreochromis niloticus*)

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Introduction

Opuntia plants and their by-products have been gaining research interest due to their contents of natural bioactive compounds, such as ascorbic acid, betalains, phenols, tannins, and flavonoids, terpenes α-amyrin, β-amyrin, oleanolic acid and cycloartenol. Despite the attributes of prickly pear fruit (*Opuntia ficus-indica*), limited information is available on its use as a feed additive in aquaculture. This experiment was conducted to evaluate the effects of prickly pear fruit (*O. ficus-indica*) peel (PFP) on growth performance, feed utilization, digestive enzymes activity, antioxidant activity, immune response and salinity tolerance of Nile tilapia (*Oreochromis niloticus*) juveniles.

Materials and methods

PFP was incorporated into four iso-nitrogenous (280 g kg^{-1} protein) and iso-energetic (18.62 MJ/Kg DM) diets at 0, 1, 2 and 4 g kg^{-1} diet. The diets were fed to *O. niloticus* juveniles (9.69 ± 0.2 g) for 75 days.

Results

The growth rates, feed utilization efficiency, body protein, digestive enzymes activities, liver function enzymes, immunological responses, and antioxidant status were all improved with increasing supplemental PFP up to 1 g kg^{-1} diet, followed by significant retardation with further increase in dietary PFP levels (Tables 1 and 2). However, the quadratic regression analyses of the results revealed that the maximum performance and the optimal fish health status occurred at about 2 g kg^{-1} of dietary PFP (Fig. 1). Moreover, salinity challenge showed that PFP supplementation at 1 g kg^{-1} diet significantly decreased stress indicators in fish. These results suggest that dry PFP can play a significant role in feed digestion and absorption and enhancing fish performance and innate immune response. About 2 g kg^{-1} diet would be sufficient for the optimum performance and health status of juvenile Nile tilapia.

Table 1. Digestive enzymes activity (U mg^{-1} protein) of Nile tilapia (*O. niloticus*) juveniles fed on the experimental diets

Parameter	PFP0	PFP1	PFP2	PFP4
Protease	8.05 ± 0.10^{d}	12.43 ± 0.21^{a}	12.00 ± 0.30^{b}	9.71 ± 0.35^{c}
Lipase	6.40 ± 0.14^{d}	11.00 ± 0.10^{a}	8.54 ± 0.11^{b}	6.70 ± 0.21^{c}
Amylase	3.80 ± 0.22^{c}	7.00 ± 0.14^{a}	6.50 ± 0.35^{a}	5.43 ± 0.15^{b}

Data are presented as mean ± SD. Data in the same row with different superscripts significantly differ ($P < 0.05$).

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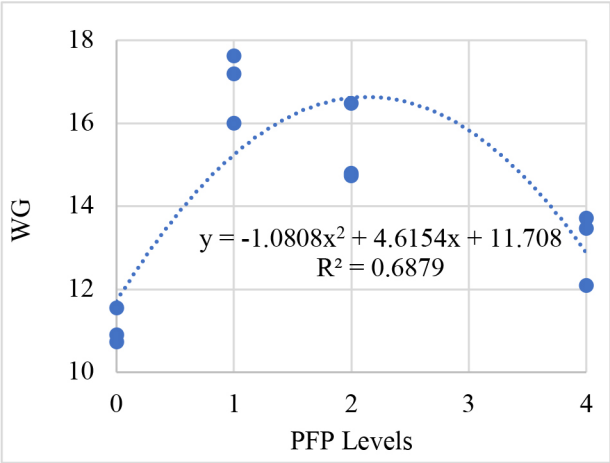

Fig. 1 Second-degree polynomial regression of the weight gain of Nile tilapia juveniles fed on the experimental diets

Table 2. Serum immunity parameters of Nile tilapia (*O. niloticus*) juveniles fed on the experimental diets

Parameter	**PFP0**	**PFP1**	**PFP2**	**PFP4**
Lysozyme (U mg^{-1} protein)	4.04 ± 0.15^{c}	7.60 ± 0.25^{a}	6.70 ± 0.21^{b}	4.40 ± 0.20^{c}
ACH50 (units ml^{-1})	19.71 ± 0.24^{c}	26.45 ± 0.13^{a}	23.60 ± 0.23^{b}	23.20 ± 0.30^{b}
PA (U mg^{-1} protein)	35.64 ± 0.10^{d}	42.05 ± 0.30^{b}	42.70 ± 0.36^{a}	38.50 ± 0.24^{c}
RB (%)	1.30 ± 0.20^{c}	2.83 ± 0.32^{a}	2.40 ± 0.20^{b}	2.10 ± 0.10^{b}

Data are presented as mean values ± standard deviation of the mean. Data in the same column with different superscripts significantly differ ($P < 0.05$).

AQUAPONICS R&D AT CSIRO: BEGINNING OF A NEW JOURNEY, CHALLENGES AND OPPORTUNITIES

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Aquaponics production is growing either as an alternative for aquaculture diversification, or as a new farming 'close-to-market' system, enabling high-quality low carbon footprint food production. As a powerful 'bio-converter', Aquaponics is a tool to enhance the circularity in Agri-Aqua food systems, and allows growers to save water, maximise production from their farm footprint and make productive use of nutrient-rich effluents as a fertiliser source. As a result of these benefits, commercial aquaponics farms are growing at a rate of 17%/year worldwide. However, high construction and running costs (e.g. labor, aquafeeds, electricity) are some of the main constraints for further development. The proper nutrient ratio and feed loads will dictate the size of the 'aquaculture component', heavily influencing the construction costs.

In 2021 CSIRO started applied research in Aquaponics with the aim of generating knowledge to help the industry grow and scale. The key focuses so far have been (i) creation of a R&D foundation and new capabilities, and (ii) establish connection, understanding and dialague with the industry. To date, six experimental trials have been conducted with barramundi (*Lates calcarifer)* and Jade perch (*Scortum barcoo*) juveniles, exploring the nursery phase (~10 to 30g as initial weights, over ~6-8 weeks). We evaluated the fish performance in aquaponics (AQP) versus conventional recirculating aquaculture systems (RAS); and the plant performance (butterhead lettuce *Lactuca sativa*, as an initial biological model) in aquaponics versus conventional hydroponics (HYD). Later experiments explored different plant species (spring onions, basil and parsley), loop approaches (coupled versus decoupled), nutrient ratios (e.g. 1 to 3g grams of fish feed/plant/day) and key mineral supplementation (including aerobic digested fish sluge-based one); assessing impact on plant performance and the incidence of visual symptoms of plant nutrient deficiencies. The system design in RAS consisted of a fish tank, media-based clarifier and biological filter. In AQP, RAS design was applied with the addition of a floating bed hydroponic units. In HYD, floating bed and reservoir (same fish tank) were utilised. The results demonstrated performance differences for both fish and plant; according to systems, loop approaches, feeding ratios and mineral supplementation. We also conducted an industry survey aiming to understand the barriers of commercial aquaponics in Australia. In addition, during the past 20 months, several industry engagements were carried-out aiming to better understand the field constraints and potential needs for R&D.

Looking towards the future, a pilot-scale Aquaponics facility is being built at CSIRO and will enable research at scale, allowing us to produce and collect "real-world" data to feed into economic-sustainabilily models & Decision Support Tools to facilitate industry development and growth.

Investigation of Proximate and Micromineral Compositions of Rapana Whelk (*Rapana venosa*) Meal for Fish Feed Industry

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aquaculture europe 23

Introduction

Rapana whelk (*Rapana venosa*) is an invasive mollusc species distributed in the sea bed of Marmara and Black Sea, Turkey. It has high potential for aquaculture feed industry due to its nutritional value. In this study, we evaluated proximate, mineral (Cu, Fe, Zn, Se, Mn, I) composition of Rapana whelk (*Rapana venosa*) caught in Black Sea. Fish meal and fish oil are the major feed ingredients which provides protein and lipids. In order to sustainable aquaculture, alternative protein sources should be introduced in aquaculture feed industry. For that purpose, we analyzed nutritional composition of rapana whelk meal for future utilization as marine fish diet ingredient.

Material & Method

Rapana venosa

0.25 g of samples
+
0.5 mL of Milli-Q Water
+
2 mL of concentrated nitric acid (HNO_3)

Milestone Ultrawave

Diluted to 25 mL with Milli-Q Water

ICP-MS
(Inductively Coupled Plasma Mass Spectrometry

Results & Discussion

Rapana whelk (*Rapana venosa*) is invasive gastropod species in Black Sea and Marmara Sea. It has been showed that it can be used as fishmeal supplement for rainbow trout broodstock diet affecting egg fatty acid composition (Şahin et al., 2021). Its utilization with a level of 50g kg-1 has been announced possible without any harmful effect in rainbow trout fry (Sahin and Ergün, 2021). In addition to its nutritional value, Rapana can improve the water-holding capacity and gelatinization properties of animal feeds, which can improve feed pellet quality and reduce feed waste (Gaspar-Pintiliescu et al., 2019; Ranasinghe et al., 2022). In this study, we aimed to investigate proximate and micromineral compositions of Rapana meal in comparison to fish meal. The crude lipid content of the rapana meal (1.86%) was found lower whereas crude protein content had higher than fish meal ($p<0.05$). Recent studies have focused on importance of minerals in larval fish nutrition due to their functional properties in mineralization of skeleton, major components of metalloenzymes (Izquierdo et al., 2017; Eryalçın et al., 2020). Therefore, all ingredients should be completely investigated whether if it meets requirements of fish before used. In terms of mineral contents, rapana meal contained of high level of Copper (Cu) compared to other microminerals ($p<0.05$). Cu is very important micromineral that have positive affect on growth and survival of fish. Moreover, the elevation of dietary Cu levels increased the whole-body contents in DHA, EPA and n-3 PUFA which is very important at early life period of fish (Yiyen et al., 2023).

Table 1. Proximate and mineral composition of Rapana whelk (*Rapana venosa*).

Proximate Composition (%)	Rapana meal	Fish meal
Crude protein	66.03±0.05^{a}	62.25±1.12^{b}
Crude lipid	1.86±0.03^{b}	7.48±0.07^{a}
Crude Ash	5.23±0.11^{b}	23.75±1.06^{a}
Dry matter	28.18±0.02^{b}	91.86±0.21^{a}
Mineral Composition (mg kg^{-1})		
Copper	21.47±0.18^{a}	9.0±0.02^{b}
Zinc	65.40±0.14^{b}	103.0±2.11^{a}
Iron	110.20±0.36^{b}	220.0±1.12^{a}
Manganese	2.70±0.51^{b}	10.0±0.78^{a}
Selenium	1.97±0.11*	-
Iodine	5.93±0.22*	-

Note: Values represent means ± standard deviation of triplicates. Values within each row not sharing a common superscript letter are significantly different ($p<0.05$).

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Conclusion

Rapana whelk is the high protein source and potential ingredient for replacing fish meal. Moreover, it contains high level of essential microminerals such as Copper, Selenium and Iodine. Future trials with rapana meal inclusion in formulated diets should be evaluated for marine fish species.

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PROBIOTICS AND POST-BIOTICS FROM SPPDP11 ON THE MITOCHONDRIAL ENERGY EXPENDITURE DISTRIBUTION IN HEPATOCYTES AND FIBROBLASTS FROM GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

The mitochondrion is a crucial cellular organelle that organises a wide range of biological processes (Basu et al., 2021). The status of mitochondria is also relevant to the phenotype and function of immune cells. Moreover, *Shewanella putrefaciens Pdp11* is a strain isolated from healthy sea bream (*Sparus aurata* L.) and is described as a probiotic for use in aquaculture. Its use has been shown to exert a stimulatory effect on the immune system in fish (Lobo et al., 2014). It has also been shown to modulate fatty acid mobilisation in the liver in fish. The way in which the probiotic affects the metabolism of sea bream may be of great interest when using the probiotic *in vivo*.

Material and methods

Under this premise, six sea bream were sacrificed and liver samples were obtained and preserved in supplemented L-15 medium. They were then mechanically disintegrated and after different washes, the liver cells were obtained. In parallel, the sea bream fibroblast cell line (SAF-1) was cultured using routine methods (Espinosa-Ruiz et al., 2022). Liver cells and SAF-1 were quantified and arranged in 96-well plates prepared for mitochondrial stress assay. SpPdp11 was routinely placed in culture (Lobo et al., 2014). The bacteria were quantified and adjusted to 10^7 cfu. On the one hand, filtered culture medium (22μms) was obtained, containing all the extracellular products (ECPs) generated by the probiotic. At the same time, the bacteria were inactivated by incubating at 60ºC for one hour. Then, cells obtained from sea bream liver, and SAF-1 cells were incubated for 24 hours with these two extracts (ECPs and inactivated SpPdp11). A mitochondrial stress test was then performed to assess the bioenergetic capacities of the cells after incubation with the extracts.

Results and discussion

The results show that SAF-1 cells were hardly affected, with only the respiratory turnover capacity of the mitochondria being increased by incubation with the ECPs. These results suggest that the ECPs gave the SAF-1 cells an increased ability to carry out respiratory processes (Espinosa-Ruiz et al., 2022). In a stressful situation, characterised by an increased need for energy or reducing power (e.g. oxidative stress, toxicity, hypoxia...), these results suggest that the cells could be more responsive (Azevedo et al., 2020).

As for liver cells, the parameters of maximal respiration and non-mitochondrial oxygen consumption were significantly increased by both extracts. In general, the incubation with Pdp11 extracts enhanced the mitochondrial capacities of liver cells related to anaerobic processes (Espinosa-Ruiz et al., 2022). In parallel, inactivated Pdp11 increased the energetic responsiveness of liver cells (including maximal respiration, spare respiratory capacity and coupling efficiency) suggesting an increased mitochondrial plasticity and stress responsiveness.

Overall, liver cell mitochondria were more susceptible to be affected by the different SpPdp11 extracts. These results hold promise for future studies, whether toxicological, metabolic or otherwise, to further investigate the mechanisms by which SpPdp11 mobilises fatty acids in fish liver.

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Acknowledgements
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EXPLORATION OF MICROALGAE CULTIVATION UNDER DIFFERENT CONDITIONS: FATTY ACID PROFILE ANALYSIS FOR NUTRITIONAL APPLICATIONS AND SUSTAINABILITY

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Introduction

Microalgae play a crucial role in aquaculture nutrition, which is the farming of aquatic organisms such as fish, shrimp, molluscs and others in controlled environments. These organisms are rich in essential nutrients, such as proteins, lipids, vitamins, minerals, etc. Among these, the omega 3 fatty acid content (such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) have a key role in cardiovascular health, nervous system development and brain function in aquatic organisms, as well as in humans who consume aquaculture products. Interestingly, the microalgae fatty acid profile could change depending of the culture conditions.

Material and methods

Under this premise, one litre of some species of microalgae belonging to the family of the Bacillariophyte, Chlorophyte and Rhodophyte were cultured under different conditions in marine sea water. Different source of nitrogen (NH_3 or NO_3) and different temperature (15 or 25ºC) were used during the trial. After 15 days, the microalgae were collected by centrifugation (3000g 4ºC 45 min). The lipid content was extracted using the method of Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared by acid-catalysed transesterification of total lipids according to the method of Christie (2003). All analysis were carried out by triplicate.

Results and discussion

The production of fatty acids in microalgae was significantly affected by the temperature difference and the nitrogen source in their culture environment. These two factors play a key role in the lipid and fatty acid composition of microalgae. Some strains of microalgae grown at 25°C increased the production of fatty acids. In this sense, temperature could affect the metabolic activity of microalgae because, under warmer conditions, metabolic rates may increase, which may influence lipid accumulation and thus fatty acid production. Furthermore, under heat stress conditions, some microalgae species may accumulate lipids as a protective response (Katayama et al., 2020). This may result in an increase in the concentration of fatty acids in the stored lipids. Finally, lower temperatures may promote the accumulation of saturated fatty acids (Katayama et al., 2020), while warmer temperatures may stimulate the production of unsaturated fatty acids, such as omega-3 fatty acids. Our data seem to be in agreement with these observations.

As for the change in nitrogen source, nitrogen is essential for microalgae growth, and the source of available nitrogen can influence the metabolic pathway (Ahmad et al., 2022). Different nitrogen sources can trigger different lipid and fatty acid synthesis pathways (Katayama et al., 2020). Certain nitrogen sources, such as nitrate, can promote lipid accumulation and thus influence fatty acid production. Stress conditions, such as the nitrogen limitation that occurred in our experiment, can increase the accumulation of fatty acid-rich lipids.

In summary, both temperature and nitrogen source in the culture environment had a significant impact on fatty acid production in microalgae. Understanding how these factors influence lipid and fatty acid composition is essential for optimising culture conditions to obtain desirable fatty acid profiles for nutritional, pharmaceutical and bioindustrial applications.

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Acknowledgements
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THE LONGER ROAD TO FUNCTIONAL ANNOTATION: THE USE OF FULL-LENGTH NANOPORE RNA-SEQ FOR ALTERNATIVE ISOFORM DISCOVERY IN ATLANTIC SALMON (*Salmo salar)* EMBRYOGENESIS

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Introduction

Atlantic salmon is an important commercial aquaculture species worth billions to the global economy. The generation of a high-quality reference genome for Atlantic salmon (Lien et al., 2016) and the increased availability of 'omic tools has led to a significant drive towards the annotation of functional genome elements and causative genetic variants underpinning phenotypic traits (Macqueen et al., 2017; Houston & Macqueen, 2019).

Transcriptomics via RNA-seq is a powerful tool for genome functional annotation. Whilst highly accurate, short-read RNA-seq methods such as Illumina struggle to properly capture exonic chaining and identify alternative splice sites, which can lead to inaccurate gene and transcript annotation. Long-read Nanopore RNA-seq captures the full-length of transcripts in a single molecule. Compared with short-read RNA-seq, this aids identification of alternative splice sites, thus allowing better characterisation of transcript diversity and identification of novel isoforms (Kuo et al., 2017; Kuo et al., 2020). Thus supporting the discovery of causative gene and isoform variants through improved transcriptome annotation.

Embryogenesis is a critical stage of ontogeny where many cell types arise and differentiate. As such, the embryonic transcriptome is a valuable resource for functional annotation due to the high diversity of cell types and patterns of gene expression present during early development.

This study falls within the framework of the European project AQUA-FAANG, which aims to develop functional annotation maps for 6 commercial aquaculture species, including Atlantic salmon. Long-read nanopore RNA-seq was performed on Atlantic salmon embryos, at 6 key stages of development (mid-blastula, mid-gastrula, early-, mid-, late-somitogenesis and eyed stage) as defined by the AQUA-FAANG consortium.

Methods

Total RNA was extracted using a phenol-chloroform method before mRNA isolation via Dynabeads mRNA isolation kit. Sequencing libraries were prepared in accordance with protocols detailed in ONT Direct cDNA Sequencing kit (SQK-DCS109). Samples were barcoded before sequencing for 72h on a PromethION device using R9.4.1 chemistry.

Basecalling and demultiplexing was carried out with Guppy_v5.0.11. Reads with q-score <7 were filtered from the data using NanoFilt_v2.7.1. Full-length reads were identified using Pychopper_v2.5.0 and then mapped to the latest Ssal_v3.1 genome assembly using minimap2_v2.22 (Li et al., 2018). Reads were collapsed into consensus transcript models using TAMA (Kuo et al., 2020). Then, single-exon transcript models with read support <50, and all models with read support <3 were discarded. The final long-read transcriptome was compared with the reference annotation using SQANTI3 (Tardiguila et al., 2018).

Results

Approximately 50 million raw reads comprising 56 Gb were obtained in 72 hours from a single PromethION flowcell. Of those, approximately 10 million high-quality, full-length reads (N50 = 1,366 bp) were obtained. 31,230 genes and 243,991 unique isoforms were characterised by the long-read transcriptome. 78% (189,751) of isoforms were deemed be novel by SQANTI3 possessing either a new combination of known splice sites or at least one novel splice site. An example of the identification of novel transcript isoforms can be seen in Figure 1.

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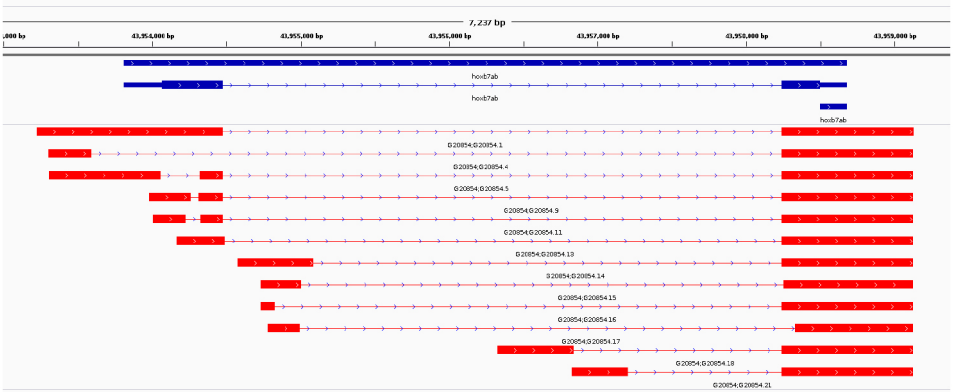

Fig. 1: IGV track comparing transcript isoforms identified in the nanopore transcriptome (red) compared with existing reference annotation (blue). Gene shown is homeobox protein gene hoxb7ab (Ensembl ID: ENSSSAG00000005981).

Discussion

Long-read RNA-seq is a powerful tool for isoform discovery. In this study, the average number of isoforms per gene doubled, from 3.9 in the reference Ensembl annotation, to 7.8 in the long-read annotation, thus describing a wealth of previously unannotated transcripts. Such improvements in genome annotation could aid in identification of causative genes and variants for traits of relevance to salmonid production and welfare, as well as traits of ecological importance.

Further investigation will examine differential transcript isoform usage across the 6 developmental stages. This data could contribute to future improvement in publicly available transcriptome annotations through the AQUA-FAANG project.

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IDENTIFICATION OF BEHAVIOUR OF FARMED ATLANTIC SALMON (*Salmo salar*) UNDER THE INFLUENCE OF EXTERNAL IMPACT FACTORS

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Introduction

In this abstract, results are presented from the study of the behaviour change of Atlantic salmon (*Salmo Salar*) in an industry scale fish farm when exposed several different influence factors. Data is gathered to study the fish behaviour change when exposed to structures with varying shape, size, light, color and sound, shown in Figure 1. Utilizing these data, the fish reaction to the structure can be studied for different case studies to identify and quantify the distance that the fish keep from the different structures and how the fish reacts when exposed to the different impact factors.

This work was financed by the Research Council of Norway through the project: CHANGE and RACE Fish Machine Interaction [1].

Methods

The structure was in each case equipped a stereo camera, and two BlueRobotics Ping360 echo sounders set to collect data in 360° swipes with a range of 5 meters. Four EK15 echo sounders and an underwater speaker were also installed on the structure for parts of the trials. Stereo camera videos and echo-sounder data have been obtained from cages in an industrial scale fish farm which is part of SINTEF ACE [2] during field trials in the fall of 2022 and compared to outcomes from results obtained in 2021.

Sound tests were carried out using the big yellow cylinder structure, which was lowered to 8 meters depth within the fish cage, as illustrated in Figure 1. The fish was then exposed to frequencies of 200 Hz and 600 Hz, as well as propeller sounds, for a period 1 minute, followed by 10 minutes of silence between tests. Furthermore, experiments for light were done for the base structure without the yellow or white outer layer, as shown in Figure 1, with additional EK15 echo sounders installed. The structure was lowered to 8 meters depth within the fish cage and four different light intensities were tested to see the reaction of the fish. Finally, tests for shape, size and color were done using the six different structures shown to the right in Figure 1.

Results

Sound tests from previous field trials showed that the fish generally had a flight response to 200 Hz and 400Hz when played for a shorter period (10 seconds). As it can be seen in Figure 2, the distribution of the fish in much smaller when the sound is activated. These observations were also supported from statistical analysis outcomes and behavioural biologist analysis. However, based on the observations made from watching the stereo camera feed and 360 ping data during the trials in 2022, it was not possible to see any clear flight response or other reaction from the fish in any of the sound tests, see Figure 3. The same goes for the light tests, the fish did generally not show any clear reaction based on what can be said from the visual observations from the stereo camera feed. Processing of the echo sounder data and the stereo camera videos can show if there was in fact any change in the general behaviour of the fish and the distance it kept to the structure that did not show clearly on the video feed.

Conclusion

Large data sets for different case studies have been collected from field trials in industrial scale fish farms to study the fish behaviour change under the influence of different impact factors. Both acoustic and video data were observed to check the fish behaviour change, however, the fish did not show any clear reaction to the sound, light or motion. Based on previous field trials, Atlantic salmon seemingly change behaviour when exposed to audio signals with a frequency between 200 Hz and 400 Hz. It is therefore interesting to investigate further why the fish did not show any clear reaction to the same frequency for the case studies presented here. If the data processing of the sonar data and formal statistical analysis gives the same results, the next step could be to look more closely into the size of the fish, as well as the time of day when the field trials were performed.

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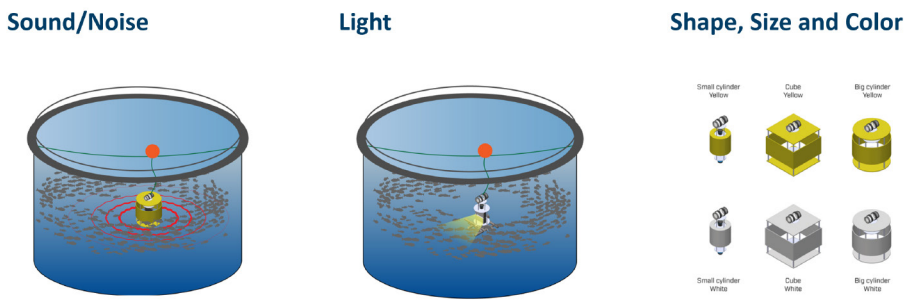

Figure 1: Illustration of the experimental case studies. Illustrations by Mats Mulelid.

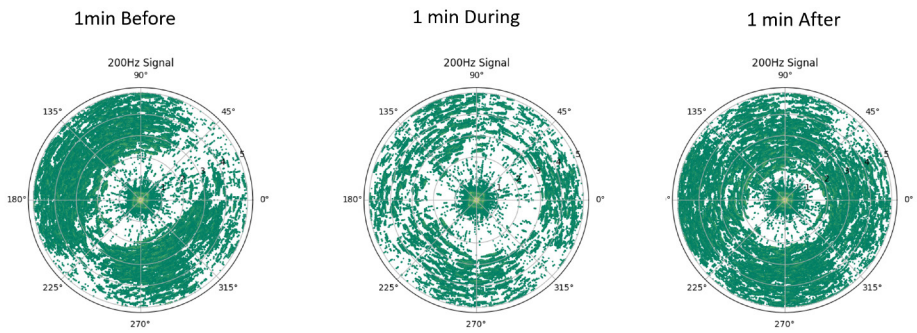

Figure 2 Response of fish during 200Hz sound experiments in 2021.

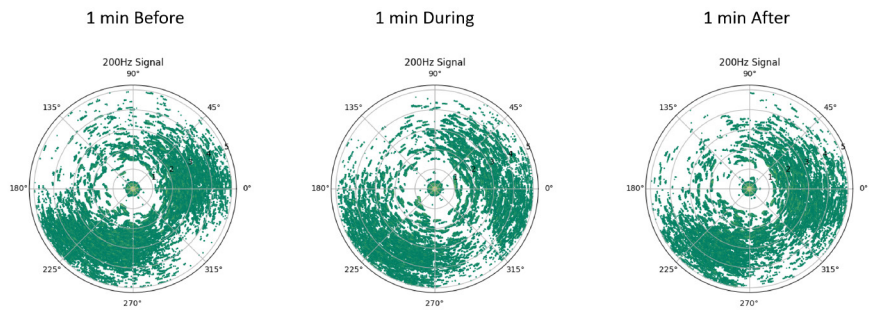

Figure 3 Response of fish during 200Hz sound experiments in 2022.

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INTEGRATING FUNCTIONAL ANNOTATION DATA IN GENOMIC PREDICTION MODELS FOR VIRAL NERVOUS NECROSIS RESISTANCE IN EUROPEAN SEA BASS

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Introduction

In 1991, the first paper reporting a viral infection associated with mass mortalities in European sea bass (*Dicentrarchus labrax* L.) was published (Breuil et al. 1991). The viral infection, presently known as viral nervous necrosis (VNN), still represents a major threat for the sea bass industry. The lack of chemotherapeutics and vaccines that can be effectively used to control the disease and the significant additive genetic variation for resistance to VNN in sea bass (Doan et al. 2017; Palaiokostas et al. 2018; Faggion et al. 2021; Griot et al. 2021) make selective breeding directed to the enhancement of host resistance a promising and sustainable approach to prevent and control mortality derived from VNN outbreaks. Traditional selective breeding approaches rely on estimated breeding values (EBV) predicted using individual phenotypes of the breeding candidates or their relatives through estimated additive genetic relationships based on their pedigrees (Zenger et al. 2019). Routine individual phenotyping for complex traits such as disease resistance is either difficult or unfeasible due to high costs and time requirements, and the implementation of genomic selection procedures might be greatly beneficial. Recently, the integration of functional genomic information into genomic prediction models has been proposed as a strategy to improve genomic prediction accuracy: expressed and regulatory genomic regions are characterized and all the obtained resources are used to efficiently predict phenotypes or EBV (Clark et al. 2020). This strategy is expected to more efficiently detect causative variants and to enhance the prediction accuracy of the genetic merit of future breeding candidates across generations, when the reference population is likely to consist of gradually distant relatives of the animals to be predicted. In this study, we assessed whether the integration of functional data into genomic prediction models could improve the prediction accuracy of breeding values for VNN resistance in European sea bass.

Materials and methods

906 juvenile fish (body weight: 6 to 20 gr) produced in a full-factorial mating (25 sires × 25 dams) were subjected to a 29-days VNN challenge test. VNN resistance was recorded both as a binary trait and as time to death. The experimental fish (N = 906) were genotyped using a high-density SNP panel (Peñaloza et al. 2021; ~27,740 SNPs after quality control), while their parents (N = 50) were whole-genome sequenced and used to impute the offspring to whole-genome genotypes. A genome-wide association study was performed to identify SNP markers associated to the traits of interest. Loci that explain a fraction of the genetic variance of gene expression phenotypes were detected through an eQTL (expression quantitative trait loci) analysis, and ATAC-Seq analysis were performed to detect open chromatin regions corresponding to active regulatory or functional elements in the genome.

SNP genotypes were used as predictors of VNN resistance EBV and Bayesian models were fitted to the data. Genomic predictions were performed following different criteria: 1) pre-filtering genetic markers on the basis of their localization in open chromatin regions; 2) weighting genetic markers on the basis of their localization in regulatory regions; 3) weighting genetic markers on the basis of the p-value of eQTLs; 4) weighting genetic markers on the basis of chromatin status score; 5) combining all the aforementioned criteria in an index and using it to filter the SNPs.

Accuracies of the models were assessed in a cross-validation approach generating training and testing sets consisting of animals of varying genetic relatedness according to the genomic relationship matrix, to mimic genomic selection scenarios where the genomic prediction equation is generated by training models using data from a reference population that is either more closely or more distantly related to the animals to be predicted.

Results

Genome-wide association analyses revealed a major QTL associated with VNN resistance phenotypes on linkage group 12. A total of 528 and 578 SNP markers were identified as significantly associated with VNN resistance phenotypes, both as a binary trait and time to death (false discovery rate, FDR, < 0.05). Using evidence from eQTLs and chromatin accessibility data, a putative causal variant was identified.

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Discussion

Results from the GWAS are consistent with those reported in the literature (Griot et al. 2021; Vela-Avitúa et al. 2022). Prediction accuracy of complex traits, such as disease resistance, can be increased by filtering genetic markers depending on whether the genetic variations are located in functional sequences and by developing prediction models that can take into account the biological priors. Integrating functional information into genomic prediction models have also the potential to deal with the decrease of predictive accuracy which occurs when the reference population consists of distant relatives of the animals to be predicted.

Research efforts on genome and functional annotation can be effectively made accessible and translated into application, promoting and facilitating the global implementation of genomic selection, either in the sea bass industry to enhance VNN resistance or in other aquaculture species for complex traits of economic importance.

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SPERMIATION ENHANCEMENT OF F1 GREATER AMBERJACK (*Seriola dumerili*) IN RESPONSE TO GnRHa OR hCG

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Introduction

The greater amberjack (*Seriola dumerili*) in the Mediterranean is a species with high potential for the diversification of the aquaculture production, however, reproductive dysfunctions in captivity have been observed in wild-caught breeders. Milt production was decreased compared to the wild, and lower fertilization success has been recorded in greater amberjack compared to other farmed species. Agonists of gonadotropin-releasing hormone (GnRHa) and human chorionic gonadotropin (hCG) have been used to overcome reproductive problems in many fishes, and in the present study we i) compared these hormonal spermiation enhancement methods in terms of plasma sex steroid production and sperm quality, and ii) applied the best spermiation enhancement method in an industrial egg production scenario.

Materials and methods

Four-year-old hatchery-produced (First generation – F1) male breeders were maintained in a sea cage (Salamina, Greece) and were fed with a broodstock diet (Skretting, Vitalis Cal, 22mm). On May 18th 2021, after blood and sperm collection, the fish (7.4±0.7 kg, mean±SD) were PIT tagged and were treated either with a GnRHa implant (n=6) with an effective dose of 139±17 μg GnRHa kg^{-1} or GnRHa injection (n=6) with an effective dose of 24±7 μg GnRHa kg^{-1} or hCG injection (n=6) with an effective dose of 972±120 IU kg^{-1} or left untreated (n=6)(day 0). Fish were sampled again for blood and sperm on day 7 and 13 after the hormonal treatments.

For egg production, and based on the result of the above experiment, wild-captured breeders were maintained similarly in a sea cage (Salamina, Greece). On June 7th 2022, after reproductive stage evaluation through ovarian biopsies and sperm collection, the males were treated either with a hCG injection (n=5) with an effective dose of 1083±93 IU kg^{-1} (day 0) or GnRHa implants (n=5) with an effective dose of 118±10 μg GnRHa kg^{-1}. Six females were implanted with GnRHa at 57±8 μg GnRHa kg^{-1}, while two females at the oocyte maturation stage were left untreated; all females were equally divided in two 70-m^{3}tanks (OUT 1: with hCG treated males, OUT 2: with GnRHa implanted males). For the following days, egg production and quality were evaluated and on day 13 males were sampled again for blood and sperm, and females for oocyte diameter through ovarian biopsies. Plasma testosterone, 11-ketotestosterone, 17-β estradiol, 17α,20β-dihydroxy-4-pregnen-3-one and cortisol were quantified with the use of liquid chromatography tandem mass spectrometry (LC-MS/MS). Sperm quantity was evaluated according to their spermiation condition –which is a measure of the available milt in the testes- after gentle abdominal pressure was applied, determined by a subjective scale from 0 to 3, as follows: Spermiation index S0 = no milt released, S1= only a drop of milt released after multiple stripping attempts, S2= milt was released easily after the first stripping attempt and S3= copious amounts of milt released with very little pressure. The sperm quality parameters that were evaluated using computer assisted sperm analysis (CASA) included: (a) sperm density (number of spermatozoa ml^{-1} of milt), (b) survival of spermatozoa under cold storage at 4°C (days), (c) motile spermatozoa immediately after activation (%), (d) progressive motile spermatozoa (%), (e) rapid motile spermatozoa (%), (f) straight-line velocity (VSL, μm sec^{-1}), (g) curvilinear velocity (VCL, μm sec^{-1}), (h) average-path velocity (VAP, μm sec^{-1}) and (i) straightness (STR, %).

Results and discussion

In 2021, neither plasma sex steroids (ANOVA, P>0.05) nor sperm quality parameters were statistically different among the four groups (Control, GnRHa implants, GnRHa injections, hCG), while plasma cortisol was elevated in all fish after handling. However, all the hCG treated males were spermiating on day 13 compared to only 33% of those treated with GnRHa implants or injections, while no spermiating male was found in the Control group. In 2022, no statistical differences were observed in egg production and quality-even though 11 spawns were recorded in OUT 1 compared to the 7 spawns in OUT 2 tank- and plasma sex steroids on day 13 between the two groups. However, the hCG treated males had significantly higher percentage of motile, progressive and rapid spermatozoa, compared to those treated with GnRHa implants (ANOVA, P<0.05) (Fig.1). The study demonstrated that hCG was better compared to GnRHa in enhancing sperm quality parameters, but this enhancement was not translated to improved spawning performance (egg fecundity and fertilization) in greater amberjack in the Mediterranean.

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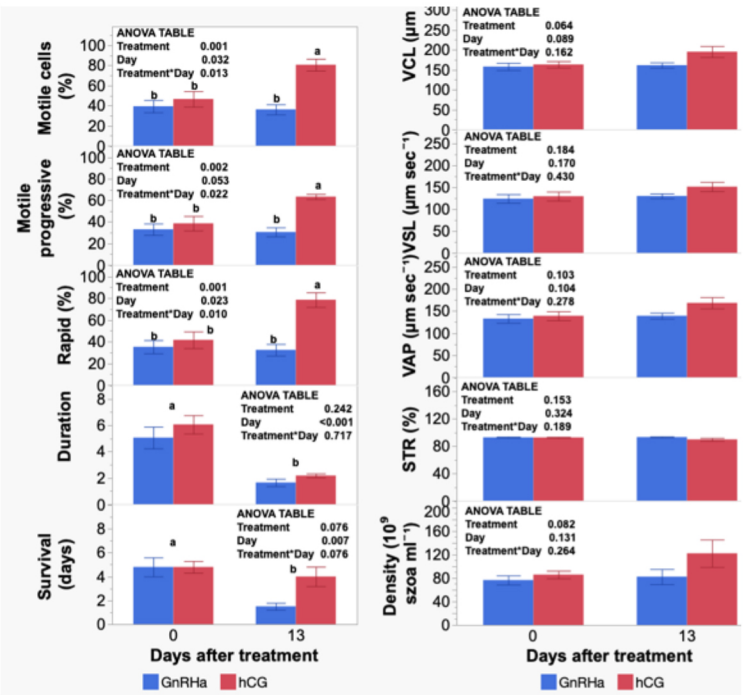

Fig.1. Mean (± SEM) percentage of motile spermatozoa (szoa), motile progressive szoa, rapidly-moving szoa, motility duration (min), survival at 4° (days), curvilinear velocity (VCL, µm sec^{-1}), straight-line velocity (VSL, µm sec^{-1}), average-path velocity (VAP, µm sec^{-1}), straightness (STR, %) and density (10^9szoa ml^{-1}) of greater amberjack males treated on day 0 with hCG injection or GnRHa implant. Lowercase letters indicate significant differences (2-way ANOVA, Tukey HSD, $P < 0.05$).

Acknowledgments

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APPETITE FOR DIVERSIFICATION: INFLUENCE OF CLIMATE CHANGE ON MARINE AQUACULTURE SPECIES IN NORWAY

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Introduction

The Intergovernmental Panel on Climate Change (IPCC) has again emphasised that there are widespread and rapid changes occurring in the atmosphere, cryosphere, oceans and across the land (IPCC, 2023). Climate change will directly and indirectly affect many different aspects of aquaculture production and the wider supply chain (Falconer et al., 2022). Species diversification has been suggested as a potential adaptation response to changing conditions, allowing national economies and local communities to build resilience through a range of activities rather than heavy reliance on single species. Establishing a new commercial aquaculture species requires considerable knowledge, time, and resources. To ensure such investments are worthwhile, it is important to assess the long-term potential, so information is required on how climate change may affect the suitability of a location for a particular species.

Norway is one of the world's top aquaculture producers, with over 1.6 million tonnes produced in 2021, worth over 80 billion NOK (Directorate of Fisheries, 2022). However, Norway also has the lowest species diversification amongst major producing countries, since approximately 95% of Norwegian aquaculture production is Atlantic salmon (*Salmo salar*). The aim of this study was to evaluate how changing temperatures under different IPCC scenarios may influence marine aquaculture species diversification in Norway.

Materials and Methods

This study used future temperature projections for three of the Shared Socioeconomic Pathways (SSP) climate scenarios (SSP1-2.6, SSP2-4.5, SSP5-8.5). The temperature projections were obtained from a regional downscaling of the Norwegian Earth System Model (NorESM) that had been forced with the NEMO ocean model (Hordoir et al, 2022). The temperature projections were calibrated to farm-level (Falconer et al., 2020), for twelve aquaculture sites in four geographic areas (South, West, North and Arctic). The temperature projections for 2020 – 2099 were then reclassified to a Challenging Conditions Index (CCI) based on thermal tolerances and preferred aquaculture temperatures for 35 different species (including fish, bivalves, crustaceans, echinoderms, and algae).

Results and Discussion

The results revealed differences in challenging conditions (cold and hot temperatures) for different species between the geographic areas, and between sites within each area, emphasising the importance of considering location and site characteristics. There are also differences between the scenarios, particularly in mid to end of the century. This study only focused on temperature, but the results still show that climate change will have important consequences for species diversification.

Funding

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INTERACTION BETWEEN DIETARY ZINC AND FAT ON ZINC UTILIZATION IN ATLANTIC SALMON (*Salmo salar*): THE EVALUATION BY SHORT-TERM AND LONG-TERM NUTRITIONAL INTERVENTION

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Introduction

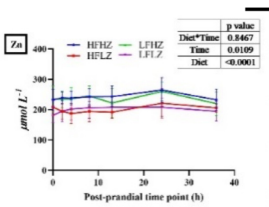
Zinc (Zn) plays the essential role in vertebrates including fish as it could exert various physiological function such as catalytic, structural and regulatory (Lall and Kaushik, 2021). Due to the limited availability of marine resources, aquatic feed increasingly utilizes plant-based resources as ingredients substitutes. However, compared to fish meal, plant protein contains lower Zn level. Besides, plant-based resources contain antinutritional factors such as phytate, which can bind with Zn^{2+} during absorption and thus reduces the Zn utilization of fish (Read et al., 2014). On the background of applying plant-based sources in aquatic feeds, there are two strategies to meet the Zn requirements of Atlantic salmon: increasing the level of dietary Zn and increasing the availability of Zn. Increased dietary Zn levels can improve the welfare of Atlantic salmon, but this strategy poses environmental challenges since less than 10% of the dietary Zn is retained in the body (Prabhu et al., 2019). Therefore, improving the knowledge on dietary Zn availability of Atlantic salmon has gained importance. In this regard, the present study aimed to investigate the interaction between dietary Zn and fat on Zn utilization in Atlantic salmon, by a short-term and a long-term nutritional intervention.

Material and Methods

Four experimental diets were produced by BioMar with difference levels of fat (high fat (HF, 35%) or low fat (32%)) and Zn (high zinc (HZ, 180 mg kg -1) or low Zn (LZ, 120 mg kg -1)), coded as HFHZ, HFLZ, LFHZ and LFLZ respectively. In trial 1, Atlantic salmon (initial weight: 741 ± 77.3g) was fed one of four experiment diets at Matre Research Station by indoor tank for 4 weeks and then the fish plasma were collected at different post-prandial time points (0, 2, 4, 8, 14, 24, 36h) for Zn level analysis. In trial 2, Atlantic salmon were fed with above description feeds by outdoor net pens from approximately 1.5kg to 2.5kg. At the terminal of trial 2, whole fish and different tissues such as plasma, liver, head kidney, muscle, bone were obtained for Zn retention analysis. Two experimental fish were fed twice a day to apparent satiation and feed intake monitored. During the start and at the end of the feeding periods, length and weight were recorded. The Zn analyses of the feed and fish samples were performed through ICP-MS. The calculating method of area under the curve (AUC) in trial 1 was based on the principle of trapezoidal rule (Prabhu et al., 2014).

Results

In trial 1, the final weight of Atlantic salmon among groups was 888 ± 108.2 g and they did not differ significantly. As presented in figure 1 and table 1, the higher baseline of post-prandial plasma Zn was observed in high-Zn supplementation group than those in low-Zn group. Furthermore, higher AUC value was found in low-fat diet group compared to high-fat diet group. In trial 2, The Atlantic salmon in high-Zn group obtained the high Zn level in their plasma, liver, muscle and bone, whereas fish in high-fat group obtain the low Zn level in their plasma and liver (Table 1). In two experiments, no interactive effects between dietary Zn and fat were observed in terms of Zn levels.



	HFHZ	HFLZ	LFHZ	LFLZ	p value Zinc	Fat	Interaction
rial 1: Post-prandial plasma Zn curves indexes							
Zn baseline (μmmol/L)	232 ± 34.9	208 ± 42.8	235 ± 27.3	181 ± 23.7	**0.05**	0.39	0.47
Zn AUC (μmmol/L)	536 ± 153.2	754 ± 153.2	1148 ± 154.9	800 ± 15.7	0.57	**0.04**	0.05
rial 2: Tissues and whole fish Zn retention							
Plasma Zn (μmmol/L)	208±17.9	98±11.5	239±42.3	140±16.8	**0.00**	**0.01**	0.95
Liver Zn (mg/kg, ww)	20.9±2.00	18.0±0.60	21.6±0.20	20.1±0.80	**0.00**	**0.00**	0.68
ead kidney Zn (mg/kg, ww)	18.3±2.50	17.4±0.09	18.9±1.95	15.4±1.78	0.07	0.54	0.25
Muscle Zn (mg/kg, dw)	14.3±0.3	13.1±1.2	15.5±0.6	13.4±0.1	**0.00**	0.11	0.32
Bone Zn (mg/kg, dw)	79.3±7.3	57.6±7.6	83.8±8.5	69.3±20	**0.03**	0.28	0.62
Whole fish Zn (mg/kg, ww)	30.9±2.41	27.6±8.18	34.8±1.82	28.8±6.45	0.17	0.44	0.68

Figure 1: The post-prandial plasma Zn curves of Atlantic salmon in trial 1; Table 1: Different indexes related to Zn level in Atlantic salmon in two experiments.

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Discussion and conclusion

Sub-optimal dietary Zn supplementation may not reduce the fish growth, but it did impact on the Zn level in tissues and whole fish (Antony Jesu Prabhu et al., 2018). Therefore, growth may not be the only criteria on Zn statues evaluation in fish. Antony Jesu Prabhu et al.,(Antony Jesu Prabhu et al., 2016; Prabhu et al., 2014) proposed that the AUC of post-prandial plasma Zn could reflect the absorption and utilization of minerals while Zn retention in whole-body and vertebra might be the better indexes in long-term experiment. In the present study, compared to the high-fat diet group, low-fat dietary intervention fish obtained the higher AUC value of post-prandial plasma Zn in trial 1 as well as higher plasma and liver Zn concentration in trail 2, indicating low-fat diet could enhance the Zn absorption and utilization in Atlantic salmon. Similarly, the reduction of plasma and femur Zn caused by high-fat diet treatment also reported in weanling rats (Weigand and Boesch□Saadatmandi, 2012). The present study provides a new perspective on improving Zn utilization in Atlantic salmon. Other findings in this experiment will be presented along with further data from ongoing analyses.

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SUPPRESSION OF REPRODUCTION BY RNA INTERFERENCE IN THE SEA LICE *Caligus rogercresseyi*

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Introduction

A substantial factor that makes parasites a serious pest is their high reproductive ability. Therefore, understanding how parasites produce plentiful offspring and how their reproductive capability can be suppressed constitutes a major subject in pest control and management research. Sea lice are ectoparasitic copepods that cause significant economic losses in both wild and farmed marine finfish by feeding on the hosts' mucus and blood, resulting in skin injuries and increased susceptibility to secondary infections. Among these parasites, *Caligus rogercresseyi* is the most important species affecting salmon aquaculture in the southern hemisphere. To mitigate the adverse impacts caused by this species, it is critical to identify the molecular mechanisms involved in its reproductive and developmental processes. Therefore, this research aims to achieve two goals: i) to identify the expression patterns and localization of selected genes putatively involved in germ cell differentiation and gonad development in *C. rogercresseyi,* and ii) to investigate the effects of RNAi-induced gene silencing of candidate targets on the reproductive output of this sea lice species.

Materials and Methods

We characterized a panel of gene homologs that are putatively involved in reproduction and gonad development in this species. RNA-seq and qPCR were used to profile the expression levels of these genes at different developmental stages and between sexes. To clarify the anatomy of the reproductive system and gonadal organization of this parasite, we performed histological and immunohistochemical assessments. We identified the localization of candidate reproductive genes, including *Vasa*, *Ecdysone Receptor* (*EcR*), and *Retinoid X Receptor* (*RXR*), using *in situ* hybridization (ISH). We designed and synthesized dsRNA for the gene silencing of *Vasa* and *RXR*, and we adapted a method for subcuticular hemocelic microinjection of dsRNA for the first time in this species. Finally, we assessed the effects RNAi-induced gene silencing on the egg strings formation and embryo development and examined the ovarian organization of treated females using histology.

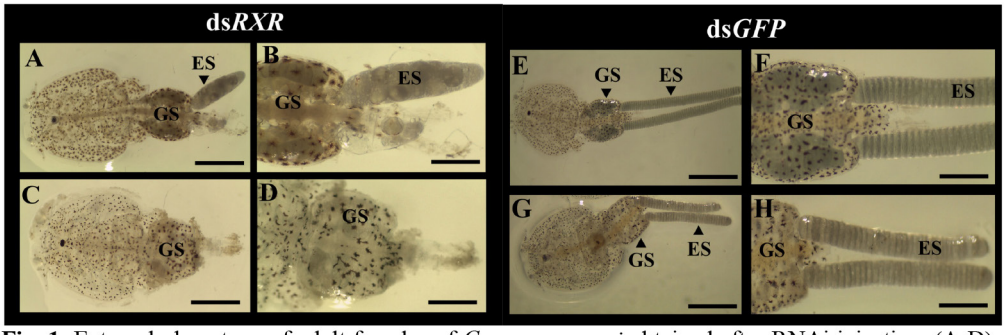

Fig. 1. External phenotype of adult females of *C. rogercresseyi* obtained after RNAi injection. (A-D): Females microinjected with ds*RXR*. (*E*-H): Females microinjected with ds*GFP* (control). (A, B): Short egg strings (ES) with abundant inclusions were commonly observed. (C, D) Females without formed egg strings were also detected. (E-H): Females microinjected with ds*GFP*, exhibited longer normal egg strings with antero-posteriorly compressed embryos (F, H). GS = Genital segment. (A, C, E, G) Bar = 2 mm. (B, D, F, H) Bar = 0.6 mm.

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Results

The expression of a panel of 18 nuclear receptors (NRs) transcript sequences was identified using RNA-seq and RT-qPCR data. Higher relative expression was found in females compared to chalimus and male stages for *Cr-RXR*, *Cr-EcR* and *hormone receptor 3* (*Cr-HR3*). Localization of *Cr-RXR* and *Cr-EcR* transcripts was examined using ISH, showing strong labeling in ovaries, oocytes, and intestine of sea lice. Furthermore, RNAi-induced gene silencing of *Cr-RXR* caused delayed egg string production, severely reduced fecundity, and generated abnormal gonad and embryo development (Bustos et. al 2023) (Fig. 1). The *Cr-Vasa* gene expression patterns were assessed by qPCR, and the results showed a significantly higher relative expression level in adult females compared to copepodid, chalimus, and adult male stages. In situ hybridization revealed strong positive signal in male testes, but also in the intestine and cuticle, while in females, it was observed in the ovaries, oocytes, cuticle, intestine, and egg strings. RNAi-mediated gene silencing of *Cr-Vasa* impacted embryonic development and reproductive output in adult female lice. Females from the ds*Vasa*-treated group displayed unusual phenotypes, including shorter egg strings with numerous extra-embryonic inclusions, irregularly shaped abnormal embryos, and aborted egg strings.

Discussion

The study expands the knowledge of NRs in parasitic copepods, examining their diversity, expression, and localization in *C. rogercresseyi*. The findings suggest a physiological role for NRs in *C. rogercresseyi* reproductive tract development, expanding our knowledge of genes involved in reproduction and development in sea lice. *Cr-Vasa* gene expression was significantly higher in adult females compared to copepodid, chalimus and adult male stages, indicating a potential role in female reproductive development. Tissue-specific localization of *Cr-Vasa* mRNA showed strong expression in the testes and ovaries of male and female lice, respectively, as well as in other tissues such as the cuticle and intestine. Finally, RNAi-mediated gene silencing of *Cr-Vasa* resulted in abnormal embryo development and impaired reproductive output, indicating the crucial role of *Cr-Vasa* in *C. rogercresseyi* embryonic and ovarian development.

Conclusions

This research provides insight into the molecular basis of reproduction in *C. rogercresseyi*, enhancing the value of this copepod as a novel model for molecular research. Furthermore, the functional characterization of specific genes involved in reproduction and development might be useful to identify potential targets for novel therapeutics approaches to control sea lice infestation in aquaculture.

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EVALUATION OF BOTH *Hermetia illucens* AND *Tenebrio molitor* IN THE DIET OF ATLANTIC SALMON (*Salmo salar)*

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Introduction

Since insect meals have begun to take center stage as next generation feed ingredients, there has been a pressing need to validate its effects on popular species such as Atlantic salmon (*Salmo salar*). Currently, there are only a few published trials evaluating the inclusion of black soldier fly larvae meal (*Hermetia illucens*) to replace fish meal in the diet of salmonids (Fisher et al., 2020; Weththasinghe et al., 2021), yet the substitution of soy protein concentrate (SPC) and the use of full-fat yellow mealworm (*Tenebrio molitor*) remains novel. Thus, the aim of this trial was to evaluate the impact these two insect meals have on the growth performance and nutrient utilization of post-smolt Atlantic salmon.

Material & Methods

An 11-week trial was conducted at the Mørkvedbukta Research Station, Nord University (Bodø, Norway) under a continuous light regime (24L: 0D) with 520 fish with an average initial weight of 143.05 ± 12.89 g divided into 4 replicates of 26 fish per diet group randomly assigned to one of 20 tanks (870 cm^3) in a saltwater flow-through system equipped with feed collectors. The experimental diets consisted of a control (CTRL) diet based on 20% FM, 14.46% wheat gluten, and 20% SPC as primary protein sources, two diets substituting wheat gluten and SPC with 5 and 10% full-fat black soldier fly larvae meal (BSF5 and BSF10), and two diets containing 15 and 30% full-fat mealworm (MW15 and MW30), respectively. FM inclusion of 20% was maintained constant in all formulations. Fish were fed by automatic feeders at a rate of 1.6% body weight. Uneaten feeds were collected twice daily. Biometric data of all fish was recorded prior to and at the end of the experiment. In addition, samples for chemical composition (whole fish and feces) were collected to study digestibility and nutrient retention.

Results

Results based on data collected at the end of the trial indicated that the inclusion of insect meal in the diet of post-smolt Atlantic salmon had no significant effects on growth performance or organosomatic indices (weight gain, condition factor, feed conversion ratio, specific growth rate, hepatosomatic index and viscerosomatic index). All groups underwent a roughly three-fold increase in weight with no mortality during the experimental period. Additionally, there were no significant differences observed in macronutrient retention with an average percent amongst the groups of 60.5, 58.3, and 47.8% for lipid, protein, and energy, respectively. Lastly, no significant differences were observed in the apparent digestibility coefficients (ADCs) of dry matter (DM), ash, or energy. However, protein digestibility in the MW30 diet (80.4%) was significantly lower ($P \leq 0.05$) compared to the CTRL diet (84.5%). The ADC of lipid will be available at the time of the conference.

Discussion

The growth performance data revealed no significant differences between the dietary treatment groups, with weight gain following a similar trend as that of other trials that investigated the replacement of FM with BSF meal (Belghit et al., 2019; Li et al., 2020), and the absence of mortality during the trial period. Our growth results are in agreement with previous studies on inclusion of BSF meal; in Atlantic salmon diets up to 12.5% (Weththasinghe et al., 2021) and up to 15% (Belghit et al., 2019), and in rainbow trout (*Onchorhynchus mykiss*) diets up to 18% (Melenchón et al., 2022). Similarly, inclusion of MW meal at 18% in rainbow trout diet (Melenchón et al., 2022) did not negatively affect growth. As observed in our study, previous studies by Weththasinghe et al., (2021) and Fawole et al., (2021) have also reported that insect meal inclusion does not influence protein and lipid retention, respectively in Atlantic salmon. Lastly, results of protein ADC in the experimental diets showed significantly lower digestibility in the MW30 diet compared to the control. There are few studies for comparison which utilized meal worm in salmonid feeds. The closest trial by Rema et al., (2019)we studied the effect of a gradual replacement of fishmeal with insect meal from yellow mealworm on juvenile rainbow trout performance. Overall, fish grew faster with the incorporation of the insect meal in the feed, and their capacity to convert feed into fish

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biomass also increased. The retention of ingested key nutrients also increased with the incorporation of insect meal in the feed. In summary, juvenile trout fed an insect-based diet grew faster and required lower feed intake to grow than juvenile trout fed on a common diet with standard levels of fishmeal. These results support the transition of fishmeal to insect meal in aquafeeds, which will improve the sustainability of the aquaculture industry. Abstract: Insects are emerging as a sustainable alternative to fishmeal and fish oil in aquafeeds. This study assessed the effect of graded incorporation levels of defatted yellow mealworm (Tenebrio molitor found that up to 100% (25% FM) could be replaced without any effect on protein digestibility. However, our trial differs in that it replaces non FM protein sources with full-fat insect meal rather than defatted meal.

Conclusion

The overall results of this study show that the inclusion of either BSF meal or MW meal in the diet of Atlantic salmon does not have any significant negative effects on growth performance, nutrient retention. However, there were differences in protein ADC with 30% inclusion of mealworm. This demonstrates that insect meals can be used as an alternative to, not only fish meal, as has been validated in previous studies, but also to plant derived sources as well. These results contribute to the growing body of knowledge on insect meal inclusion and thus the increased diversification of aquafeed ingredients for greater sustainability in the aquaculture sector.

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FORMING A BREEDING STOCK OF THICKLIP GREY MULLET *Chelon labrosus*: SEX IDENTIFICATION AND MONITORING OF GONADAL DEVELOPMENT PRIOR TO HORMONE INDUCTION

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Introduction

The establishment of a breeding stock requires the sexing of the specimens -by non-invasive or minimally invasive methods- as an essential step in the configuration and management of breeding groups. For that, the most reliable and widely used method is gonadal biopsy. However, this method is not always suitable outside the period of sexual maturation, having to resort to other methods. In this study we evaluate the use of sex steroid hormone levels in blood plasma -11-keto testosterone (11-KT) and estradiol (E2)- as an alternative method to gonadal biopsy for sex identification in *Chelon labrosus* during the non-breeding season. On the other hand, the determination of reproductive maturity stage is key in breeding programmes involving hormone induction. So, we also monitored the gonadal development in captive males and females of *C. labrosus* to verify the appropriate time to carry out hormonal induction.

Material and methods

With the aim of creating a broodstock of *C. labrosus*, adults and sub-adults specimens (0.3 – 2.0 kg) were captured in the wild during the non-breeding season (June-October) in 2021 and 2022. After one month of adaptation to captivity, fish were anaesthetized (clove oil: eugenol 80%) and PIT-tagged, and a blood sample was taken from the caudal vein, the plasma was extracted by centrifugation and the levels of 11-KT and E2 were determined by ELISA methods. According to de las Heras et al. (2012), fish were initially considered as males when 11-KT levels were higher than those of E2, while fish with E2 levels higher than 11-KT levels and with 11-KT/ E2ratio << 1 were categorized as females. The specimens caught in 2021 were not biopsied in the 2022 breeding season, but in 2023 together with those captured in 2022. In the 2023 breeding season, we begin to monitor gonadal maturation by gonadal biopsy in 11 females and 7 males (Bw > 1.0 kg). From mid-January, a gentle abdominal pressure was performed to males to determine if they were fluent, and the Spermiation Index (SI: 0-3) was recorded. Females underwent gonadal biopsy by cannulation. From that initial sampling until mid-May, gonadal biopsies were performed biweekly in females, and 3 times (beginning, middle and end of the breeding season) in males. The oocytes were examined under a microscope and measured for diameter (n = 20 per female).

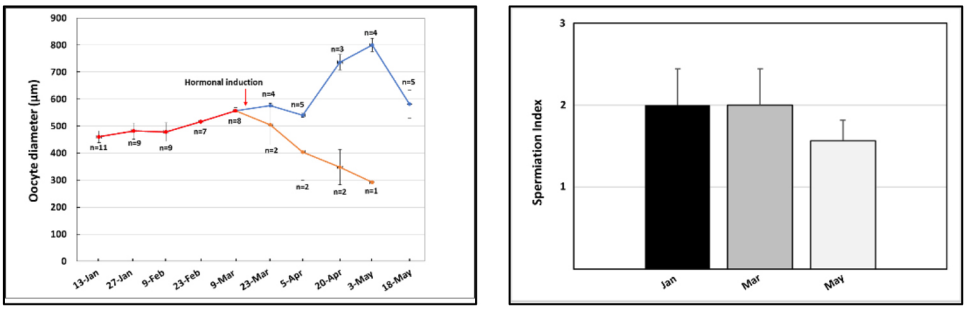

Fig. 1. Oocyte diameter and Spermiation Index throughout the 2023 breeding season (bars = standard error of the mean).

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Results and Discussion

A total of 27 specimens were subjected to steroid hormone analysis and subsequent gonadal biopsy. According to de las Heras et al (2012), 13 individuals were categorized as males and 14 as females after hormone analysis. All individuals that were sexed as female after gonadal biopsy had higher levels of E2 than KT, and a 11-KT/ E2 ratio < 0.4 (13/13: 100 % success rate), and all of them weighed more than 1 kg and had a size larger than 40 cm. All fish sexed as males through gonadal biopsy had a 11-KT/ E2 ratio > 0.4, but not all of them had KT levels higher than those of E2. Only 3 male identifications after hormone analysis were erroneous after gonadal biopsy (11/14: 78.6 % success rate). The common factor in these contradictory cases was that they all sized less than 35.5 cm, the length at first sexual maturity according to Ben-Tuvia (1986), and two of them weighed less than 350 g when the blood sample was taken. The other misleading case was sexed as a male after hormone analysis when it weighed less than 0.7 kg (35 cm in size), whereas when it was identified as a female through gonadal biopsy two seasons later it weighed almost 1.9 kg (48 cm in size). It can therefore be concluded that sexing by steroid hormone analysis in *C. labrosus* outside the breeding season provides a 100 % success rate in females, as well as in males when their size is above the size of first sexual maturity. It can also be concluded that a value above or below 0.4 in the 11-KT/E2 index is applicable to distinguish males from females, respectively.

As shown in Fig. 1, there was a clear trend towards increasing oocyte diameter as the breeding season progressed. However, from the middle of season the oocytes diameter of about half of the females began to diminish showing signs of atresia, while the oocytes from the other half of the females continued to mature. The number of fluent males was high at the beginning (6/7) and middle (6/7) of the season. SI was about 2 in most males early to mid-season. However, at the end of the season, although the number of flowing males was high SI decreased significantly.

According to Besbes et al. (2020), the right time to induce egg-laying in females is when they reach an advanced stage of vitellogenesis, once mean oocytes diameter is about 550 µm, which occurred in mid-season. At that time, hormonal induction was performed in 4 adult females stocked in a 8 m^3 tank with 5 fluent males, according to Besbes et al (2020): a priming dose of 10.000 IU kg^{-1} of human chorionic gonadotropin (hCG) (on March 15th) and a resolving dose of 10.000 IU kg^{-1} hCG + 100 µg kg^{-1} of Luteinizing Hormone-Releasing (LHRH) 5 days later. One of the hormonally induced females swelled extremely a few days after resolving dose. Spawning occurred on March 25th, and we suspect that the fertilized oocytes came only from this female. No spontaneous spawning occurred in any of the broodstock batches under natural temperature and photoperiod conditions.

Monitoring of gonadal development showed that not all females progress in gonadal maturation in the same way and at the same time. Also, no spontaneous spawning was obtained, so hormonal induction for spawning is necessary for the time being. But it would be necessary to identify, at the level of individual females, the most suitable timing for induction, as well as the protocolization of sperm cryopreservation for this species for a correct planning of potential artificial fertilization.

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AGRI-FOOD INDUSTRY WASTE FEEDS INFLUENCE FATTY ACID COMPOSITION IN *Hediste diversicolor* PRELIMNARY RESULTS

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Introduction

The use of nereid polychaetes as fishing bait or for gonadal maturation in finfish and shrimp farming is widespread. Additionally, the recognised capacity to *de novo* synthesise long-chain polyunsaturated fatty acids (LC-PUFA) in some nereid species such as *Hediste diversicolor* (HD) (Kabeya et al., 2018) represents an opportunity to investigate new alternative nutritional uses in aquaculture. It is known that rearing conditions and diet can modulate LC-PUFA production in HD. The consolidation of HD production as a business involves, among other issues, the use of a low-value feed that provides good growth and an adequate resulting nutritional profile. From a circular economy perspective, the use of by-products from agri-food industries would increase the sustainability of HD farming of sustainability to the HD farming. Following a decoupled integrated multitrophic aquaculture scheme, we used several agri-food industry wastes to feed HD assessing their influence on growth and final fatty acid composition.

Material and methods

HD juveniles taken form de COST-IEO stock with a mean body weight (BW) of 38.4 mg were placed in *ad hoc* experimental units (Rasines et al., 2023) at a density of 1000 individuals m^{-2} within a RAS system. Five groups in triplicate were each fed one of the following diets: i) brewers spent grain (BSG), ii) crushed corn plant (CP), iii) fish (*Chelon labrosus*) faeces (FF), iv) fishmeal waste (FM), and v) fish feed as a control diet (CD). Feed was provided *ad libitum* on a daily basis for 2 months. Every two weeks all specimens of each replicate were weighed and weight gain was recorded and plotted. Differences in weight over time (*t*) among diet treatments was checked using one-way ANOVA with *t* as a repeated measures factor, and Tukey's honest significance distance (HSD) test for *post-hoc* comparisons. The fatty acid (FA) profile was analysed in the five diets and HD at the end of the experiment. Differences in body weight over time among diets was checked using one-way ANOVA with *t* as a repeated measures factor. PCA was used to select those FA contributing the most to the explained variance. Then, FA percentage values were expressed over 100% of the selected FA, divided by their respective 18:0 (stearic acid) values for normalization and log-transformed (Wang et al., 2007). Differences in FA composition between in HD fed different diets was performed by mean of permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarities.

Results and Discussion

Variations in weight over time for the different diets given were significant (ANOVA $P < 0.01$; Fig. 1). Notably, the CD diet exhibited the highest average body weight increase, followed by the BSG and FM diets, which yielded similar results. The FF diet led to a comparatively lower weight increase, while the CP diet resulted in the lowest

Similarly, feed type influenced significantly the resulting FA composition in HD (PERMANOVA, $P< 0.001$) Once the FAs were grouped into the abovementioned FA classes and subjected to n-MDS analysis, a greater similarity was observed between HD fed the CD and FM diets (Fig. 2). This observation seems reasonable since FM is a typical raw material used to produce CD.

Evaluation of the difference in FA classes between diets and the subsequent composition in HD fed these diets (Fig. 3) showed that HD transformed the lipid profile in the diets by increasing the proportion of PUFA´s, specifically ω3 and ω6, while decreasing the levels of SFA´s, MUFA´s and ω9. This effect was particularly evident in HD fed the FF diet.

These results indicate that agri-food industry wastes can serve as suitable feed for HD. The high capacity of HD to biotransform their diet resulted in an improved lipid profile, particularly in terms of sought-after PUFA´s and **ω3**. Notably, residues from the beer and aquaculture industry, such as BSG and FF, provided satisfactory growth and an improved FA composition, respectively. Further research is required to optimize the diet formulation for HD, aiming to achieve similar or even higher growth rate than that obtained with CD, as well as improving their FA composition.

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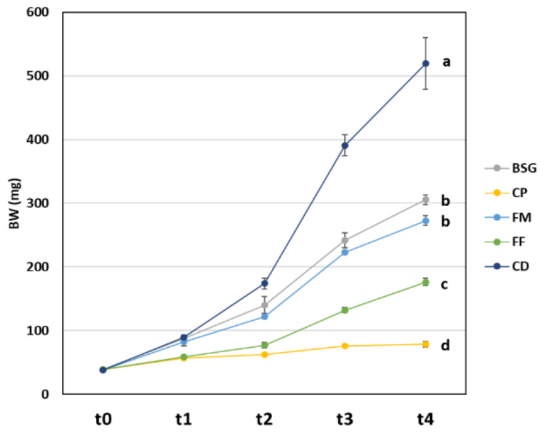

Fig 1. Body weight evolution in HD fed different diets. Letters indicate homogeneous groups (Tukey P<0.05).

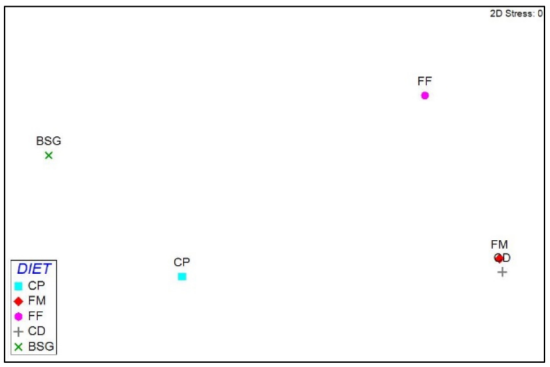

Fig 2. n-MDS plot for the FA classes in the different diets.

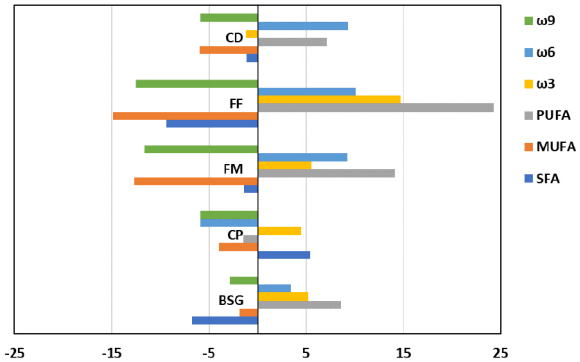

Fig 3. Difference (%) in the FA classes between the given diet and the resulting FA profile in HD.

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ENDOGENOUS AND EXOGENOUS MELATONIN EFFECTS ON SPERM QUALITY OF FIRST-GENERATION SENEGALESE SOLE

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Introduction

Melatonin is not just a time-keeping hormone that controls circadian and seasonal functions, such as reproduction. Our team has previously shown that this potent antioxidant is also present in fish seminal plasma (Félix et al., 2023). The use of melatonin as a supplement has been increasing, inclusive in cryopreservation processes. Especially for Senegalese sole (*Solea senegalensis*), a species with reproductive constraints whose production has been growing on the Iberian Peninsula, would be interesting to study if melatonin could increase the quality of sperm, either fresh or cryopreserved. This study comprehends a set of experiments that aimed to better understand the endogenous and exogenous effects of melatonin on first-generation (G1) Senegalese sole sperm quality.

Methodology

An established broodstock of Senegalese sole from the CCMAR research facilities - *Ramalhete* (Faro, Portugal), was used for this study. Fish were distributed within 6 fiberglass tanks, with 18 animals each, kept on a semi-closed system with a 2:1 sex ratio (male:female). Males were sampled in two consecutive reproductive seasons. First, sperm samples were collected at mid-light (ML, n=42) and mid-dark (MD, n=36) daytimes, to evaluate the effects of endogenous melatonin on sperm motility. In the second year, samples were collected at ML to evaluate the exogenous melatonin toxicity and its potential usage as an antioxidant during a cryopreservation assay. A toxicity test (n=11) was performed using different melatonin concentrations (0.01, 0.1, 1, and 10 mM) and exposure times (3, 5, 15 and 30 min), and sperm motility parameters were registered (TM, PM, VCL, VSL, LIN) using the CASA system. Since only melatonin concentrations influenced sperm motility, the best conditions from the toxicity test (0.1 and 10 mM) were used to evaluate the protective effect of supplemented melatonin during the cryopreservation assay (n=11), performed according to the protocol established by Riesco et al. (2017). Therefore, a set of quality analyses were performed [motility, viability, Reactive Oxygen Species (ROS) (flow cytometry), lipid peroxidation (MDA), and DNA fragmentation (Comet assay)]. Using confocal microscopy, a sperm sample was stained with FITC-melatonin to determine if melatonin enters fish spermatozoa. Statistical analysis was performed on SPSS software, and significant differences were considered when $p<0.05$.

Results

Motility results analyzed at ML and MD revealed significant differences in all parameters, but especially in the velocities (VCL, VSL, VAP) that were significantly higher at MD from the beginning of the activation until the end of the movement. In what supplemented melatonin is concerned, it did not influence spermatozoa motility, MDA content, or DNA fragmentation, although a lower percentage of viable cells was obtained on the 10 mM treatment. Altogether, it is suggested that supplemented melatonin did not confer extra protection to spermatozoa during cryopreservation. Even though, it was demonstrated that melatonin enters the nucleus and mitochondria area of fish spermatozoa.

Discussion

Overall, the obtained results suggest that endogenous melatonin can improve the motility parameters of *Senegalese sole* sperm, which can be of interest to the aquaculture industry to explore the possibility to obtain good gamete quality in a non-invasive way. Moreover, regarding exogenous melatonin, its toxicity to spermatozoa was proved to depend mainly on the concentration of this compound, and supplementing melatonin to the cryoprotectant medium did not confer extra spermatozoa protection against oxidative stress, as hypothesized. Even though, at the confocal microscopy, it was demonstrated that melatonin easily enters the cell. Further research is being done in order to understand if endogenous melatonin is produced by the gonads or up taken from the bloodstream.

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Acknowledgments
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ASSESSING EARLY UTILISATION OF VEGETABLE-BASED FEED AND ITS INTERACTION WITH GENOTYPE AND EPIGENETICS IN ATLANTIC SALMON

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Introduction

The proportion of plant-based ingredients being used to replace marine resources in Atlantic salmon (*Salmo salar*) feed is in constant growth. Hence, the lipid profile of the diets is changing, what can in turn affect the performance and health of fish. According to Lucas (1991), exposure to specific conditions during critical early life periods can lead to stable and long-lasting changes in the DNA, resulting in potential lifetime consequences during adult life. The application of nutritional programming in freshwater stages of Atlantic salmon has shown benefits in feed efficiency and nutrient retention when using plant ingredients in a stimulus diet compared to marine during first feeding (Clarkson et al., 2017). Moreover, physiological adaptations have been described at gene expression level in vegetable stimulated groups after challenged (Vera et al., 2017). These changes could have improved the use of nutrients as well as enhanced the fish tolerance to vegetable-based feed. However, it is important to determine whether these benefits can be extrapolated to seawater stage and if a "booster" is needed. Methylation of the DNA is one of the most important epigenetic modifications, and it is well characterized that various environmental factors, such as diet or stress, can result in changes in DNA methylation which can persist through life and even become hereditary (Skjærven et al., 2022). The aim of the present study was to validate the long-term effect of nutritional programming and the possible interactions of nutrition with factors such as genotype and epigenetics.

Materials and methods

A trial was performed with Atlantic salmon from six families characterised by either high or low pigmentation genotypes. Fish were distributed into four groups and exposed to two stimulus diets; with either marine or vegetable ingredients, both delivered within the first three weeks of exogenous feeding. After that period, they were maintained with a commercial marine diet until 14 weeks after seawater transfer. All the groups were then challenged with a vegetable-based feed which was delivered from week 66 until the end of the trial when they reached harvest size. Body weight and total fork length were recorded before fish were processed for sample extraction. Samples were taken during freshwater and in four sampling points through seawater. Nutritional analyses included proximate and fatty acid composition of feed, whole fish and tissue samples. Additionally, DNA from liver fragments was extracted and used for the Reduced Representation Bisulphite Sequencing (RRBS) libraries which were sequenced for all the samples.

Results

Genotype had a significant effect on body weight at the start of the marine phase, with high pigment group showing larger weights compared to low pigment, however, these differences disappear at the end of the intermediate phase. The same factor dictated differences for SGR throughout the seawater phase and for nutrient retention. In the last, genotype explained the variations between all the nutrients analysed (protein, lipid, LA, ARA, ALA, EPA, DPA, DHA), yet again, these differences weren't extended until the end of the challenge. On regards of biochemical composition for whole fish during freshwater, both dry matter and lipid content were affected by the stimulus diet, with vegetable stimulated fish presenting larger values compared to those marine stimulated groups. Lipid content and dry matter also showed differences in seawater during the intermediate phase, although differences were explained by genotype rather than by stimulus diet. All differences disappeared by the mid challenge sampling point. In general, changes observed in growth parameters and nutritional composition were related to genotype rather than to a specific stimulus diet and differences disappeared at the end of the challenge. The exception was anterior intestine from which differences in the proportions of ARA, DHA, n-3 PUFA and total PUFA were explained by the nutritional stimulus and the effects maintained until the end of the challenge.

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When looking at the impact of nutritional stimulus in DNA methylation, only a couple of differentially methylated CpG (DMCpG) sites were found after a 3-week exposition to stimulus feed, with 1 showing increased methylation levels (hypermethylation) and 1 presenting decreased levels (hypomethylation) in the marine compared to the vegetable treatment. In seawater, and before the vegetable challenge started, the number of DMCpG sites increased, with 109 hypermethylated and 21 hypomethylated in the marine-stimulated group relative to the vegetable-stimulated. After 14 weeks of challenge in seawater the number of sites were 4-fold above the previous values, with 112 hypermethylated and 508 hypomethylated in the marine stimulated group relative to the vegetable stimulated. Finally, after 36 weeks of vegetable challenge in seawater, the number of DMCpG sites decreased compared to the last sampling with a total of 21 sites identified with 17 hypermethylated and 4 hypomethylated in marine relative to vegetable stimulated groups. The same trend is shown when looking at the differences between low and high pigment retention genotypes. These preliminary results show that nutritional stimulus had a clear impact on the methylome of the fish. Differences between marine and vegetable stimulated fish became larger during the challenge diet phase, suggesting that nutritional programming may alter the response of the fish to a vegetable-based diet via epigenetic mechanisms. The ability of the fish to retain pigment also has an impact on the epigenetic response to the diet, suggesting that different genotypes may be more adaptable to vegetable diets.

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ESTIMATING THE POTENTIAL DIRECT COSTS OF TWO IMPORTANT DISEASE OUTBREAKS AND THEIR ECONOMIC IMPACT IN GROW-OUT FARMS OF EUROPEAN SEA BASS

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Introduction

Infectious diseases represent a bottleneck for the sustainable development of the aquaculture industry. They are associated with an increase of mortality, a feed conversion worsening, and a decline in animal welfare, hampering the farms' production and their economic viability (Lama et al., 2020). Understanding the economic impact they may have is pivotal for a proper investment on health management measures. One of the most important infectious diseases in Mediterranean aquaculture is viral encephalopathy and retinopathy (VER), previously described as viral nervous necrosis (VNN). The causative agent of this disease is the nervous necrosis virus or betanodavirus (Bellance and Gallet de Saint-Aurin, 1988; Glazebrook et al, 1990; Breuil et al., 1991). Due to its virulence and rapid spreading, VER outbreaks are associated with growth reduction and high mortality of fish, affecting mostly juveniles (Vendramin et al., 2016; Lama et al., 2020; Muniesa et al., 2020). In addition, the infections of bacterial disease vibriosis, photobacteriosis and tenacibaculum spp. are also considered among the most important diseases for European sea bass (Zrncic and Pavlinec, 2020).

The aim of this work is to evaluate the direct costs of a nodavirus and vibriosis outbreak as well as their economic impact on a typical Mediterranean grow-out farm culturing European sea bass of 450g with two different sizes according to the biomass produced: small farm (540 tons/year) and large farm (2,250 tons/year). For it, we have employed a deterministic static model proposed by Fernández Sánchez et al. (2022) as well as the economic framework proposed by McInerney et al. (1992) to evaluate the direct costs caused by these disease outbreaks (see Table 2) and their economic impact on the annual income statement of the farms (see Table 3). Our estimations show that the higher is the mortality and as the larger is the farm, the larger are the direct cost of a disease outbreak. However, the economic impact on the net operating profit is significantly worse in smaller farms, which points out the importance of investing on disease prevention and control in small scale aquaculture. Finally, we have included an estimation with the improvement of the net operating profit depending on different reductions in the disease mortality (see Table 4) to facilitate a future break-even analysis of different decisions related to the implementation of vaccination, treatments, or health management in these farms.

Table 1. Estimated direct costs for each farm size (th.€/year)

Cost type	**Nodavirus** (outbreak period: 30 days)				**Vibriosis** (outbreak period: 7 days)			
	More virulent Mortality[a]: +42% FCR impact: +0.02 Growth delay[b]: +2		*Less virulent* Mortality[a]: +21% FCR impact: +0.02 Growth delay[b]: +2		*More virulent* Mortality[a]: +45% FCR impact: +0.20 Growth delay[b]: +0.5		*Less virulent* Mortality[a]: +22.5% FCR impact: +0.20 Growth delay[b]: +0.5	
	Small farm	*Large farm*	*Small farm*	*Large farm*	*Small farm*	*Large farm*	*Small farm*	*Large farm*
Lost value of dead fish	1,031	4,296	515	2,148	1,105	4,602	552	2,301
Collecting mortalities/waste[1]	3	3	3	3	1	1	1	1
Disposal of dead fish[2]	1	1	1	1	1	1	1	1
Growth retardation	20	82	58	243	4	15	14	60
FCR degradation	8	32	9	37	80	334	93	386
Veterinarian diagnostic[3]	2	2	2	2	2	2	2	2
Direct costs of disease	**1,065**	**4,416**	**588**	**2,434**	**1,193**	**4,955**	**663**	**2,751**

[1]An expense of 100 €/person per day (one person). [2]An expense of 75 €/tn of dead fish. [3]An expense of 850 €/day (2 days).
[a]Mortality is based on no treatments or fingerlings' vaccination and is added to the baseline mortality rate. [b]Production delay measured in months.
Note: It has been assumed that the disease outbreak is always suffered at the beginning of the production batch.

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Table 2. Economic impact of a disease outbreak in a typical grow-out farm of European sea bass

Economic variable	Scenario	**Nodavirus** (outbreak period: 30 days)				**Vibriosis** (outbreak period: 7 days)			
		More virulent		*Less virulent*		*More virulent*		*Less virulent*	
		Small farm	*Large farm*	*Small farm*	*Large farm*	*Small farm*	*Large farm*	*Small farm*	*Large farm*
Net operating profit (th.€/year)	Farm without a disease outbreak	642	2,675	642	2,675	642	2,675	642	2,675
	Farm with a disease outbreak	-423	-1,741	54	241	-551	-2,280	-21	-76
Average operating cost (€/kg)	Farm without a disease outbreak	4.48	4.48	4.48	4.48	4.48	4.48	4.48	4.48
	Farm with a disease outbreak	7.56	7.54	5.64	5.63	8.10	8.10	5.86	5.85

Note: Financial and fiscal expenses are not considered in this analysis whereby the effect of diseases on farms' results would be larger depending on their capital structure and location.

Table 3. Increase in the net operating profit depending on different reductions in the disease mortality caused by a nodavirus or vibriosis outbreak (th.€/year)

Reduction in mortality	**Nodavirus**		**Vibriosis**	
	Small farm	*Large farm*	*Small farm*	*Large farm*
1% of reduction	23	94	24	98
2% of reduction	45	189	47	196
3% of reduction	68	283	71	294
4% of reduction	91	378	94	392
5% of reduction	113	472	118	490

Note: Because the reduction in mortality is based on percentual points instead of percentages of variation, the degree of disease virulence has not impact on these results.

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PROFITABILITY OF THE GREEK AND TURKISH MARINE AQUACULTURE FIRMS: A COMPARATIVE ANALYSIS OVER THE PERIOD 2011-2020

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Introduction

More than 95% of the world's seabream and seabass production comes from aquaculture, of which 97% is produced by Mediterranean countries. The main producers are Turkey and Greece, while the main consumers are Spain, France, and Italy (Carvalho and Guillen, 2021). The Greek aquaculture industry is highly export oriented as approximately 80% of production is exported, while the remaining 20% is sold in the domestic market. On the other hand, Turkey is the biggest non-EU competitor in the Mediterranean species (Boufidis-Asimakopoulos, 2019). Both countries are neighbors whereby they share the same water and consequently the have similar potential in this kind of species production (Boufidis-Asimakopoulos, 2019). However, being outside the European Union, Turkey's aquaculture is not subjected to the European Commission Directives addressing the mandatory standards for such activities, the ban of subsidies for exports, and extra tariffs on imports (Boufidis-Asimakopoulos, 2019). This fact gives a significant advantage for Turkish products with lower production costs and lower prices.

The purpose of this work is to analyze the differences in profitability between the Greek and Turkish marine aquaculture firms, as well as which are the most relevant factors to explain firms' profitability. This analysis is important to evaluate the competitiveness of the Greek aquaculture industry and to plan future policy actions. Different variables should be jointly merged to capture the overall complexity of the firm performance since firm competitiveness is considered as "multi-faceted" in nature (Dvouletý and Blažková, 2021). Thus, profitability is subjected to simultaneous effects of many factors (internal and external) and jointly determine the competitive strength of an individual firm and, consequently, its financial performance (Szymańska, 2017). In accordance with theoretical perspectives of the industrial organization and the strategic management (e.g., the structure-conduct-performance paradigm, the market-based view, or the resource-based view), different empirical studies have been carried out to explore the determinants of firm profitability. Their findings indicate that there are three different categories that those determinants can be classified into (Slade, 2004; Pervan et al., 2019): (i) firm-specific variables, (ii) industry-specific variables, and (iii) macro-economic variables. A sample of marine aquaculture firms from Greece and Turkey operating over the 2011-2020 period have been identified in the Orbis database and selected for this research. Annual economic and financial data of those firms was collected to carry out our analysis (see Table 1). In addition, we also use industry (meso) and country (macro) data to complete this analysis (see Table 2). The results obtained with our profitability model are showed in Table 3.

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Table 1. Differences of profitability and internal factors between Greek and Turkish firms (Average values for the period 2011-2020)

Firm-specific variable	Unit	All firms		Small firms		Large firms	
		Greek firms	*Turkish firms*	*Greek firms*	*Turkish firms*	*Greek firms*	*Turkish firms*
Number of firms	#	67	32	60	23	7	9
Profitability (ROA)	%	-0.88	7.81	-0.95	6.91	-0.32	10.03
Profitability (ROE)	%	18.15	15.44	21.30	14.22	-8.88	18.42
Age (operating years)	#	23	23	22	21	25	24
Size (employees)	#	45	133	15	NA	305	133
Size (total assets)	th.€	12,448	23,672	4,473	4,969	80,806	71,468
Size (production)	tons	1,544	4,887	576	1,167	9,844	14,394
Financial structure (capital ratio)	%	38.96	40.23	39.81	36.02	32.28	51.00
Cost of debt	%	1.23	9.80	1.05	7.84	2.82	14.60
Unit operating cost	€/kg	5.20	3.75	5.20	3.84	5.16	3.54
Total efficiency	%	67.00	89.98	67.26	98.45	64.70	69.28
Technical efficiency	%	64.15	67.35	63.02	65.40	73.87	72.35
Productivity	kg/€	19.84	27.32	19.84	26.71	19.90	28.81
Profit margin	%	-2.65	7.77	-2.66	5.69	-2.53	12.86
Asset turnover	%	66.11	95.96	66.31	102.48	64.39	79.31
Revenues from exports	%	42.28	12.70	41.65	3.89	47.70	34.23

Source: Orbis database.

Table 2. Differences in external factors (Average values for the period 2011-2020)

External factor	Variable	Unit	Greece	Turkey
Industry (meso) factors	Concentration (CR7)	%	55.69	33.52
	Production	tons/year	115,793	165,007
	Growth	%	1.32	10.08
	Cost of debt	%	1.37	12.34
	Processed fish	%	16.95	72.34
Country (macro) factors	Population	mil. persons	10.85	79.73
	Economic development	constant US$ per capita	18,279	10,880
	Annual fish consumption	kg/person	18.79	5.53
	Inflation	%	0.08	10.21
	Interest rate	%	0.63	11.63
	Labor cost	€/year	21,382	8,743

Source: Orbis, FAO, and World Bank databases.

Table 3. Regression results (dependent variable: %ROA)

Explicative variable	Pooled OLS	GLS random effects
Intercept	22.2695	24.5124
Country dummy (Turkey)	14.4882	12.7924
Age	-0.0125	-0.0163
Size	0.0743***	0.0782***
Unit operating cost	-9.8897***	-9.6368***
Efficiency	1.6655	1.1712
Productivity	-22.3311	-21.8409
Revenues from exports	-0.0085	-0.0076
Financial structure	0.0398***	0.0439***
Industry concentration	0.1301**	0.1012*
Industry growth	0.0610	0.0705
Economic development	0.0014	0.0013
Inflation	-0.7204	-0.6608
Year filter	Included	Included
Observations	486	486
Groups (firms)	92	92
R-square	0.6704	0.6693
Model significance (F test/Wald test)	21.14***	389.08***

Note: Clustered robust standard errors by firm have been used for testing parameter and model significance.
***Significant at the 1% level. **Significant at the 5% level. *Significant at the 10% level.

BACTERIA AND ARCHAEA COMMUNITIES IN COMMERCIAL RECIRCULATING AQUACULTURE SYSTEM PRODUCING SMOLT OF ATLANTIC SALMON *Salmo salar*

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Introduction

The crucial roles of prokaryotes in the recirculating aquaculture system (RAS) highlight the need to characterize the prokaryotic communities in the system. Understanding the communities can lead to improvements in system control, system function, and future system design of RAS, and therefore are indispensable steps. Using two universal primer pairs and two Illumina sequencing runs, we characterized bacteria and archaea communities sampled from eight different sampling points from two identically designed commercial RAS systems (Fig 1).

Results

In this short abstract, we only shared the two most interesting findings. Firstly, we found that the bacterial communities in the biofilm and the water were different both in term of abundance and species present (Fig. 2). The finding contrast with the general belief that recirculation of water in the system would promote the dispersal of bacteria communities between RAS compartments, which the biofilter may serve as the primary bacteria source through continuous seeding from the shedding of old biofilm. Secondly, the diversity of archaeal communities was not high as previously thought. We found only 30 ASVs, with Nitrosopumilus as the dominant group (Fig. 3). This study further supplements our existing knowledge on bacterial and the archaeal community in RAS.

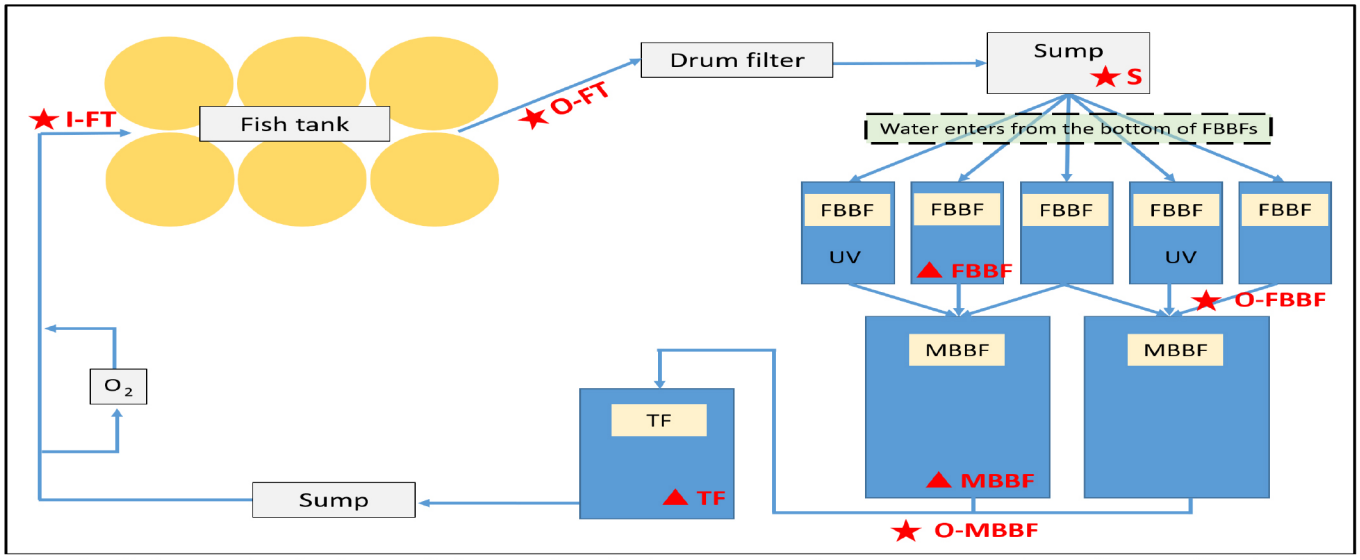

Fig. 1. Schematic design of the recirculating aquaculture system (not for scaling). The solid star symbols represent five different water sampling points: I-FT = inlet of fish tanks, O-FT = outlet of fish tanks, S = sump, O-FBBF = outlet of fixed-bed biofilters, and O-MBBF = outlet of moving-bed biofilters. The solid triangle symbols represent three different biofilm sampling points: FBBF = fixed-bed biofilters, MBBF = moving-bed biofilters, and TF = tricking filter.

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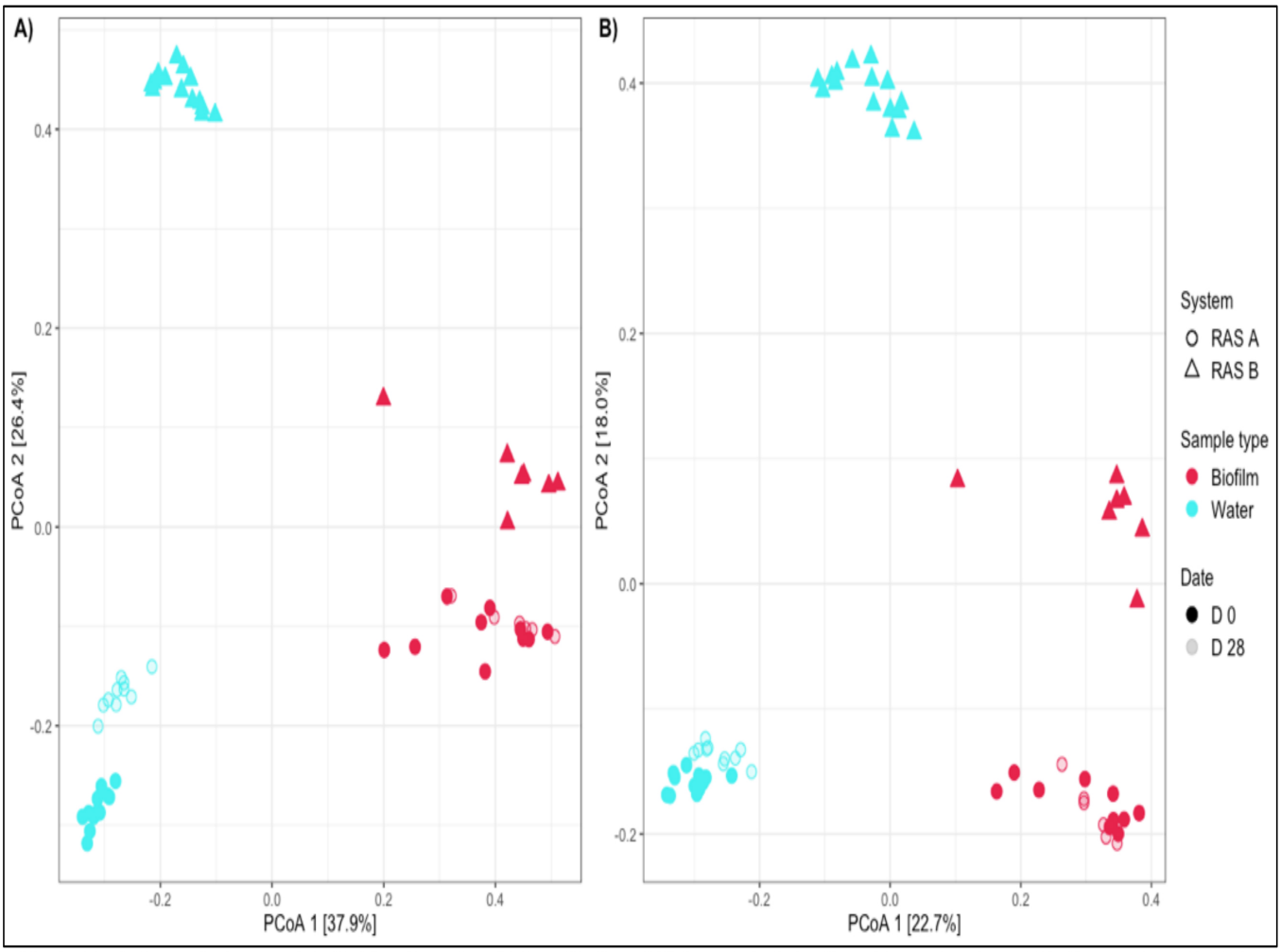

Fig. 2. Principal coordinate analysis (PCoA) plot based on A) Bray-Curtis and B) Jaccard dissimilarity matrix of all water and biofilm samples collected from two different RAS systems on different sampling dates.

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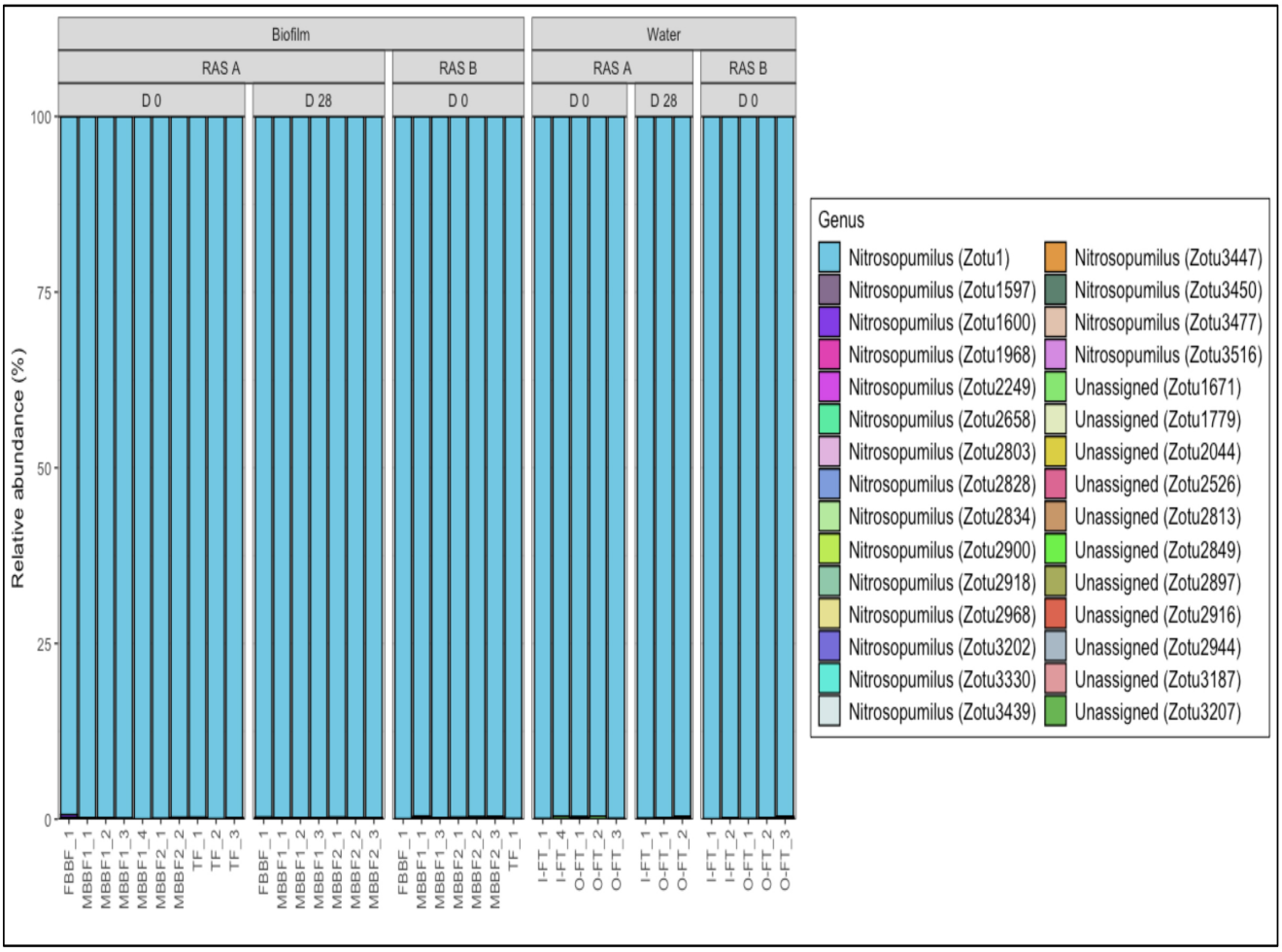

Fig. 3. Composition of archaeal communities at ASV level in the water and biofilm samples.

EFFECT OF FEEDING RATE AND DIET PROTEIN CONTENT ON GROWTH PERFORMANCE IN *Pennaeus Vannamei* UNDER BIOFLOC TECHNOLOGY PRODUCTION (BFT)

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Introduction

White shrimp (*Penaeus vannamei*) production is the most relevant aquaculture sector with a production of 4,966 million kg in 2020 and a commercial value of 28,782 million U$. Biofloc Technology (BFT) is a sustainable aquaculture system based on the principle of recycling waste nutrients, particularly nitrogen, into microbial biomass, which can be used *in situ* by the animals produced or collected and processed. It should be noted that the protein content of feed and feeding regimes can affect not only the growth performance, but also influence water quality through nitrogen excretion, thereby inducing eutrophication. *Penaeus vannamei* requires 30– 50% crude protein (CP) in its diet, but it is important to take into account the supplementary nutrients supplied by biofloc, that it has been demonstrated that dietary protein levels of 24% gave comparable results to 32 or 40% protein (Panigrahi et al., 2019). The feeding regime is a controversial research field that needs to be more studied. Up today, most farmers fed shrimp based on conventional tables, which consider the size and biomass of the organisms to adjust the feeding rate. Tables do not consider the availability of natural productivity of BFT, resulting in a possible overfeeding or underfeeding that eventually might lead to adverse consequences. As consequence, the aim of the present study was to optimize the protein feeding rate using feeds with low protein content at different feeding regimes.

Material and Methods

In the present study, five protein content levels: 30, 34, 38, 42, and 46% were assayed using three feeding rates: 100, 85, and 70% using as reference the feed intake (FI) proposed by Kureshy and Davis (2002) as 100% group. Each group had 4 replicates, therefore a total of 60 experimental units. The feeds were manufactured by adjusting the carbohydrate (Panigrahi et al., 2019) and dietary lipid levels to 10%. All experimental groups were manually fed three times a day. Once shrimp reached an average weight of 0.5 g, they were introduced into the tanks at final densities of 350 shrimp/m^2 (super-intensive production conditions). To assess both survival and growth performance, shrimp were sampled weekly (Kuhn et al., 2010). The animals were housed in 90 L tanks at a salinity of 21±0.15 g/L, temperature of 28 ºC, pH 7.5-8.5, oxygen >5 mg/L, and alkalinity >150 mg/L monitored daily by the multiparametric HANNA equipment, HI19829 Model. Ammonium, nitrite, nitrate, alkalinity, and phosphate values were measured weekly using colorimetric kits in a spectrophotometer (Hanna Instruments). Total suspended solids (TSS) were determined following the protocol of Strickland and Parsons (1972), by weighing the solids retained on glass-fiber Whatman filters (0.45 microns) after filtering the water samples.

Results and discussion

The water quality parameters behaved as expected within the pre-established values, showing tolerable levels for white shrimp production. Regarding the evolution of nitrogenous compounds, as expected in a culture with a mature biofloc, it assimilated the ammonium, nitrite, and nitrate resulting from shrimp production, and the levels remained below 0.09, 0.06, and 170 mg/L, respectively.

In terms of growth performance, the evolution of weight based on protein content or feding rate showed similar results, finding significant differences mainly at the end of the trial (Figure 1).

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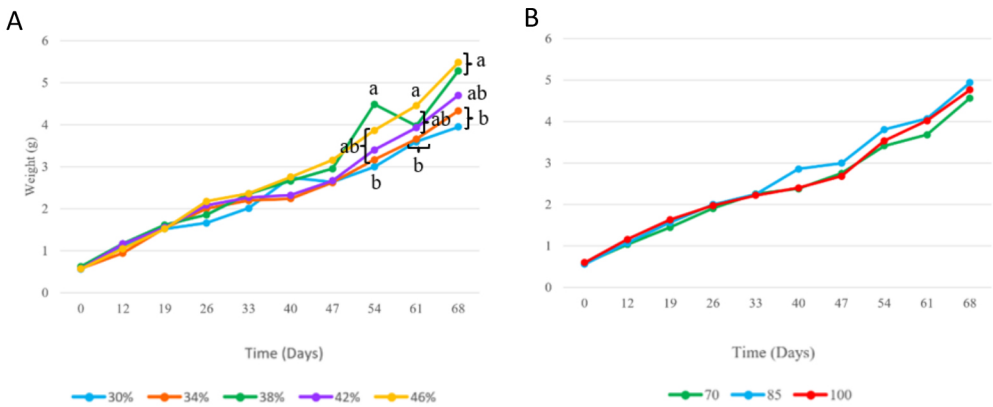

Figure 1. Evolution of average weight (A-B) by protein content and feeding rate respectively. Letters represent significant differences among diets (1A) or feeding rates (1B) at the same time point.

Table 1. Growth performance of *P.vannamei* fed with different protein content diets and feeding rates. Letters represent significant differences among diets or feeding rates.

		Final Weight (g)	SGR (%/day)	FI (g/100g fish day)	FCR
Protein content	**30/10**	3.9 ± 0.2 **b**	2.9 ± 0.08 **b**	5.4 ± 0.02	2.8 ± 0.05
	34/10	3.5 ± 0.6 **b**	2.9 ± 0.2 **b**	5.1 ± 0.2	1.8 ± 0.8
	38/10	4.4 ± 0.6 **ab**	3.1 ± 0.1 **ab**	5.2 ± 0.2	2.1 ± 0.6
Diet	**42/10**	4.7 ± 0.2 **a**	3.1 ± 0.07 **ab**	5.4 ± 0.02	2.7 ± 0.05
	46/10	5.5 ± 0.3 **a**	3.3 ± 0.07 **a**	5.5 ± 0.02	2.6 ± 0.03
Feeding rate	**70%**	3.8 ± 0.4	3 ± 0.08	5.2 ± 0.1	2 ± 0.5
	85%	4.7 ± 0.4	3.2 ± 0.1	5.3 ± 0.08	2.4 ± 0.3
	100%	4.8 ± 0.2	3 ± 0.06	5.4 ± 0.02	2.8 ± 0.04

As is expected feeds with a higher protein content (46, 42 and 38%) achieved the highest final weights (5.5 ± 0.3, 4.4 ± 0.6, and 4.7± 0.2; Table 1), in agreement with Correira *et.al* (2014), with higher growth in diets containing 40% protein. In addition, no distinctions were related to growth according to feeding rate, which may be explained by the consume of natural microbiomase present at Biofloc. In contrast, Kureshy and Davis (2002), obtained better growth with higher feeding rates; and determined that due to a restriction of feed intake and consequently protein intake, diets with low protein content did not support maximum weight gain. No significant differences were observed in relation to survival between experimental groups (64%).

In conclusion, up to 5g in super-intensive production a reduction of 70% is possible, what is translate not only into better productivity but also water quality management.

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CHROMATIN STATE DYNAMICS DURING IMMUNE RESPONSE TO VIRAL-LIKE STIMULATION IN THE EUROPEAN SEABASS

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Introduction

The European seabass (*Dicentrarchus labrax*) is a marine fish of great importance for Mediterranean aquaculture. Disease outbreaks, especially viral infections, represent severe threats that can interfere with the progress and sustainability of intensive aquaculture systems. Knowledge of the complex molecular mechanisms and signaling pathways underlying host response to pathogen is of crucial importance to improve understanding of genome function and regulation. Changes in chromatin structure, in terms of chromatin accessibility for transcription machinery, and histone modifications (HMs) are considered to play a key role in transcriptional regulation.

As part of the AQUA-FAANG (www.aqua-faang.eu) project, the present work aims to profile *in vivo* and *in vitro* response to stimulation with viral (Poly I:C) mimics. RNA-seq, ATAC-seq and ChIP-seq methodologies were applied in order to define a comprehensive genome-wide map of chromatin states and gene expression upon stimulation with viral mimics.

Material and methods

In vitro challenge was conducted on head kidney isolated leucocytes stimulated with PBS or Poly I:C (6 replicates/condition). Cell cultures were collected 12 hours post-infection (hpi) and employed for RNA-seq, ATAC-seq and ChIP-seq library preparation. For *in vivo* challenge, adult individuals were stimulated by injection with PBS or Poly:IC (6 replicates/condition). Animals were sacrificed 24 hpi and head kidney sampled for RNA-seq, ATAC-seq and ChIP-seq library preparation. The histone marks investigated: H3K4me3 (promoter regions), H3K27ac (active enhancer and promoter regions) and H3K27me3 (associated with Polycomb repression). All sequencing data analyses were conducted by using dedicated NF-core pipelines (https://nf-co.re). Differentially expressed genes (DEGs) analysis was conducted with EdgeR and functional enrichment analysis on the DEGs were performed using the package clusterProfiler. ATAC-seq data were employed in order to investigate for differential DNA accessibility between PBS and Poly:IC stimulated samples by using DiffBind package. ATAC-seq and ChIP-seq peaks from the *in vivo* and the *in vitro* experiments were employed to predict genome-wide chromatin states using the software ChromHMM.

Results

Differential expression analysis identified a total of 516 and 1467 DEGs from *in vitro* and *in vivo* datasets, respectively. The two challenges showed peculiar response not only in terms of number of DEGs, but also in terms of enriched biological pathways. The top GO terms in over-expressed genes in *in vitro* stimulated leucocytes were mainly associated with immune system activation (i.e. GO:006955-immune response, GO:0098542 – defense response to other organism), in this context IFN-stimulated genes and IFN regulatory factors are highly significant. *In vivo* challenged head kidney showed a great enrichment on biological processes involved in DNA replication and RNA processing while immune related pathways are associated to down-regulated genes.

Analysis of ATAC-seq peaks revealed weak or no differential DNA accessibility between treatments for *in vivo* and *in vitro* challenges, respectively. Accessible chromatin regions were found to cover up to the 20% of the genome with variations mainly due to cell source rather than treatment indicating that those regions are transcriptionally enabled prior to stimulation.

HMs analysis through ChromHMM software allowed to define the first chromatin state map of non-stimulated and Poly I:C stimulated cells. Comparison between groups highlighted a 4-fold increase of active state regions in Poly I:C stimulated samples.

Conclusion

The present study provides the first genome-wide functional annotation map of the European seabass response to immune stimulation. This represent a significant increase on our fundamental understanding of the genomic basis for immune function and disease resistance in an important aquaculture species.

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THE FINS (FARMING IN NATURAL SYSTEMS) FRAMEWORK FOR AQUACULTURE MANAGEMENT

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Introduction

Marine areas in the coastal zone have a variety of uses including fisheries, aquaculture, tourism, recreation, and other activities, all dependent on ecosystem services. Marine spatial planning (MSP) seeks to optimize coastal resource use to minimize conflict and maximize sustainability. In practical application, most MSP has been a GIS exercise in which the boundaries of various activities are mapped and perhaps zoned in order to reduce conflict.

However, these multiple activities have an influence on the environment that goes beyond, and is often not indicated by, their simple location. Moving beyond static GIS layers to a more realistic portrayal of influences and footprints in the ecosystem provides much greater potential for management and is necessary in order to address carrying capacity and sustainability. Among the activities amenable to such management, aquaculture has huge potential for dynamic planning since (a) farm boundaries are specifically defined, (b) stocking density and biomass of cultured organisms is tightly controlled, (c) there is abundant scope for management operations including site location, stocking, harvesting, and fallowing, (d) environmental interactions such as waste dispersion can be modelled and measured.

This is particularly the case for areas such as North America and Western Europe, where the legislative and governance frameworks are both established and well-tested, but the core principles that underpin sustainable aquaculture management are key to the development of the industry across the whole world and are a fundamental part of the drive for food security and food safety in light of population expansion and climate change.

We present the Farming In Natural Systems (FINS) framework, developed to address the key management questions related to carrying capacity.

The FINS framework includes models to represent the production and ecological pillars (e.g. Inglis et al, 2000; McKindsey et al, 2006) of carrying capacity, including water circulation, harvestable biomass of cultivated species, organic deposition and diagenesis, and dispersal of inorganic waste.

The main objectives of FINS are:

1. To produce a software platform for practitioners (i.e. farmers and managers) that allows for dynamic review of alternatives for siting, stocking, and general husbandry practice, taking into account both the environmental consequences of aquaculture and the ways in which the environment conditions aquaculture;
2. To include and model a range of key performance indicators (KPI), ranging from compliance with loading thresholds to pathogen management and eutrophication, addressing both near- and far-field effects;
3. To test the scientific validity of the framework and the degree to which accuracy and usability can be jointly optimised in order to deliver a robust product for practitioners.

Approach

FINS is used by means of the application shown in Fig. 1. Liverpool Bay, in eastern Canada, is shown in this example, together with various layers such as water circulation and dispersal of ammonia from the cage grid, a medium to far-field effect that is often ignored but a key driver for water column eutrophication.
The following set of FINS capabilities is highlighted:

1. Georeferenced display of bathymetry for any area of the world. In the example in Fig. 1, a high-resolution dataset is used; in other parts of the world; sources such as GEBCO might be required;

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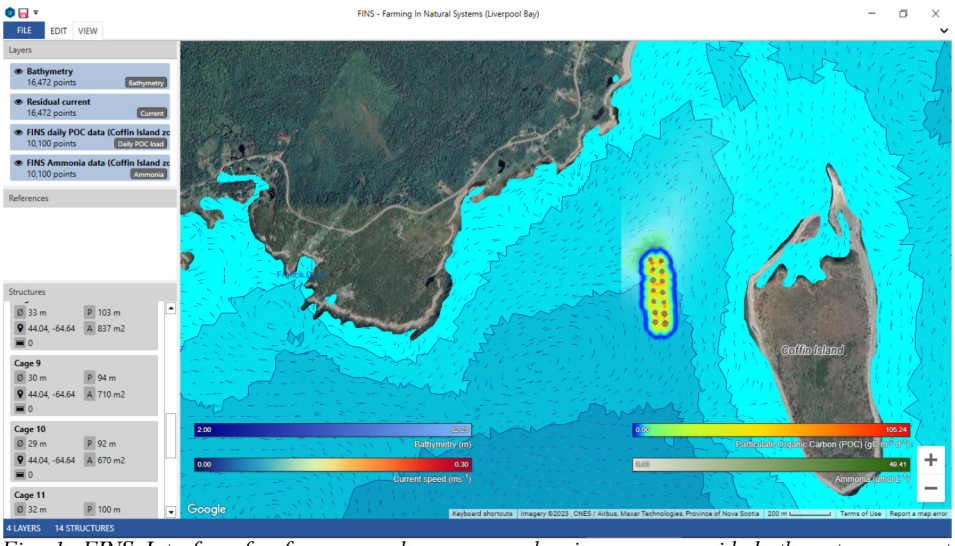

Fig. 1. FINS Interface for farmers and managers showing a cage grid, bathymetry, current velocity, deposition of particulate organic carbon (POC) and ammonia dispersal.

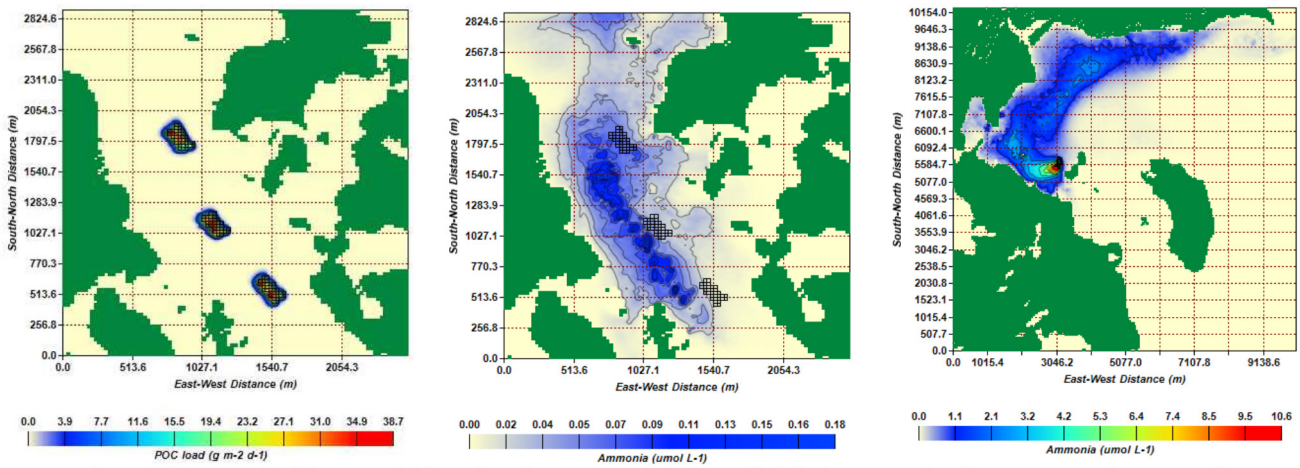

Fig. 2. Outputs from the ORGANIX model based on data exported from FINS: left, deposition for three conceptual 8-cage salmon grids in Whitehead Bay; centre, ammonia released by three conceptual mussel farms in Whitehead Bay; right, emission and dispersal of ammonia from a legacy trout grid in Port Mouton (background NH_4^+ of 1 µM).

2. Display and animation of current velocities obtained from mathematical models and/or measurements from e.g. ADCP moorings; the bathymetry and current velocities are key to drive other models in the framework that simulate specific aspects of the aquaculture activity;
3. Positioning and editing of aquaculture structures, definition of stocked species, stocking status, stocking density; in the example shown and results presented, the cultures species is Atlantic salmon (*Salmo salar*);
4. Multiple grids can be placed in any coastal area (or in a lake) to examine the interactions at a wider scale;
5. Application of models to simulate growth of finfish (salmon, trout, seabass, seabream, tilapia etc) and shellfish (oysters, mussels, clams etc) in order to determine farm production and environmental externalities;
6. Definition and export of a zone of interest for application of specific models to determine deposition, diagenesis, shellfish food depletion, and other KPI;
7. Import of outputs from such models for display and analysis by FINS users. Fig. 1. shows imported layers for loading of POC to the sediment and for dispersal of NH_4^+ by advection and diffusion to a broad area;

The ORGANIX model (Cubillo et al, 2016) was designed to simulate loading of POC to the sediment, using both Eulerian and Lagrangian approaches. In addition, ORGANIX simulates emission and dispersion of ammonia, oxygen demand, and for bivalve shellfish, chlorophyll uptake and its consequences for food depletion.
The FINS framework has been applied to a number of ecosystems on the Canadian east coast, including Liverpool Bay, Whitehead Bay, Port Mouton, and Saddle Island. Hydrodynamic inputs were generated through the application of the FVCOM model and optimised for offline coupling, since one of the priorities for FINS is a very fast execution.

(Continued on next page)

Results and Discussion

The results provided in this paper are divided into two parts: (i) the first provides an illustration of the outputs from the ORGANIX model; and (ii) the second provides details of validation of outputs by comparison to previously published work.

The results in Fig. 2 (left) show the loading of POC to the sediment for three conceptual salmon grids, using an Eulerian model. As expected, the deposition fields in this relatively shallow bay are approximately bounded by the grid area; each cage is 30 m in diameter and stocked with 50,000 fish at an initial weight of 80 g, grown for 500 days, resulting in a final individual weight of 5 kg, with an FCR of 1.13.

In the central pane of Fig. 2, the same conceptual grids are used to grow mussels (1,500,000 individuals per structure). The culture starts with 1 g (live weight) seed and the animals grow to 19 g over a 730 day period. Each individual will clear 7.4 m3 of water during this period and remove (net) 26.3 mg of chlorophyll. The figure shows that there are ammonia peaks to the west of the structures, and unlike the deposition profile, a clear interaction can be seen among the three grids, although the ammonia concentrations at peak are very low (0.18 mM).

The right pane shows ammonia concentrations resulting from a trout (*Oncorhynchus mykiss*) grid at Port Mouton—this farm is no longer active, but a comparison can be made between these results and other work to provide validation for the FINS framework.

It should be noted that in all three examples given, the loading varies in time as determined by the underlying growth model—in other words the FINS approach does not use averaging for emissions, for both finfish and shellfish.

The modelling framework was validated for several bays in eastern Canada, including Liverpool Bay, Port Mouton and Whitehead Bay. Some results of validation for ammonia emissions from a legacy trout site in Port Mouton are shown below.

The dynamics of dissolved nitrogen were simulated for a historic farm in Port Mouton that usually held 400,000 steelhead trout over a production cycle of 16 months. Fish were typically stocked in cages at 150 g in April and were harvested at 2 kg during July or August of the following year. During the growout period, the fish were fed to near satiety.

The pens usually occupied the top 8-10 m of the water column, which averages 10-12 m depth at the farming site. This aquaculture scenario was simulated using two approaches: 1) a fully-spatial dynamic hydrodynamic model in which the mass of dissolved nitrogen released was estimated using a nutrient loading model (Reid et al. 2017), and 2) FINS, which uses the residual currents for the study area and the AquaFish individual growth model to estimate the release of nitrogen. In both simulations, the dissolved nitrogen is considered a conservative tracer in which the only source of nitrogen comes from fish excretion, and the only sink is the tracer that is exchanged through the boundary.

The outcomes of both simulations (Filgueira et al. 2021 and Figure 2 – right pane, for the hydrodynamic and FINS approaches, respectively) differ slightly in the shape of the footprint. While both approaches predict the major concentration of dissolved nitrogen on the eastern side of the vicinity of the farm, FINS predicts a low-concentration plume that extends beyond this area towards the northeast of the bay. The different shapes could be a consequence of comparing the output of a dynamic hydrodynamic model (Filgueira et al. 2021) with the integrated temporal pattern generated using residual currents (FINS).

Despite the difference in shape, it is important to highlight that the maximum values were in both cases comparable, with maximum values between 9 and 10 μM. Further, the total area with a concentration over 3 μM was similar and, in both cases, restricted to the vicinity of the farm. The similar outcomes suggest that the management decisions that could stem from both approaches would be similar. In this particular case, they would indicate the maximum concentration of dissolved nitrogen is below the toxicity levels for seagrass.

Although not shown due to space constraints, we present also simulations of pathogen spread using particle tracking models, and results on the application and validation of diagenetic models driven by POC loads simulated in ORGANIX. The representation of these results is shown in the FINS application.

FINS is applied to eight coastal systems in the Canadian Maritimes, in order to support management, increase sustainability, help create jobs, and contribute to food security.

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Acknowledgements
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SYSTEMATIC REVIEW AND BIBLIOMETRIC ANALYSIS ABOUT ISKNV (SPLEEN AND KIDNEY INFECTIOUS NECROSIS VIRUS) AND CELL LINEAGES

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Introduction

Pisciculture is an economic activity that has being growing worldwide in the last few decades becoming an important source of food and income for numerous producers. Although China is the world's largest fish producer in total production volume, Brazil ranks fourth in the world in tilapia production, indicating a Brazilian tendency to expand its participation in the global fish market. This can be explained by the fact that fish in general are a healthier source of protein than other kinds of meat.

In recent years, emergent viral diseases have been worrying fish farmers due to the great economic losses they may cause. Among them is the infectious spleen and kidney necrosis disease (ISKN), whose etiological agent is a DNA virus (ISKNV) belonging to the *Iridoviridae* family. ISKNV is not classified as mandatory notification in Brazil but can easily decimate big populations of fingerlings. The diagnosis can be done by observing clinical signs or by PCR. Additionally, to study the virulence of the strains, isolation in cell culture is essential. In this regard, the number of scientific papers involving cell cultures for viral agents of importance in pisciculture has increased. Despite being a laborious and relatively expensive method, it has also been applied to the study of the modulation of gene expression, mainly related to immune responses, characterization of cytopathic effects, and obtaining adequate amounts of viral load for next generation nucleic acid sequencing. Furthermore, prior to the implementation of experimental protocols, the selection of the best methodology to be applied is a necessary step, and for this purpose, systematic literature reviews adequately meet this objective.

Objective

The objective of the present study was to conduct an extensive survey of the literature associated with the use of cell cultures for isolation of ISKNV/Megalocytiviruses, considering publications from 2000 to 2022.

Material and Methods

We used PRISMA methodology and worked with secondary data taken from the platforms Scopus, Web of Science and CABDirect. A central keyword was stablished (ISKNV) and several others secondary words (n=15) were used in an advance search with a time frame of 22 years (from 01/01/2000 to 31/10/2022). We also used articles both in Portuguese and English. To assist our search, we used the software Rayyan and only included scientific articles which presented information about ISKNV or its family and details about the viral isolation process fish cell line cultures. All other papers that investigated tracking, genomic expression or phylogeny were discarded.

Results

Following the settled criteria during the first step we gathered 6006 articles which were submitted to Rayyan. A total of 4969 of them were found to be exact duplicates with 100% similarity and were excluded. From the 1037 articles left, we manually searched for other non-exact duplicates and found another 408 papers which were also excluded leaving us with only 629 files to be sorted by reading both title and abstract. In this step, we discarded 586 articles that were not related to our question remaining 43 papers which were eligible and included in our qualitative synthesis to answer our objective. We found that a great majority of the articles selected were from Asian groups which can be explained by the great relevance the aquaculture has in the Asian countries. The oldest article among ones that were eligible was dated from 2008. Most of the studies aimed to investigate different kinds of cell cultures in different viruses to set which type of cell linage would fit best for the cultivation of different pathogens. There were a total of 17 different cell linages including: MFF-1 (Mandarin fish fry); GF (Grouper fin cell); CBP (Chinese perch brain); YFSB (Yellowfin seabream); PSF (Pearlspot fin); ARB8 (*Aequidens rivulatus* brain); MSH (*Micropterus salmoides* heart); SCSC e SCC (*Siniperca chuatsi*); GP (Giant gourami); GB e GS-1 (orange-spotted grouper); RoBE-4 (rock bream embryo); OmB (primary cell culture); EAGS e EAGSB (grouper *Epinephelus akaara*); PMF (Pagrus major fin); ELHK (kidney grouper); SBP, GBC1 e GBC4 (brain

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tissue) and; SK-9 (Cellosaurus-oncogenic). Most studies were combining new or existing cell lines with different viruses to study cell-virus interactions. The most used medium was Leibovitz-15 with 10% fetal bovine serum and temperature around 25-30ºC. The most present cell lineage was the MFF, found in 10 different papers, followed by CPB (8) and GF (6). There were also a few studies that used DMEM as the cell medium and others that tried to use different concentrations of fetal bovine serum. We also found a systematic review from 2011 that compilated studies that were published since the last systematic review in 1994. Some papers searched the apoptotic roles of the cell in viral replication, demonstrating the association of ISKNV with apoptotic involvement in cell cultures infected by this viral agent.

Conclusion

We verified the predominance of cells of fibroblast origin in cell culture isolation procedures for ISKNV. Asian research groups are also a constant in this systematic review, which can be explained by the great cultural relevance of this continent in fish production. We observed a great effort in the attempt to produce new strains of different fish cells, which demonstrate that we are still trying to find the most suitable cell strains for each type of virus that we know.

SCREENING OF ISKNV (SPLEEN AND KIDNEY INFECTIOUS NECROSIS VIRUS) IN TILAPIA (*Oreochromis niloticus*) IN THE SÃO PAULO STATE, BRAZIL

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Introduction

The production of farmed fish has been growing at an average rate of 5.5% per year in Brazil since 2017, with Nile tilapia (*Oreochromis niloticus*) being the highest-producing organism in the country, reaching approximately 550 tons in 2022. Despite working within the guidelines of good practices, there has been an emergence of emerging pathogens in some fish farms in the Southeast and South regions of the country, leading to economic losses. In 2021, the infectious spleen and kidney necrosis virus (ISKNV) was officially reported in Brazil. This virus belongs to the family Iridoviridae, which has seven genera: Megalocytivirus, Lymphocystivirus, Ranavirus, Chloriridovirus, Iridovirus, Daphniairidovirus, Decapodiridovirus (the latter two recently described). Outbreaks of ISKNV have been reported in several countries such as China, Israel, Africa, Australia, and India. It is not a notifiable disease (OIE), but it is potentially a cause of significant losses in aquaculture, as it can infect both marine and freshwater fish.

Objective

The objective of this study was to detect circulating strains of ISKNV (infectious spleen and kidney necrosis virus) in the Southwest region of the state of São Paulo, Brazil.

Materials and Methods

During the month of April 2023 (autumn in the Southern Hemisphere), 171 specimens of tilapia (*O. niloticus*) weighing between 17.12 + 8.42g were collected from a fish farm in the Upper Paraná Basin in the city of Ilha Solteira, SP. Samples were taken from net cages, prioritizing symptomatic fish. Euthanasia was performed using eugenol, and necropsy was directed towards the collection of the target organs for ISKNV (*i.e*. spleen and kidney). Organ pools were made with tissues from three animals and stored in 99% alcohol for molecular analyses. The samples were stored in a refrigerator. Total DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (A1120 Promega), followed by conventional PCR and agarose gel electrophoresis. The primer sequences used are described in Table 1.

Table 1: Primers used for ISKNV (infectious spleen and kidney necrosis virus) detection. Samples from Ilha Solteira, SP, Brazil (2023).

Virus	Primers
Megalocytivirus (RSBIV, ISKNV, TRBIV – 777 pb	MCP-uni332-F3
	(5'-aggtgtcggtgtcattaacgacctg-3')
Major capsid protein - MCP	MCP-uni1108-R8
	(5'-tctcaggcatgctgggcgcaaag-3')
ISKNV (415 pb) major capsid protein	MCP-specI465F3
	(5'-ggtggccggcatcaccaacggc-3')
	MCP-specI879-R6
	(5'-cacggggtgactgaacctg-3')

Source: Kurita & Nakajima (2012).

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The positive samples were subjected to Sanger sequencing, and the obtained sequences were aligned using BioEdit software to fill gaps in the genome present in the databases.

Results

Molecular analyses showed positivity in 38.60% of the samples analyzed for ISKNV, with 52.70% being detected in fish of higher weight (21.33 + 6.95). Several collected animals showed ascites, exophthalmia, and low mucus secretion. Reports from fish farmers indicate that tilapia fingerlings undergo a weakening process resulting in high mortality after 21 days of being stocked in net cages within the reservoir. This mortality is more significant during the transition between seasons.

Discussion and Conclusions

We conclude that the ISKNV virus is present in the Ilha Solteira reservoir, Southwest region of the state of São Paulo, Brazil. This raises interest and concern since virus transmission can occur through water. Further investigations are needed regarding the native ichthyofauna (spillover), as this virus can infect a wide variety of species. This is the first official report of this Iridovirus in the state of São Paulo in the year 2023.

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HUMAN SENSORY CHARACTERISTICS OF KALE WINTERBOR F1 HYBRID *Brassica oleracea* GROWN IN DECOUPLED BRACKISH-WATER AQUAPONICS SYSTEMS AT VARIOUS SALINITIES

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Inland brackish-water recirculating aquaculture systems (RAS) must reuse water as much as possible due to challenges such as saline effluent discharge and inhibitory salt costs. Brackish water aquaponics systems using kale have been shown to reduce nitrate and phosphate that build up over time in RAS. Kale is marketable, but marketability of kale grown in saltwater is unknown. This trial analyzed sensory characteristics and preferences using a panel of volunteers who tasted Winterbor F1 kale grown in a range of salinities (0, 5, 10, 15 and 20 ppt salinity) in decoupled brackish water aquaponics systems containing reused shrimp culture water.

Participants (n=112) were presented with five raw kale samples (one from each salinity) and instructed to taste them in a counter-balanced order and rate their overall liking, taste liking, texture liking, and aftertaste liking on a 9-point hedonic scale anchored by “dislike extremely” and “like extremely”. Participants then rated their perceived intensity of basic tastes (sweet, salty, bitter) using a 0-100 line scale anchored by “not at all” and “extremely”. Following the sample evaluation, participants were presented with a brief description about aquaponics and asked whether this influenced their opinion and willingness-to-pay. Differences in liking and intensity were assessed using one-way ANOVA followed by Tukey’s post-hoc comparisons.

The 5 ppt kale was significantly more liked (overall liking, taste liking, and texture liking) than then 0 ppt kale. Focusing only on taste liking, the 5 ppt was also liked significantly more than both the 15 ppt and 20 ppt samples. No sample was liked less than the 0 ppt control. However, a closer examination of liking ratings revealed a bimodal distribution of the 20 ppt but not 5 ppt samples; in other words, participants were either “likers” or “dislikers” of the 20 ppt sample and very few provided a neutral rating. Aftertaste liking of kale samples grown in any salt concentration was significantly higher than the control 0 ppt sample. Saltiness intensity ratings increased in a dose-response manner, with the 10 ppt, 15 ppt, and 20 ppt samples all rated as significantly saltier than both the 0 ppt and 5 ppt samples. The 5 ppt sample was rated as significantly less bitter than the 0 ppt sample. Together, the saltiness, bitterness, aftertaste, and liking ratings suggest that a low level of saltiness in the kale samples masked the bitter kale flavor, which softened the aftertaste and improved taste liking. Information about aquaponics growing conditions resulted in an average willingness-to-pay of $2.06 USD per bunch, a 38% increase above the reference price of $1.49 USD. Overall, this study demonstrates that kale grown in brackish-water aquaponics is likely to be accepted by the consumer.

EMPLOYING INDIGENOUS MICROALGAE BIOMASS PRODUCTION FOR THE VALORIZATION OF HYDROPONIC WATER EFFLUENTS

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Introduction

Hydroponic farming produces large amounts of wastewater overloaded with nutrients, mineral pollutants, and organic matter. Thus, disposal of agricultural effluents is an emerging challenge of important environmental and economic impact. Exploring means to effectively recycle and reutilize wastewaters, microalgae offer an innovative solution for viable bioremediation, while simultaneously producing high value bioproducts with various biotechnological applications[1]. In the present study, effluent water from hydroponic cultures was used for isolating naturally present microalgal species that may possibly be of biochemical interest and play a crucial role in wastewater treatment.

Material and Methods

The isolation process of indigenous microalgae strains from hydroponic effluents was roughly divided in five successive steps: size separation, general enrichment and 3 separation stages including serial dilution, agar plating, agar streaking and micropipette isolation. Four different strains were isolated and characterized with morphological, biokinetic and molecular taxonomy methods. The microalgal strains were subsequently cultured under steady conditions in both commonly used media and hydroponic water effluents in order to estimate a) the potentiality of hydroponic wastewater as an alternative medium for microalgae b) microalgae exploitation for wastewater bioremediation c) the valorization of the biomass derived and its potent *in vitro* bioactivities. Nutrient removal from the microalgae cultures was measured as well as total biochemical characterization (primary and secondary metabolites) was determined to microalgae biomass. Custom made extracts were also produced in order to evaluate *in vitro* potential bioactivity on human cell line Caco-2.

Results

Our results revealed that all microalgae species included in the study were able to efficiently grow in hydroponic water effluents utilizing the available nutrients (**Fig.1**)

Using the *TufA* and *RBCL* genes from the isolated microalgae strains and the corresponding sequences from reference strains deposited in NCBI a concatenated phylogenetic tree was created using the Maximum Likelihood method (Bootstrap 1000, Kimura 2-parameter) (**Fig. 2**). Based on the results, it's safe to state that PR2 exhibits strong resemblance to *C. reinhardtii* and PR4 strongly resembles *S. reticulata* and *S. rubescens.*

Biochemical characterization of microalgae biomass (**Table 1**) highlighted the high-efficiency biomass of the isolated strains using hydroponic effluents. In order to obtain more data on the accumulation of a wide range of compounds spanning both primary and secondary metabolism, GC-MS based metabolomic platform was also used.

Custom produced extracts from the above microalgae presented no cytotoxicity on Caco2 cells whereas PR4 (0.1-2.5μg/ml) exhibited even greater viability of Caco-2 vs to control (**Fig 3**). The ability of these extracts to protect Caco2 cells against H_2O_2 induced oxidative stress was also demonstrated (**Fig. 4**). To this end, transcriptome analysis of Caco2 cells pre-treated with PR2 (0,5μg/ml) and PR4 (0,1μg/ml) followed by H_2O_2 induced stress revealed significant alternation at several human genes expression.

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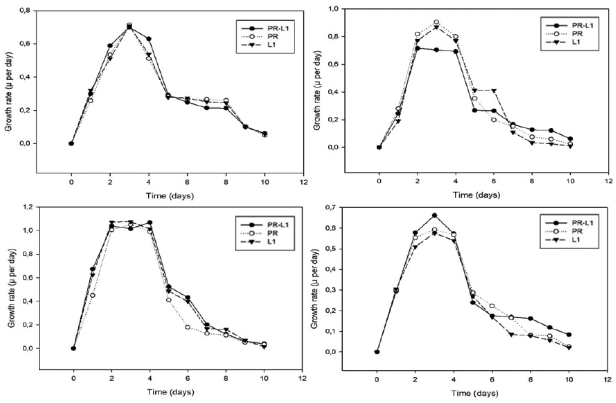

Fig.1 Growth rates of isolated microalgae strains (PR1, PR2, PR3 and PR4) using 3 different culture media; **L1**: commonly used culture media, **PR**: hydroponic effluent, **PR + L1** trace: consists of autoclaved hydroponic effluent enriched with the micronutrient mixture used to create the nutrient medium L1.

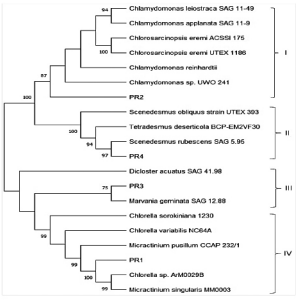

Fig. 2: Concatenated tree for *TufA* and *RBCL* genes (Maximum likelihood, Bootstrap 1000, Kimura 2-parameter)

	PR2 common media	PR2 Hydroponic	PR4 common media	PR4 Hydroponic
FRAP (mg/gDW)[a]	6,34 ± 0,141	7,86 ± 0,148	2,40 ± 0,255	5,52 ± 0,093
TEAC (mg/gDW)[b]	0,46 ± 0,003	0,54 ± 0,004	0,10 ± 0,005	0,47 ± 0,008
Total phenolics (mg/gDW)[c]	3,99 ± 0,058	5,01 ± 0,072	0,87 ± 0,040	3,28 ± 0,063
Total flavonoids (mg/gDW)[d]	203,19 ± 2,89	312,30 ± 3,13	62,95 ± 11,55	151,97 ± 4,57
Total protein content (%)	23,2	39,69	24,4	38,6
Total polysaccharides (%)	28,9	8	43,4	16,1

Table 2 Values are given as mean ± SE (n=3), [a]As ascorbic acid equivalent, [b]As trolox equivalent, [c]As gallic acid equivalent; [d]As quercetine equivalent.

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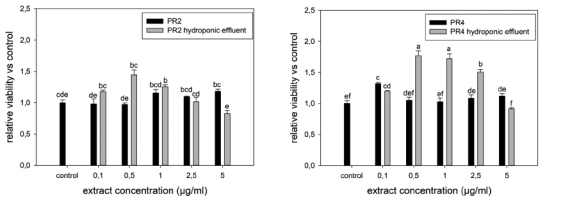

Fig 3 Cell viability by MTT assay on Caco2 cells after incubation with PR2/PR4 extracts (0.1-5 µg/ml). Letters indicate significant differences according Tukey's range test ($p < 0.05$).

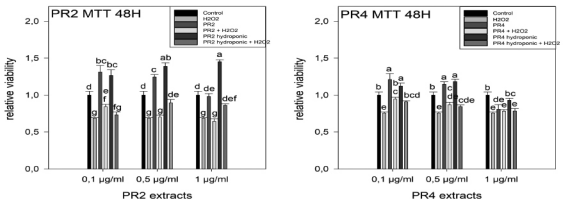

Fig. 4 MTT assay on Caco2 cells after 48h incubation with PR2 and PR4 extracts under 0.05 mM H_2O_2 (3h incubation). Letters indicate significant differences according Tukey's range test ($p < 0.05$).

NOVEL CELL CULTURE AND SINGLE-NUCLEI RNA-SEQUENCING METHODOLOGIES FOR THE STUDY OF WSSV RESPONSE IN PACIFIC WHITELEG SHRIMP

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Introduction

Shrimp are one of the most important groups of animals in global aquaculture. The Pacific whiteleg shrimp (*Litopenaeus vannamei*) is the most cultured shrimp species, accounting for over 50% of worldwide production. Unfortunately, the industry's efficiency and sustainability are threatened by significant annual losses due to infectious diseases, 60% of which are caused by viral pathogens such as white spot syndrome virus (WSSV). Efforts to limit the impact of disease caused by this pathogen are impeded by a lack of effective treatments available.

Materials and methods

In order to establish whiteleg shrimp primary cell cultures, we extracted haemolymph, lymphoid organ and hepatopancreas samples from sterile shrimp and cultured them as dissociated cells or explants in a range of cell media mixes while monitoring the growth and survivability of cells. For RNA sequencing, we isolated nuclei from snap-frozen hepatopancreas tissue and processed it using 10x Chromium Next GEM Single Cell Dual Index kits. The sequenced samples have been analysed and the cell atlas was built using cell cluster marker genes.

Results

Our team has established an *in vivo* infection model in juvenile and adult stage shrimp and developed primary cell culture systems for the study of host response to WSSV using haemocytes, hepatopancreas and lymphoid organ tissues from adult *L. vannamei* (Figure 1). Additionally, we have establish a novel protocol for nuclei isolation and single nuclei RNA-sequencing, and in doing so, create a hepatopancreas cell atlas for the species (Figure 2).

Conclusion

The novel protocols will help us use high throughput transcriptomic analysis to identify priority candidate genes for WSSV resistance via single-nuclei RNA sequencing of WSSV-infected and non-infected shrimp. The results will serve as a foundation for future studies using *in vitro* models and single nuclei sequencing in whiteleg shrimp and set a new foundation for developing therapeutic strategies to combat WSSV in shrimp aquaculture.

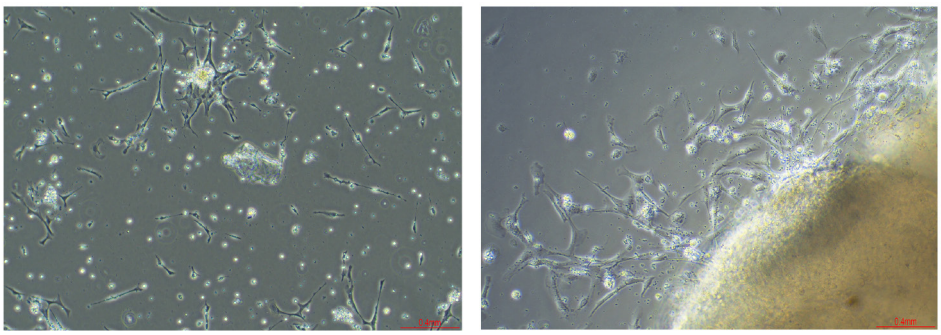

Figure 1. Microscope images of *L. vannamei* primary cell culture. Left: Haemocyte cells dividing seven days post-seeding; Right: Lymphoid (Oka) organ cells emerging from a seeded explant 10 days post-extraction.

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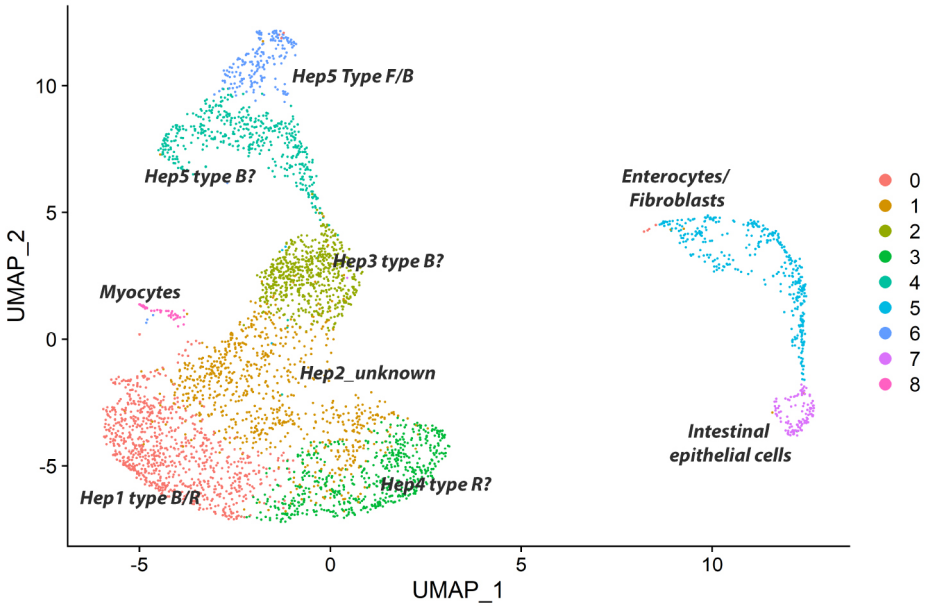

Figure 2. UMAP showing the clustering of *L. vannamei* hepatopancreatic (Fibrillar 'F', reserve 'R' and blister 'B' cells) and adjacent cells. The clustering was generated from 10PCs with a resolution of 0.4. The analysis protocol was adapted from SatijaLab (https://satijalab.org/) and generated using Seurat V4 (Hao*, Hao*, *et al.*, Cell 2021).

Tenacibaculum maritimum AS A FISH HEALTH MODEL FOR THE EVALUATION OF NEW PREVENTIVE STRATEGIES IN MEDITERRANEAN SPECIES

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Introduction

Due to current restrictions on the use of chemicals or antibiotics in aquaculture production, effective and sustainable preventive treatments against systemic infections in cultured species are requiered (Yilmaz *et al*. 2022). To evaluate its effectiveness, the development of new standardized fish health models is needed. In this scenario, a bath infection model for European sea bass (*Dicentrarchus labrax)* against *Tenacibaculum maritimum* has been developed in this study. This bacterium, represents one of the most worrying pathogens for Mediterranean aquaculture species. It is a gram-negative filamentous bacterium which, on top of producing high mortality rates, increases fish susceptibility to other pathogens in a variety of marine aquaculture species. Specifically in this study, this model has been used to test a probiotic (*Vibrio lentus*) characterized, *in vitro* analyzed and cultured by AZTI. The use of probiotics as a method for prophylaxis and control of disease outbreaks in aquaculture has been proposed in recent years (Wanka *et al*. 2018). These compounds included in functional diets have a huge potential optimizing growth and feed conversion and boosting the immune responses, increasing the resistance of fish to diseases. Therefore, in this study, fish performance parameters and innate immune indicators have been evaluated as well as fish disease resistance through a new standardized fish health model consisting in exposing fish to *T. maritimum* and comparing relative percentage of survival (RPS) against a control group (non-probiotic diet). Complementing these results, a cohabitation infection model with the same bacterial strain is under development. Cohabitation models simulate more accurately disease outbreaks in production farms. However, their development is generally more complex as they are more susceptible to other variables such as fish density or ratio naive/infected fish. Preliminary results are very hopeful so a European sea bass against *T. maritimum* cohabitation model is expected to be standardized in forthcoming studies.

Materials and methods

European sea bass were fed during 6 weeks with a diet including probiotic *V. lentus* at 0,5% (probiotic group) or a control diet without probiotic (control group). At the end of the treatment phase, survival, weight gain, specific growth rate and feed conversion factor were calculated. In addition, kidney, plasma and mucus samples were collected to evaluate innate immune indicators using microscopy and absorbance and/or fluorescence techniques. Specifically, phagocytic activity, lysozyme activity, protease activity, anti-protease activity and total proteins have been analyzed. After the treatment phase, fish were exposed to different concentrations of *T. maritimum* to evaluate the RPS. The strain used in this challenge pertains to CTAQUA strains collection (CT0013 *T. maritimum* strain) and prior to fish exposure, it has been cultured in FMM (*Flexibacter maritimum medium*) at 22 °C and 250 rpm during 24 hours (Pazos *et al*., 1996). The infection by bath has been carried out in 10-litre buckets, in which fish have remained for 1 hour with aeration exposed to different concentrations of the pathogen ($7.50x10^4$, $5.00x10^5$ and $1.00x10^6$ CFU/ml, in triplicates). Each concentration has been obtained by diluting the original bacterial culture in seawater with a starting concentration of $1.00x10^9$ CFU/mL. After exposure to the pathogen, fish were transferred the tanks of the RAS (Recirculation Aquaculture System) holding system, controlling all culture parameters throughout the test. For the cohabitation tests, infected a non-infected fish were allocated in the same tanks in triplicate in a 1:1 ratio (fish density 10 Kg/m^3). Infected fish acted as infection vectors and were previously exposed to the pathogen strain following the methodology described above for the bath model. In this case, after infection ($1.00x10^8$, $1.00x10^7$ and $1.00x10^6$ CFU/ml), fish were transferred to the system tanks together with no-infected fish). In order to differentiate the infected/naive populations, naive fish were marked with visible implant elastomer (VIE).

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CT0013 concentration (CFU/ml)	Control group (%)	Probiotic group (%)
$7.50x10^4$	10.00±8.66	8.33± 10.41
$5.00x10^5$	98.00±4.00	80.00±13.00
$1.00x10^6$	100.00±0.00	92.00±3.00

Table 1. Mortality percentage at different pathogen concentrations in control and probiotic group expressed as means ± standard deviation.

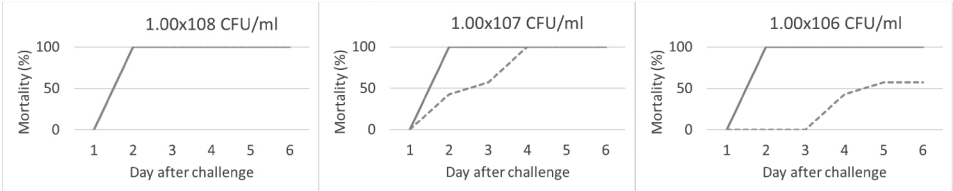

Figure 1. Mortality percentage at different pathogen concentrations. DPC: day post-challenge.

Results

Results did not show differences in growth and feed conversion neither in activity of immune system indicators evaluated between probiotic group and control group but there were differences in the percentage of survival after exposure to the pathogen between groups (table 1). For the development of cohabitation model, we have observed a relation between pathogen concentration and mortality percentage (figure 1).

In sight of the results, it can be concluded that bath infection model with *T.* maritimum has allowed the validation of the probiotic, which has a potential effect as a preventive treatment. Blue line: infected. Orange line: naive.

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Acknowledgement

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USE OF BIOCONTROL AND FUNGICIDES IN AQUAPONICS; IMPLICATIONS FOR FISH AND BIOFILTER
USE OF FUNGICIDES AND BIOCONTROL IN AQUAPONICS; IMPLICATIONS FOR FISH AND NITRIFYING BACTERIA

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Introduction

Aquaponics is a sustainable food production system combining aquaculture with hydroponics to simultaneously produce fish and economic crops. Disease infestation is a critical challenge in aquaponics due to the limited available safe curative methods. Biological control and natural fungicides can be crucial to sustainable control of diseases in aquaponics. Therefore, we examined the use of entomopathogenic fungi (*Isaria fumosorosea and Lecanicillium attenuatum*), mycoparasitic fungus (*Trichoderma virens*) and the risks associated with the use of natural (clove oil and lecithin) and synthetic (tebuconazole) fungicides on a biofilter and Nile tilapia, *Oreochromis niloticus*, in aquaponics. Our study identified that *T. virens*, besides its biocontrol property, can improve the growth of basil plants in aquaponics at a concentration of 1 x 107 spores per ml. The foliar application of clove oil (eugenol), lecithin, and tebuconazole at recommended dosages, spray-drifted, and were detected in aquaponics water at a percentage runoff rate of 0. 3 %, 2.3 % and 0.3 % respectively. In the biofilter, tebuconazole and clove oil at the maximum runoff concentration showed no significant effects on the nitrification processes during a 96 hr exposure period. In contrast, lecithin altered the ammonium and nitrite levels by substantially increasing ammonium-nitrogen levels from an initial 5 mg L-1 at the 1st hour to ~13 mg L-1 at the 6th-hour post application. These runoff concentrations were further evaluated on the physiology of Nile tilapia in a 28-day semi-acute toxicity test. The tebuconazole-treated group showed a significant effect on hematological (haemoglobin, red blood cell, MCH, etc.), biochemical (total protein, albumin, globulin, etc.), and antioxidative (glutathione peroxidase and glutathione reductase) parameters. Eugenol, on the other hand, showed no significant effects on the fish physiology, indicating its suitability for all aquaponics systems. The use of lecithin and tebuconazole should only be limited to decoupled aquaponics due to their effects on the biofilter and fish.

Materials and Methods

For ethical concerns regarding the unmeasured effects of fungicides on fish, three experiments were conducted separately. The first, a pre-requisite experiment, was conducted on monitoring the runoff of the active ingredients of applied fungicides in the water of decoupled aquaponics over 72 hr timepoints. Using the concentrations detected in the aquaponics water in the first experiment, we exposed a matured biofilter to the maximum concentrations of the active experiment to investigate their effects on nitrification and nitrifying bacteria. In the third experiment, *Oreochromis niloticus* were exposed to the maximum concentrations in a semi-acute toxicity test over 28 days to determine subacute toxicity from the runoff concentrations.

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Result

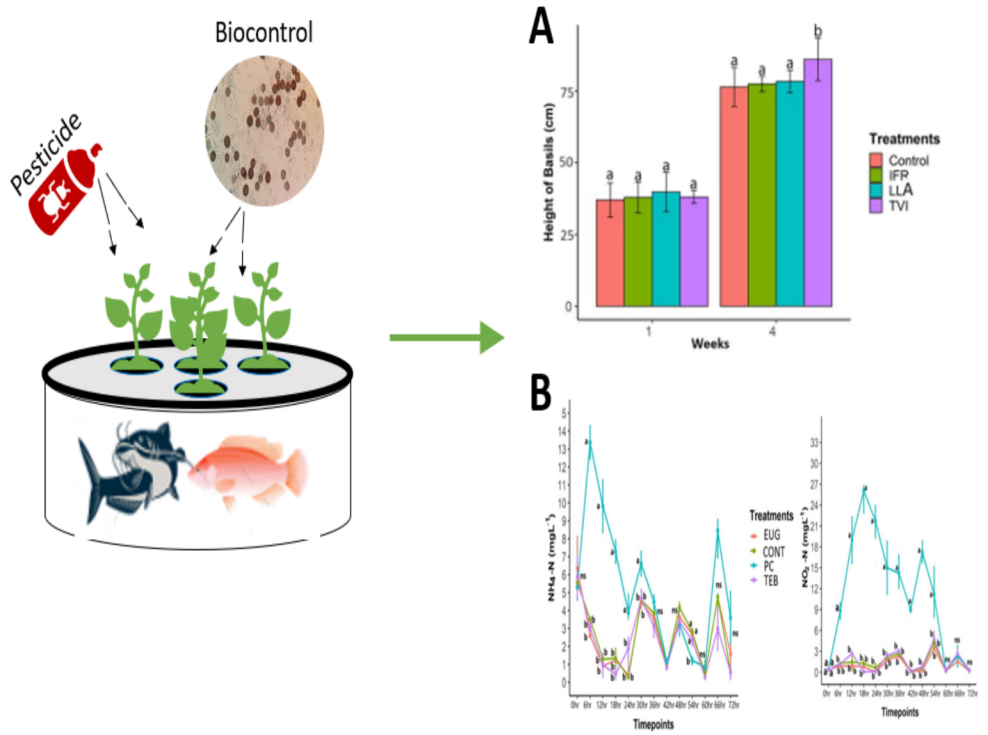

Figure 1. A graphical abstract showing the effects of disease treatments, biocontrol agents and pesticides on aquaponics basil and nitrification. Graph A shows that *T. virens* (TVI), improved the growth of basil in aquaponics over a period of 4 weeks. Graph B shows that lecithin fungicide (PC) runoff in aquaponics, significantly altered the ammonium and nitrite levels.

THE NUTRITIONAL COST OF MOUNTING THE IMMUNE RESPONSE IN A. SALMON (*Salmo salar*)

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Introduction

Mounting the immune response and maintaining a competent immune system is thought to be a nutritionally demanding process. In terrestrial animals, the influence of an immune challenge on animal growth is well established (Huntley et al., 2018). In fish, and particularly in A. salmon, it remains to be investigated. An immune challenge and the associated lower feed intake can theoretically partition energy and nutrients away from productive processes such as muscle growth, and negatively impact efficiency. In this work, two experimental trials were carried out to first estimate the nutrient costs of immune stimulated A. salmon, and second to design diets to support the adequate immune response and performance during an immune stimulation, using vaccination as challenge model.

Material and Methods

To validate vaccination as a model to estimate nutrient utilisation during immune challenge a pilot trial (trial 1) was carried out in fresh water with A.salmon from 50 to 70g. Two groups of fish were vaccinated with either commercial vaccine 1 (V1) or commercial vaccine 2 (V2), a third group of fish was injected with a saline solution (S) and a fourth group was used as a control (C). All fish were fed with the same commercial diet. At the beginning of the trial and after 24 days post vaccination fish were sampled for body composition for nutrient balance estimation. To evaluate the dietary support to the immune response, a second trial (trial 2) was carried out with fish from 65 to 100g in fresh water. Three groups of fish were immunized with a commercial vaccine after 17 days of feeding with one of the following diets: energy rich (E), amino acid rich (A) and energy and amino acids (EA). A fourth group consisted in non-injected fish (C) fed a commercial diet served as control. After 41 days post-immunisation, performance, nutrient retention, immune response, and gene expression were the response parameters measured.

Results

In trial 1, the immune stimulation response affected nutrient retention. Vaccinated fish (V1 and V2) showed between 7 to 16% lower energy retention efficiency, between 15 to 20% lower fat retention and between 4 to 13% lower protein retention, in comparison with the non-vaccinated control group (C). Moreover, V2 fish showed lower amino acid retention efficiency for most of the amino acids in comparison with the rest of fish groups. In trial 2, immunized fish ate 7% less compared to non-immunized. Increasing dietary energy improved protein retention with approximately 8%, suggesting higher energy requirement of immunized fish. Immune response was not different between dietary groups suggesting the primary importance of immune function. There were differences in gene expression regulation between dietary treatments and immune stimulated fish.

Conclusion

Overall, the results showed for the first time, the cost of mounting an immune response through vaccination in A. salmon. Nutrient retention and gene expression is modified under immune stimulation towards energy utilization, while the immune response was shown to be prioritized. It is possible to design feeds to precisely support performance under an immune challenge.

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THE EFFECT OF SHORT-CHAIN FATTY ACIDS IN THE GUT IMMUNE SYSTEM OF EUROPEAN SEA BASS (*Dicentrarchus Labrax*): AN "*EX VIVO*" APPROACH

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Introduction

The fish gut is a unique biological system that functions as a barrier against pathogens and regulates osmoregulation, immunological and inflammatory responses (Peñaranda et al., 2020).

The immune system comprises innate and acquired responses and can be modulated by functional additives like Short Chain Fatty Acids (SCFA) (Ahmadifar et al., 2019; Semple & Dixon, 2020). SCFA are bacterial fermentation products, formed by a short hydrocarbon chain (2-6 carbons) and a carboxylic acid moiety (Corrêa et al. 2016). Acetate (C2), propionate (C3), and butyrate (C4) are the most abundant SCFA in the fish intestine and are transported through intestinal cells by passive diffusion or by active transport of dissociated SCFA anions using different transporters, namely anion exchange with bicarbonate (den Besten et al., 2013). In the intestinal epithelial cells, SCFAs are partially used as a source of ATP, contributing to the maintenance of gut homeostasis, and playing an important role in the enhancement of anti-inflammatory and antimicrobial responses (Parada Venegas et al. 2019).

Ex vivo techniques allow the evaluation of tissue's response to external stimuli and reduce the number of experimental fish compared to the in vivo approach (Bello and García-Arrarás 2022).

Thus, this study aimed to evaluate the intestinal interactions between the SCFA acetate, propionate, and butyrate and pathogenic bacteria in intestinal explants of European sea bass (*Dicentrarchus labrax*) juveniles.

Material and Methods

The anterior intestine of 12 fish with an average weight of 100g and killed by excess anesthesia with 2-phenoxyethanol were sampled, sliced into pieces with an average weight of 1.3 mg, and placed in 24-well plates (3 pieces per well). The experimental treatments consisted of a control and 1mM and 10 mM of sodium acetate (SA) sodium butyrate (SB) and sodium propionate (SP), and each treatment was replicated 6 times. After being incubated with a pre-treatment medium (penstrep 500U, FBS 10%, glutamine 2mM, and glucose 5.5mM) for 1h at 22ºC and 100 rpm, one piece of tissue per well was collected (pre-treatment) and stored in RNAlater at -80ºC until analysis. The two remaining explants were incubated for 2 hours at 22ºC and 100 rpm with DMEM medium (penstrep 100U, FBS 10%, glutamine 2mM, and glucose 5.5mM) supplemented with each SCFA at a concentration of 1 or 10mM and then one piece of tissue per well was collected (SCFA treatment) and stored in RNAlater at -80ºC until analysis. The remaining explant was challenged with *Vibrio anguillarum* at 1×10^7 for 2 hours at 22ºC and 100 rpm and then collected (bacteria challenge) and stored in RNAlater at -80ºC until analysis.

The expression of pro- and anti-inflammatory genes, namely tumor necrosis factor β (TNF-β), interleukin 8 and 10 (IL-8 and IL-10), transforming growth factor β (TGF-β), caspase 3 (Casp 3), and nuclear factor k β (NF-kβ) was evaluated according to (Machado et al., 2021).

Results

Incubation with 1 or 10 mM of each SCFA increased NF-Kβ and TGF- β expression. Regardless of the concentration, incubation with sodium acetate and sodium propionate increased the expression of caspase 3 compared with sodium butyrate.

At 1mM concentration SA, SP and SB increased the expression of NF-Kβ compared with the control diet, on the other hand the higher concentration of 10 mM increased the expression of IL-8 and Caspase 3.

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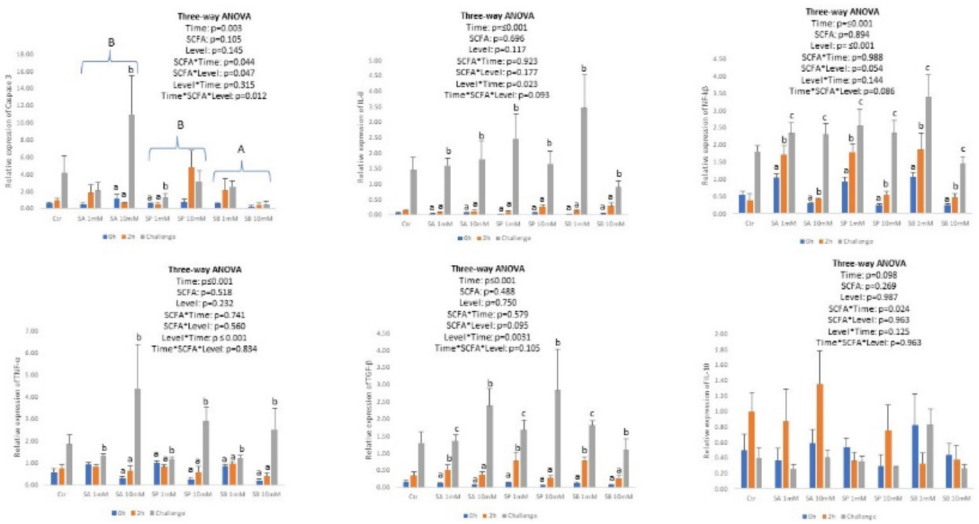

Figure 1. Quantitative expression of immune related genes in the anterior intestine of European seabass subjected to the experimental treatment and challenged with vibrio anguillarum. Values presented as mean and standard error (SE); Three-way ANOVA followed by Tukey's test. Capital letters means differences between SCFA and small letter differences between time within each treatment

Regardless of SCFA, the bacterial challenge increased the expression of all immune-related genes analyzed, except for IL-10.

Overall, the incubation with SCFA increased the anti-inflammatory NF-Kβ and TGF- β, and the bacterial challenge modulates the expression of inflammatory responses.

Acknowledgments

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BEST PRACTICES FOR PREVENTING WINTER ULCERS IN ATLANTIC SALMON *(Salmo salar L.)* IN NORTHERN NORWAY

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Introduction

Winter ulcers in Atlantic salmon generally lead to reduced welfare and downgraded product quality. Wound development is mainly linked to the winter ulcer bacteria, particularly *Moritella viscosa* or a cocktail of other bacteria, i.e., *M. viscosa*, *Tenacibaculum* spp, *Vibrio* spp. These pathogens thrive at low temperatures (< 8 °C). Naturally enough, the winter ulcers outbreak in salmon aquaculture is a significant fish health challenge in northern Norway, where temperatures are lower and production time is longer.

An essential measure against winter ulcers is avoiding handling the fish as much as possible. However, due to high salmon lice infestations, the fish must undergo delousing treatments. These treatments can be both medicinal and non-medicinal. In the case of non-medicinal delousing treatments (which are more effective), there are unavoidable injuries to the fish skin, where the outcome is usually ulcer outbreaks at low temperatures. The severity of the outcome depends on many factors, but handling intensity; among others, how hard or gently the fish is handled, is very important. From our field experience, there is a significant knowledge gap in the cause-and-effect relationships and limited knowledge about the factors associated with this variation. Results from our pilot studies show that sedating the fish during crowding and delousing operations makes fish calmer, reducing the risk of ulcer development. In addition, there is little data about which indicators can be used to assess the risk of winter ulcers. This lack of knowledge gives limited room for action to adapt the production plan or implement measures to reduce the risk.

Furthermore, there is no systematized knowledge about how crowding affects the risk of winter-ulcer outbreaks during delousing operations. We will fill in this knowledge gap by documenting the effects of handling and different crowding intensities (CI) on the susceptibility of Atlantic salmon to winter ulcer outbreaks. We will document this by answering the following questions -

1. Does the level of CI during cold seasons significantly affect ulcer outbreaks?
2. Is it primarily the physical damage to the skin and mucous layers, stress, a compromised immune system, pathogens, or combinations of all these factors that are decisive for wound development after handling?
3. Can indicators for ulcer risk be established to be used as decision support when preventive measures against ulcers are to be defined?

The main goal of this study is to identify best practices for crowding and mapping wound risks in connection with handling second autumn and winter at sea to reduce the risk of, or extent of, winter ulcer outbreaks in Atlantic salmon. Results from this project will help to shed light on what is today's best practice for crowding and how it should be adapted to a situation with low temperatures and an increased risk of wound development. The author will present and discuss the experimental design at Aquaculture Europe 2023.

Material and Methods

The project is financed by the Norwegian Seafood Research Fund (FHF 901835), with a total budget of 1,112,261 Euros[1]. The project (named ReduSår; duration: 2023-2025) will be a close collaboration between the Norwegian industry and research institutes/Universities.

The experiment will be performed at the R&D Sea facility of LetSea AS at Dønna, Norway, in October 2023. We will use mesoscale cages (n=12) in triplicates with large pit-tagged salmon (approx. 2.5 kg; n = 200 per cage). All cages will be treated uniformly, except for crowding methods during the corresponding delousing. We will test different combinations of CIs, i.e., grades 1 and 3, according to FISHWELL (referred to as **T1** and **T3**) with or without sedation (referred to as **S+** and **S-**) during a 60-minute crowding operation[2], giving us three test groups **T1 S+, T3 S-, T3 S+** and a control group (**T1 S-**). Grade 1 is an acceptable CI in the Norwegian aquaculture industry, whereas Grade 3 is an undesirable practice (see

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FISHWELL for more info). Water and fish mucus samples will be collected throughout the experiment to investigate the presence of wound bacteria; furthermore, camera-based monitoring of wounds at the fish group level will also be done. The aim is to utilize the detailed monitoring of wound development in the experiment to test indicators for predicting ("early warning") of wound outbreaks.

Results

Sedation as a measure for more gentle handling must be systematically investigated. In the short term, our results will help salmon breeders learn from each other's experiences and increase the basis for decision-making when planning and carrying out handling operations. In the longer term, knowledge developed in the project will provide a basis for optimizing production planning, methods, or measures for lice prevention or treatment.

The project will also map possible new indicators for increased winter ulcer risk or "early warning" in case of ulcer outbreaks. Such indicators can initially help to improve the decision-making basis for crowding methods or adjusting handling procedures before and during delousing. Over time, new mapping tools can be established to develop knowledge about the causes of an increased risk of winter sores. The project will be able to contribute both knowledge and new tools that can improve preventive and mitigating measures to reduce wound problems in the industry, thereby improving fish welfare, profitability, reputation, and sustainability.

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PREVENTING SPINAL DEFORMITIES IN PIKEPERCH: INVESTIGATING THE EFFECTS OF MECHANICAL IMPACT FOR IMPROVED AQUACULTURE PRACTICES

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Introduction

Pikeperch is a species with growing value for aquaculture, but its rearing has proven to be a bottleneck for the industry (Policar et al., 2019). One factor affecting larval quality is deformations, exemplarily spinal curvatures such as lordosis and scoliosis. While many factors are known to influence the occurrence of vertebral malformations (Boglione, Gavaia, et al., 2013; Boglione, Gisbert, et al., 2013; Di Biagio et al., 2022), one factor that can be influenced by the husbandry conditions are mechanical stressors as water currency and the handling, exemplarily during size sorting procedures. The resulting deformations can negatively affect pikeperch health, welfare, and performance. Consequently, understanding the underlying mechanisms and the developmental timing of vertebral formation and the impacts of vertebral deformations in pikeperch is essential for developing effective management strategies, to improve fish welfare, and optimize aquaculture practices. This study aims to investigate the occurrence and mechanisms of vertebral column deformations in pikeperch resulting from mechanical stressors.

Materials and methods

The study involved the examination of over 1000 farmed pikeperch specimens, collected from the aquaculture pikeperch rearing facility. Hereby, the sampling focused on specimens of different age stages between 17 and 40 dph to allow observation of vertebrae formation and older postlarval specimens of 67 dph to analyse malformations that may occur. The fish were examined for the presence of lordosis using visual inspection and clearing and staining procedures (Taylor & Van Dyke, 1985). Additionally, the fluorescent properties of the Alizarin red staining were utilized to verify for the formation of vertebrae and to investigate the changes in the spinal structure.

Results

Based on external observations, the specimen of the oldest group had a almost 65% occurrence rate of vertebral column defects. Among them, scoliotic deformations occurred at a higher rate (47.6%) than lordotic (30.5%). Caudal vertebral column deformations occurred in 26.7% of the specimens. Further analysis of the cleared and stained specimen in this age group showed that even some of the specimens with externally normal body shape possessed deformations of the vertebrae. In these cases, inconspicuous caudal vertebral column deformations were revealed by the clearing and staining procedure. This caudal spinal area proved to still be less ossified in the stages just before the onset of size sorting. In these, vertebral formations were present up to centrum 37-40 of the total 44-45 vertebrae. Fluorescence analysis indicated that influences on the vertebral deformations might be associated to the osteological developmental status of the larvae.

Discussion

The observed higher incidence of caudal vertebral deformations suggests that this issue might be linked to aquaculture practices, especially size sorting procedures. Generally, sorting procedures are necessary for pikeperch rearing, because of the high growth rate during larval development (Franz et al., 2021) and the occurrence of cannibalism, especially between different sized specimens. Therefore, sorting procedures are an important tool to reduce losses in pikeperch aquaculture (Kestemont et al., 2003; Szczepkowski et al., 2011; Zakęś et al., 2004). Adjusted timing and handling protocols could effectively reduce the incidence of lordosis in farmed pikeperch in this regard. In conclusion, this study sheds light on the occurrence and impacts of vertebral column deformations in pikeperch due to mechanical impact, emphasizing the need for further research and management practices aimed at reducing the incidence of this issue in the aquaculture industry.

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GENETIC ARCHITECTURE OF RESISTANCE TO SEA LICE IN ATLANTIC SALMON: CONSISTENCY ACROSS TWO SEA LICE SPECIES

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Introduction

Sea lice are parasitic crustaceans that infest Atlantic salmon (*Salmo salar*). They attach to the skin of salmons and feed on tissue, mucus and blood, causing abrasion-like lesions, open injuries and stress that in turns lead to reduced growth rates, secondary infection due to opportunistic pathogens and increased mortality. Sea lice represent one of the most important threats to salmon aquaculture and welfare, causing millions of losses worldwide. Several species of lice can affect Atlantic salmon, with *Lepeophtheirus salmonis* being predominant in the northern hemisphere and *Caligus rogercresseyi* in the southern hemisphere. Better understanding of genetic resistance to both parasites is a prerequisite to include in selective breeding strategies to improve lice resistance. Here we study resistance to both *L. salmonis* and *C. rogercresseyi* in Atlantic salmon, working with the same salmon families in the two hemispheres to assess whether resistance to both parasites has the same genetic background.

Material and methods

During three consecutive years, 2017 to 2019, a total of 4 375, 3 730 and 5 346 Atlantic salmon fish were produced, respectively, belonging to 160 to 200 full-sib families from Benchmark Genetics breeding programme. For each year-class (YC), the offspring were separated into two groups at the eyed eggs stage and sent to two different locations for rearing and disease challenge. Half of the offspring were challenged in Iceland with *L. salmonis*, while the other half were challenged in Chile using *C. rogercresseyi*. A similar challenge protocol was used across locations and year-classes. Briefly, fish were raised in separate family tanks until tagging, and then mixed. For the challenge, fish were separated into 2 to 4 tanks with a recirculating system, and 30 (in Iceland) or 40 (in Chile) copepodite of lice per fish were deposited in each tank. After 7 to 15 days, the number of lice (at sessile stage) attached to each fish were visually assessed and reported as sea lice count (SLC). The body weight (BW) of each fish was recorded before and after the challenge.

The fish were genotyped using 57K (Chile, YC2017) or 65K SNP (all other year-classes) arrays (with 33K shared SNPs). Standard quality controls on SNPs and individuals were performed using PLINK [1] in each dataset separately, and then all were merged into a single dataset. Genotype imputation using FImpute3 [2] was performed to obtain a final dataset with 61,065 SNPs.

Table 1. Heritability and genetic correlation estimates for resistance to two sea lice species and body weight measured in two locations.

	SLC 2017	SLC 2018	SLC 2019	BW 2017	BW 2018	BW 2019
h^2 (Iceland) *L. salmonis*	0.10 ±0.03	0.13 ± 0.03	0.21 ±0.03	0.59 ± 0.03	0.62 ±0.03	0.51 ±0.03
h^2 (Chile) *C. rogercresseyi*	0.26 ±0.04	0.21 ±0.03	0.15 ±0.03	0.41 ± 0.04	0.43 ±0.04	0.56 ±0.04
Rg (*Chile : Iceland)*	0.13 ±0.16	0.62 ±0.13	0.03 ±0.13	0.82 ± 0.05	0.81 ±0.05	0.63 ±0.05

h^2 = heritability (± sd) and Rg = genetic correlation (± sd), SLC= sea lice count, BW= body weight.

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Genetic parameters (variance, heritability and genetic correlation) were estimated using blupf90 [3]. Genome wide association studies (GWAS) were performed with a mixed-linear-model implemented in GCTA [4] to detect QTL associated with resistance. The animal model used for variance component estimation and GWAS included tank, sex and counter (when available) as fixed effect and body weight as covariate.

Results

Heritability of Atlantic salmon resistance to each lice species was consistent across year-classes and estimated to be low for *L. salmonis* and moderate for *C. rogercresseyi* (Table 1). A low to null genetic correlation was observed in YC2017 and YC2019 between resistance to the two lice species. In YC2018, a high positive genetic correlation was estimated for resistance to *L. salmonis* and resistance to *C. rogercresseyi.*

Body weight was highly heritable in both locations, with a higher heritability in Iceland than in Chile for YC17 and YC18. High positive genetic correlations were estimated across locations for body weight measured within a year class.

The GWAS performed showed that resistance to both sea lice species is highly polygenic. Only one QTL above the 5% genome wide significance threshold was identified, located on chromosome Ssa15 for resistance to *C. rogercresseyi* in YC2017.

Discussion and conclusion

Resistance to *L. salmonis* and resistance to *C. rogercresseyi* in Atlantic salmon are highly polygenic. It is unclear whether resistance to the two species share common genetic mechanisms. The absence of genetic correlation for resistance to the two sea lice species in YC2017 and YC2019 and the high positive correlation observed in YC2018 might be because of differences in the challenge methods and the parasite counting procedure. Indeed, the size of the fish at challenge and the number of days of challenged and the number of people involved in the counting of the lice varied according to the location and the year, reflecting normal practices in the farms. A meta-analysis combining all three year-class will be performed to better understand the genetic architecture of sea lice resistance using a powerful dataset of over 10,000 fish.

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LIFE - BOAT 4 STURGEON - AN INTEGRATIVE EFFORT TO SAVE DANUBE`S STURGEONS

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The project LIFE-Boat 4 Sturgeon builds upon the methods and results of the LIFE-Sterlet project. It has the aim to establish a genetically diverse captive broodstock of mature animals for all four remaining Danube sturgeon species in at least two locations (AT and HU). Those stocks will be maintained over the long-term to preserve the gene pool and to support all four species with genetically diverse, autochthonous and fit juveniles. In Austria, a floating rearing station in the Danube in the centre of Vienna will be built in addition to the existing LIFE-Sterlet hatchery container. The mother fish stock of all species will be constantly expanded through different genotypes and the reproduction through a studbook enables the greatest possible genetic diversity of the offspring. Existing monitoring efforts are to be continued and intensified to document the development of the populations. Furthermore, an investigation along the whole Danube for possible residual populations, will be carried out. For the whole Danube Region and other European catchments a long-term database and manual for ex situ actions and monitoring in sturgeon conservation will be provided. Further objectives are the coordination with fishing authorities and communities along the Lower Danube and Black Sea to reduce IUU fishing and to raise general public awareness.

SEA STAR MEAL AS A NOVEL PRODUCT FOR AQUAFEEDS

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Introduction

An extraordinary biomass resource in marine habitats is present in the form of sea stars, which are scarcely utilised in European marine environments. The InEVal project approaches diverse value chains related to increasing and adding value to echinoderm biomass to create new processes, products and industry. One aim is the up-valuing of waste sea star biomass from European inshore shellfish industries and mussel farms by developing and evaluating new products. Sea star biomass offer a valuable resource as a sustainable and low prized alternative protein source from sidestreams and further processed as a feed ingredient for aquafeeds. A previous study (van der Heide et al., 2018) already revealed promising nutrient compositions of the sea star species *Asterias rubens*. The aims of this study were to process sea star biomass to meal and to determine the suitability of using *A. rubens* meal as replacement for fish meal (FM) in shrimp (*Litopenaeus vannamei*) and European sea bass (*Dicentrarchus labrax*) diets.

Materials and methods

Sea stars *A. rubens* were harvested at a mussel farm (Kieler Meeresfarm, Kieler Förde, Germany), dried, ground to meal and analysed for nutrient content and amino acid profile (Table I). Diets for Whiteleg shrimp (*L. vannamei*) and European sea bass (*D. labrax*) were formulated with increasing levels of sea star meal (SM) as FM replacement. In two feeding experiments, the acceptability of SM inclusion in diets were investigated in shrimp and sea bass in a recirculating aquaculture system. Survival, growth performance, feed intake and metabolic parameters were evaluated.

Results showed an annual variation in the composition of the nutrients and amino acids in sea stars *A. rubens* showing highest crude protein, crude fat, lysine and methionine content and lowest crude ash content in spring. Shrimps accepted all diets containing SM. Growth performance increased with increasing SM in diets showing significant higher growth with 21.6% SM (One-way anova, $p < 0.05$) compared to the 0% SM diet (Figure I). Health performance was not effected by SM inclusion.

In comparison to shrimps, sea bass did not accept high inclusion of SM in diets. Feed intake and growth performance (Figure II) decreased with increasing SM content. Final weight of fish fed the 25% diet (25% FM replacement with SM) was significantly different to fish fed the 0% SM diet after 60 days of feeding. However, no significant difference in final fish growth was found in fish fed the 25% diet and the commercial feed.

Table I Nutrient composition and essential amino acids of sea star meals of *A. rubens* harvested at a mussel farm (Kieler Meeresfarm, Kieler Förde, Germany) during spring, summer, autumn and winter. DM = dry matter

Nutrient composition [% DM]	April	May	August	September	November	December
Crude protein	43.8	45.3	32.8	34.7	37.8	42.4
Crude fat	8.4	9.3	7.4	7.4	8.7	9.3
Crude ash	37.2	36.3	52.0	48.3	44.9	37.9
Calcium	12.3	11.3	15.6	16.3	13.5	12.3
Phosphor	0.89	0.87	0.43	0.53	0.58	0.70
Essential amino acids [% DM]						
Lysine	3.6	3.7	2.2	2.6	2.5	3.1
Methionine	0.89	0.87	0.66	0.70	0.72	0.82

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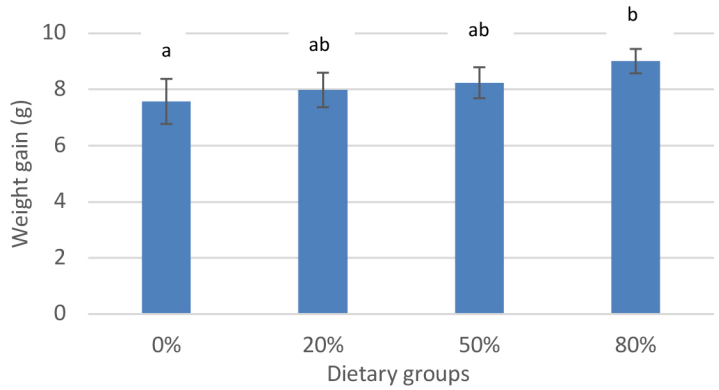

Figure I Weight gain (± SD) of shrimps (*L. vannamei*) fed four diets containing increasing levels (0%, 20%, 50% and 80%) of fishmeal substitution by sea star meal after 42 days of experiment (5.3 g mean initial shrimp weight in each treatment). Each value is mean ± S.D. (n = 4). Superscript letters indicate homogenous groups after pairwise comparison (Tukey HSD, α=0.05).

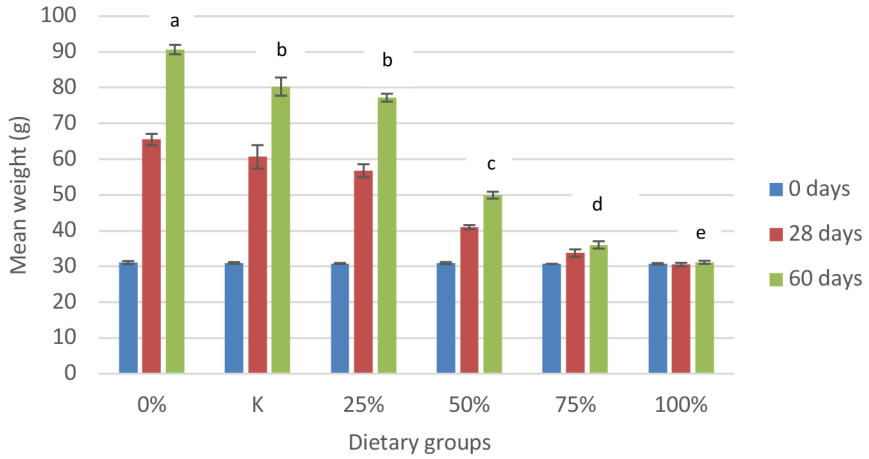

Figure II Mean weight (± SD) of sea bass fed a commercial diet (K) and the five sea bass diets containing different levels (0%, 25%, 50%, 75% and 100%) of fishmeal substitution by sea star meal at start and after 28 and 60 days. Each value is mean ± S.D. (n = 4). Superscript letters indicate homogenous groups after pairwise comparison (Tukey HSD, α=0.05).

Conclusion

Sea star biomass of *A. rubens* can very efficiently replace FM in diets for *L. vannamei* providing high-level protein and fat sources without impairing growth or health of shrimps. For sea bass diets, however, SM inclusion is limited and can replace a maximum of 20-25% of FM without significant growth or health reduction.

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SUPPLEMENTATION OF CASEIN-BASED NON-FISH MEAL DIET WITH FISH MEAL SOLUBLE FRACTION IMPROVES GROWTH PERFORMANCE AND DIGESTION IN YELLOWTAIL (*Seriola quinqueradiata*)

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Introduction

Fish meal (FM) is an important protein source in fish feed, especially for carnivorous fishes. Yellowtail, a marine carnivorous fish, is the most popular aquaculture fish in Japan, with a production of approximately 100,000 tons/year. Generally, yellowtail diets contain high levels of FM (approximately 30–50% for adult fish); therefore, reducing the FM content has become imperative for sustainable farming. However, reducing dietary FM levels sometimes results in low feed palatability (low feeding amount) and, subsequently, low growth (Murashita et al., 2019; Senzui et al., 2020). A casein-based non-FM diet supplemented with a water-soluble fraction of FM (FMS) was prepared to investigate the function of FM. Growth performance in feeding trials, and the gene expression of appetite-related hormones, digestive hormones, and digestive enzymes were evaluated.

Materials and methods

Yellowtail (mean body weight = 9.5 g) were divided into 12 × 1100 L capacity FRP tanks (15 fish/tank in triplicate). The experimental diets, i.e., an FM-based diet (FM diet), a casein-based diet (C diet), C diet supplemented with water-soluble fraction of Chilian jack mackerel meal (C+JM diet), and C diet supplemented with water-soluble fraction of Peruvian anchovy meal (C+A diet), were prepared as single moist pellets (Table 1). FMS was prepared as follows: 100 g of each FM was mixed with 600 mL of water and allowed to settle for 1 hour. The supernatant (400 mL) was used as the FMS for 1 kg of diet. The fish were fed the experimental diets once a day (6 days/week) for 6 weeks. The total fish weight per tank was measured every two weeks by counting the number of fish. Growth performance was calculated based on the total fish weight and feeding amount. At 6 weeks, the hypothalamus and pyloric ceca were collected from three fish pre-feeding and three fish post-feeding (3 h) per tank. Gene expression of appetite-related (orexigenic) hormones (neuropeptide Y [NPY] and agouti-related protein 1 [AgRP1]) in the hypothalamus and gene expression of digestive hormones (cholecystokinin 1 and 2) and digestive enzymes (trypsin and lipase) in the pyloric ceca were measured using RT-qPCR.

Table 1. Composition of experimental diets

	FM	C	C+JM	C+A
FM (Peruvian anchovy)	600	0	0	0
Casein	0	490	490	490
Schizochytrium sp.	0	150	150	150
Krill meal	100	100	100	100
Pollock oil	50	0	0	0
Taurine	0	40	40	40
Feeding stimulant*	0	5	0	0
Others (Vitamin, mineral, guar gum etc.)	250	215	220	220
Total	1000	1000	1000	1000
Solution for granulation (400 mL)	Water	Water	Water soluble fraction from Chilian jack mackerel	Water soluble fraction from Peruvian anchovy
Proximate composition (%)				
Crude protein	50.7	50.1	53.7	53.2
Crude fat	11.0	12.3	12.7	11.4

*Alanine 1.1 g, Proline 1.8 g, and Inosine 5'-monophosphate disodium salt hydrate 2.1 g

Results

Growth performance (Fig. 1): Significant differences in body weight were observed at 6 weeks in the order: C+A group > C+JM group > FM group > C group. The feed efficiency followed the same order. The total feed intake (g/fish) was significantly higher in the FM group than in the other dietary groups.

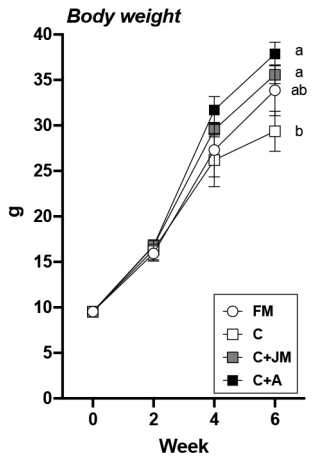

Fig. 1. Changes in average body weight (mean ± standard error, n = 3). Different letters indicate significant differences in two-way ANOVA followed by Tukey's multiple comparisons test ($P < 0.05$).

Gene expression in the hypothalamus (Fig. 2): Significant decreases were observed in *npy* mRNA expression in the FM group and *agrp1* mRNA expression in the C+A group from pre- to post-feeding. Except for group C, a decreasing trend in *agrp1* mRNA expression was observed after feeding.

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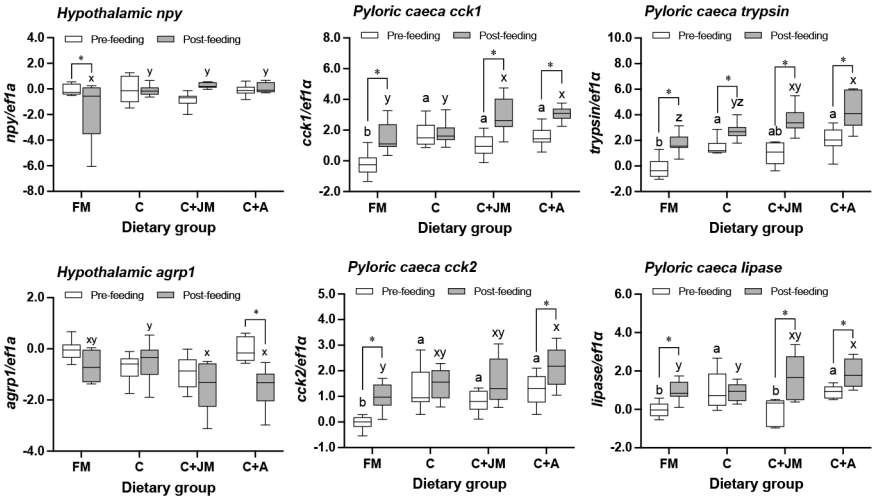

Fig. 2. Relative expression of target genes before and after feeding (*n*=7–9). Boxes indicate the 1st and 3rd quartiles. The vertical lines (whiskers) represent the maximum and minimum values. The central line marks the median. All measurements were standardized using *ef1a* expression levels. Different letters indicate sifnificant difference ($P < 0.05$).

Gene expression in the pyloric caeca (Fig. 2): A significant increase in *cck1* and *cck2* was observed from pre- to post-feeding in the FM and C+A groups. The gene expression of *lipase* increased after feeding, except in group C.

Discussion

FMS supplementation of the casein-based diet improved growth performance, indicating a better value than the FM group. The improved response of *agrp1*, digestive hormones, and digestive enzymes to FMS supplementation might have resulted in higher growth in the C+JM and C+A groups. FMS contains high levels of free amino acids, peptides, and nucleic-related substances. In our previous studies, the addition of free amino acids and FMS to rearing water affected brain NPY expression and feeding behavior (Senzui et al., 2020; Senzui and Fukada, 2023). Furthermore, oral administration of FM solution increases *cck1*, *trypsin*, and *lipase* mRNA expression (Furutani et al., 2012). FMS contains important substances that stimulate physiological responses in fish. By determining this substance, better fish diets, including low-FM or non-FM diets for carnivorous fishes, can be developed.

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UNRAVELING THE IMPACT OF PLANT-BASED SUSTAINABLE FISHFEEDS ON WHITE MUSCLE DEVELOPMENT IN GILTHEAD SEABREAM: A COMPARATIVE STUDY

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Introduction

The aquaculture sector has experienced rapid development, providing economic benefits to countries utilizing the industry. However, continuous growth and reduced wild fish stocks have led to reduced availability and increased prices of fishmeal and fish oil for fish feeds. Alternative protein sources of low ecological footprint and at affordable prices for fish feed formulation are key to the sustainable development of aquaculture. Nevertheless, they often impact fish physiology and metabolism due to the presence of various endogenous antinutritional factors and phytoestrogens (1). The deposition of proteins in the white muscle, which is the largest and continuously increasing tissue in fish and constitutes 58-80% of their total body weight, is the ultimate goal in fish farming. The growth of fish is affected by phytoestrogens and antinutritional factors, whose effects vary depending on their concentration, fish species, and other factors. Phytoestrogens may compromise growth and disrupt reproductive function, while antinutritional factors can reduce growth and cause health issues. The demonstrated negative effect of phytoestrogens on white muscle development and growth highlights the need for tools to screen for potential myostatic action of raw materials and fish feeds (2). This study combines different approaches to validate white muscle gene markers (3) in the gilthead seabream, *Sparus aurata L.,* as indicators of compromised myogenesis when fed alternative protein sources.

Materials and methods

Three diets were formulated - a soy-free diet (C), a 20% soybean meal diet (SBM), and a 20% soy protein concentrate diet (SPC) – and they were fed to triplicate groups of gilthead seabream of 27g average initial BW for two months. After the three-month period, sampling was conducted in all three groups and samples were stored in RNAlater at -20oC until RNA extraction. In a second approach, extracts of the diets and of raw materials were applied in primary cultures of myogenic progenitor cells from seabream for three different exposure times and four different quantities of the food extract (0, 2, 4 mg) following the onset of differentiation. Cells were collected at the end of the exposure and RNA was isolated. The expression levels of *mylpfa* (myosin light chain phosphorylatable, fast skeletal muscle a, associated with hypertrophy), *mylpfb* (associated with hyperplasia), and *myog* (myogenin) in white muscle were determined at the end of the experiment using real time RT-PCR. The results were analysed using the R programming language.

Results and Discussion

In teleosts, muscle growth occurs through two main mechanisms: hyperplasia, which involves the recruitment of new muscle fibers, and hypertrophy, which involves an increase in the size of existing muscle fibers. The effects of SBM and SPC on hyperplasia and hypertrophy in the feeding trial were consistent with the effects recorded *in vitro*. Hyperplasia as marked by *mylpfb* expression was significantly reduced in fish fed on SBM and SPC, whereas hypertrophy as marked by *mylpfa* expression was significantly reduced only in fish fed on SBM. Myogenin (*myog*) expression remained unaffected.

Similar results were recorded in primary cell cultures after 48h of exposure to diet extracts; both SBM and SPC were potent in downregulating *mylpfb* expression, whereas *mylpfa* expression was significantly affected only in the presence of SBM extract. Increasing the extract quantity in the SBM and SPC diets resulted in reduced expression levels for *mylpfa*. In contrast, *mylpfb* expression was not greatly affected by increasing extract quantity in SBM and SPC.

Myogenin expression remained largely unaffected by the presence of the raw materials extracts in the primary culture. However, there are noteworthy findings: *mylpfb* expression significantly increased in the presence of corn gluten extract, and *mylpfa* expression was elevated in the presence of either corn gluten or wheat gluten extract, suggesting their potential influence on muscle growth.

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To our knowledge this is the first study to demonstrate that primary cultures of myogenic progenitor cells can be used as a screening tool for potential myostatic action of raw materials and fish feeds containing alternative protein sources.

Conclusions

Our results offer new insights into the mechanisms behind myostatic action of plant raw materials rich in phytoestrogens. Differences recorded in fish growth *in vivo* could be attributed to differentiated white muscle hyperplasia and hypertrophy with the help of molecular markers. The validation of the myostatic action of raw materials and fish feeds in vitro comes with an added value as a tool for wide-scale screening in search for sustainable raw materials for fish feeds.

Acknowledgements

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INTEGRATED SUBMERSIBLE AQUACULTURE FOR WARM WATER OPEN OCEAN

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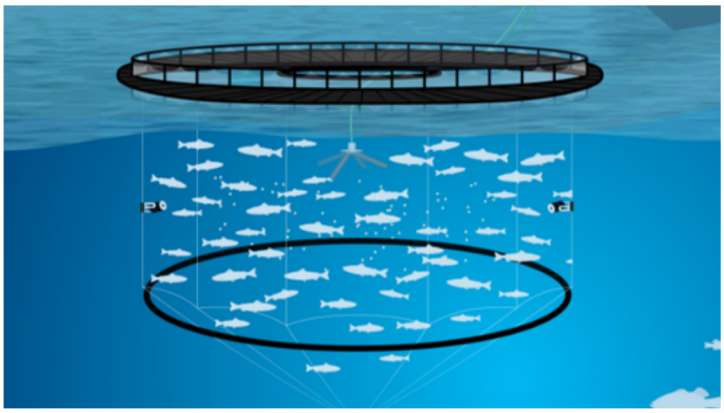
The expansion of warm water aquaculture is trending away from sheltered sites to either land-based or open ocean production. The industry consensus is that to safely farm in the open ocean, equipment needs to be submerged to avoid the surface energy of waves and storms. Other benefits of being submerged include less stakeholder conflict, optimal growing temperatures as a result of seasonal thermoclines, and the ability to evade parasites or blooms that are commonly found at the surface.

Until now there has not been an integrated suite of equipment and services capable of addressing all the key steps of fish production, from stocking to harvesting, with submerged equipment.

For the past five years, Innovasea has been developing a family of products to address the unmet needs of submerged open-ocean aquaculture. The cost-competitive SeaProtean pen provides the same walkway as traditional surface pens as well as a centered variable buoyancy chamber that affords a much more controlled linear descent and ascent than other circular submersible pens. The three discrete volumes in the buoyancy chamber allow the operator to choose from multiple depths and hold station at those depths. This is especially useful when targeting optimal growing conditions due to temperature and parasitic events.

Reliable waterborne feeding is now available at commercial volumes with the modular FlowFeeder, which delivers the pellets in a gentle flow of water using 50% less energy than air-blown systems. The feed rides in a cushion of fluid, minimizing pellet damage and improving food conversion ratios. Other benefits of the water cushion are less pipe damage and deposits of microplastics in the ocean.

Finally, AI-powered software and hardware solutions monitor underwater activity in real time to help optimize production. aquaEnvironment provides precise data on water conditions such as temperature, salinity, and dissolved oxygen in and around the pens to protect fish while BiomassPro instantly estimates the size and weight of fish stocks in real time to reduce feeding costs and better predict sales schedules.



THE EFFECT OF DIETARY VITAMIN D_3 AND PHOSPHOROUS ON GROWTH AND SKELETAL HEALTH ON JUVENILE BALLAN WRASSE

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Introduction

Farmed ballan wrasse (*Labrus bergylta*) are produced as cleaner fish in salmon aquaculture and this relatively new industry faces several challenges in its pursuit of producing sustainable, high quality, robust fish. Slow growth (Brooker et al., 2018) and a high frequency of nephrocalcinosis and skeletal abnormalities (Cavrois-Rogacki et al., 2021; Fjelldal et al., 2021) are crucial challenges. While there are likely many factors involved, identifying the nutritional requirements of this species will be essential to improving performance and skeletal health. Phosphorus is an essential element of skeletal tissue and is involved in a wide range of metabolic processes (Antony Jesu Prabhu et al., 2013) making it a key target for dietary optimisation to improve both skeletal health and growth. Vitamin D_3 (VD) plays an important role in Ca and P uptake and directly interacts with osteoblasts to regulate mineralization (Lock et al., 2010). It has also been implicated in the development of nephrocalcinosis in mammals (Letavernier and Daudon, 2018). This study aims to investigate the impact of 3 levels of P and 2 levels of VD on juvenile ballan wrasse performance and skeletal health.

Materials and Methods

Ballan wrasse (4.3 ± 1.1g) from Otter Ferry Seafish Ltd. (Tighnabruaich, Argyll, UK) were randomly distributed into 18 100L flow through tanks (140 fish per tank; 2520 fish in total). Tanks were allocated to one of six experimental diets, formulated to two different levels of VD (1500 IU/Kg or 3000 IU/Kg) and three levels of P (1.1, 1.9 and 2.7 %; Table 1) in a 2x3 factorial design. Each condition was conducted in triplicate and fish were fed the experiment diets to satiation for 10 weeks.

Table 1: Target levels of phosphorous and D_3 in the experimental diets.

		Phosphorus		
		Low 1.1%	Mid 1.9%	High 2.7%
Vitamin D_3	1500 IU/KG (0.0375 mg/kg)	Diet 1	Diet 2	Diet 3
	3000 IU/KG (0.075 mg/kg)	Diet 4	Diet 5	Diet 6

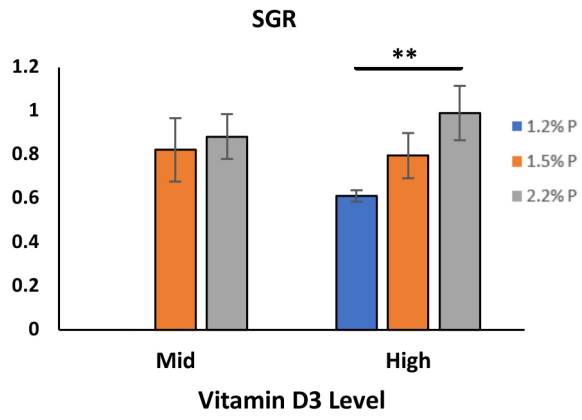

Figure 1: Weight of ballan wrasse fed different levels of vitamin D_3 and phosphorous (percentage inclusion). Data are presented as mean ± sd and were analysed by two way ANOVA.

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After 10 weeks, 33 fish per tank were weighed, measured, and welfare scored before storing in 10% neutral buffered formalin for x-ray, whole mount staining and histology analysis. Growth results were analysed with a two-way ANOVA after testing for assumptions followed by a Tukey's post-hoc test. Welfare scores were analysed using the Kruskal-Wallis test followed by a Dunn's test.

Results

After 10 weeks, growth (SGR) was significantly impacted by significantly impacted by P (two-way ANOVA, $p = 0.003$) but not VD_3 ($P = 0.599$). The highest P level (2.7%) performed the significantly better than the low P condition (figure 1).

There was a high frequency of pectoral fin erosion across all groups (90-98% with a score of 1 or greater), though in both VD groups the high P conditions scored the highest, with median scores of 1.5 compared to 1 in the other groups (Kruskal-Wallis, $p < 0.01$).

Chemical composition analysis of the final formulated diets showed lower P and higher than expected VD3, resulting in no low P/low VD3 diet.

These general performance and welfare indicators will be complemented with radiological analysis which is currently in progress to investigate vertebral health and nephrocalcinosis frequency.

Discussion

The nutrient requirements for this species have yet to be established and most commercial diets currently in use contain ~2% P and ~2000 UI/kg. This study suggests higher levels of P may improve growth performance. However, P requirements for optimum bone mineralisation are often higher than the levels required for optimum growth (Antony Jesu Prabhu et al., 2013) and pectoral erosion scores shows initial indications that this level of P may not be optimum for welfare.

Acknowledgements

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GENOMIC ASSOCIATION STUDY AND GENOMIC PREDICTION OF FILLET COLOR IN THE LOCHY STRAIN OF ATLANTIC SALMON

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Introduction

Fillet color is a key trait which determines the quality of farmed salmon, and has been identified as a polygenic trait in nature, with some QTL (quantitative trait loci) being associated with carotenoid and lipid metabolism genes. The Lochy strain of Atlantic salmon has shown high variation in fillet color between families and between males and females. Thus, identifying QTL associated with color could help improve the quality of this strain through the use of genomic selection. This study presents a genome-wide association study (GWAS) of fillet color in a domesticated population of the Lochy strain. Additionally, we assessed the potential to predict fillet color using genomic information for broodstock selection.

Materials and methods

1194 pit-tagged fish (from 200 full-sib families; 589 females and 605 males) were harvested from a marine fish farming center in Chile belonging to the salmon breeding program of Salmones Camanchaca company. Fillet color was digitally measured using the QMCOLOR software (Quality Metrics SPA), and expressed as a quantitative variable ranging from 20 (low color) to 34 (high color) (Figure 1). All fish were genotyped with a customized 62 K Affymetrix genome-wide SNP-chip. The genomic association analysis was conducted using the GMMAT package of R; then, breeding values were estimated using the Genomic Best Linear Unbiased Prediction (GBLUP) with the BGLR package of R. The predictive ability of GBLUP was obtained from a 5-fold-cross-validation with BGLR, while the ICSASG_v2 genome in the NCBI database was used to identify genes associated with color.

Results and Discussions

The average fillet color, measured in the belly region, was 24.2 ± 0.98 (Females: 24.4 ± 0.94; Males: 24.0 ± 0.98). A total of 35 SNPs were found to be significantly associated with belly color, distributed in Ssa26 (33 SNPs) and Ssa29 (2 SNPs) (Fig. 2). The heritability of this trait was 0.29, and the genetic variance explained by QTL markers ranged from 8.6%-26.4% in Ssa26 and from 6.8%-7.0% in Ssa29. Notably, Ssa26 harbors two genes, bco1 and bco1l, which are involved in the metabolism (oxidation) of carotenoids (Helgeland et al., 2019; Sae-Lim et al., 2022). The correlation between breeding value and phenotype varied from 0.72 to 0.79 for the training populations, but was only between 0.32 and 0.44 in the test populations.

Conclusion

In conclusion, two genomic regions were associated with fillet color variation in the Lochy strain of Atlantic salmon. The first, located on chromosome 26, has already been identified in other domesticated fish strains such as Mowi and Aquagen. A new chromosomal region was detected on chromosome 29 in the Lochy strain, but the explained variance was lower than that on chromosome 26. These results demonstrate that genomic selection can be used to accelerate the genetic progress of color measurement digitally in this strain.

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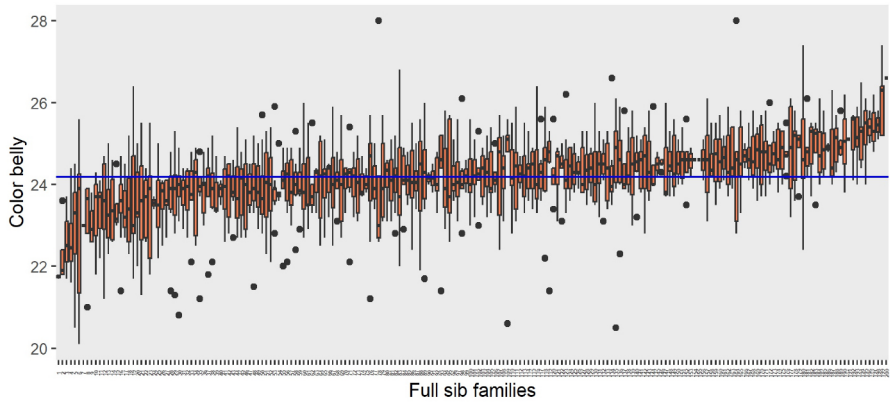

Figure 1: Variation in fillet color (belly region) across 200 full-sib families of the Lochy strain of Atlantic salmon.

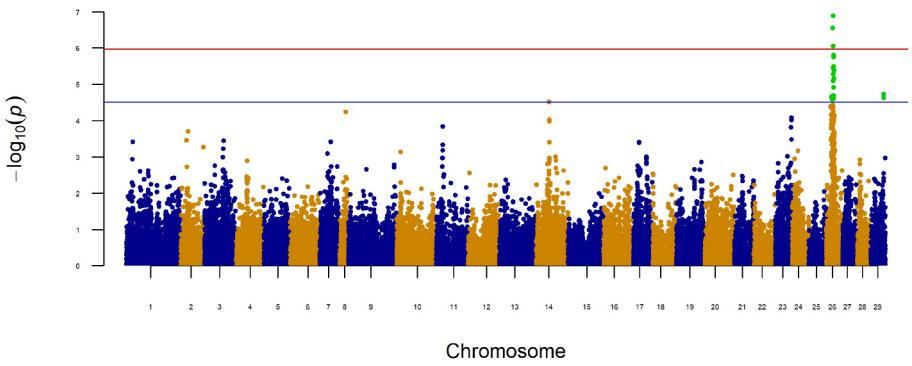

Figure 2: Manhattan plot showing the significant SNPs of Color Belly in the Lochy strain of Atlantic salmon.

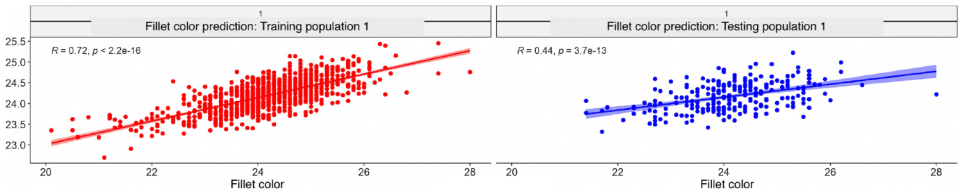

Figure 3: Fillet color prediction of the Lochy strain of Atlantic salmon in the training population (left) and the test population (right).

Acknowledgment

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COLLECTION AND TREATMENT OF WASTEWATER FROM RECIRCULATION AQUACULTURE SYSTEMS, WITH THE SCOPE OF PRODUCING BIO-BASED FERTILISERS, AS PART OF THE EUROPEAN FUNDED HORIZON PROJECT SEA2LAND

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Introduction

AquaBioTech (ABT) Group in the SEA2LAND project is responsible for providing marine aquaculture sludge for the nutrient extraction methods employed by UVIC (Universitat Central de Catalunya). Based on the circular economy model, SEA2LAND promotes the production of fertilisers in the EU from raw materials. This solution is expected to reduce the soil nutrient imbalance in Europe. However, there are still technological difficulties surrounding the concentration of sludge, , therefore there is a need to modify and change the existing concept in order to search for other ways of concentrating more sludge and perhaps of better quality (nutrient concentration).

It is well known that ozone causes clumping of solids (micro-flocculation), which facilitates their removal by foam-fractionation, filtration, and sedimentation. It was thought that ozone treatment could be effective after drum-filters or even before the drum-filter. In this case, the backwash of drum-filters could provide not just normal sludge but also more flocculated solids (treatments with ozone lead to flocculation). This will result in more sludge removed (parts filtered by the drum and solid clumps) and hopefully could solve the problem identified in the project.

Wastewater was treated with ozone on many occasions at ABT with different protocols of dosing. However, there were not any positive results in the flocculation of solids. Effective doses of ozone for such treatments were found to be above 2mg/L in literature (Bogner *et al* 2018; Ji *et al* 2019). However, when the trials were carried out, these levels could not be reached. The saturation level was reached early before ozone reached 1mg/L. Hence, after the saturation point (the highest observed was 680μg/L in 30 minutes post ozone application), ozone started to spread in the atmosphere, becoming dangerous for humans. If that was the case on an experimental level, on a bigger scale these levels of ozone could be lethal for humans. Therefore, it was decided that other alternatives should be trialled.

Specific, water-soluble polymers are widely used in wastewater treatment to remove suspended solids and/or contaminants from the water, and therefore can be found in municipal, industrial and stormwater treatment systems. The liquid/solid separation, that can take hours or days when left to gravity alone, can typically be achieved in minutes or seconds with properly prepared, activated, and applied polymers. A coagulant added to wastewater creates a coagulation process that neutralizes the particles' negative charge. Once neutralized, the particles can come together to form larger particles called micro-flocs, creating a larger particle with a higher mass-to-drag ration and hence speeding up the natural process of sedimentation.

Research shows most polymers used in wastewater come from the backwash of drum-filters. It is believed that perhaps the application of polymers in the wastewater before entering the drum-filter could flocculate solid particles, increase their size, and therefore increase the efficiency of the drum-filter in terms of solid removal which will be increased furthermore with the application of filter-bags to the wastewater leaving the filter after the backwash. In 2009, Sharrer *et al*. tried and successfully implemented the use of flocculants after the drum-filter and before geotextile bags. In 2005, Ebeling *et al*. showed 99% removal of Total Suspended Solids using commercially available polymers from various companies.

After being taken off-line and allowed to dewater and dry, a sludge cake is sufficiently dewatered, i.e., to approximately 20% solids dry weight (Sharrer *et al*. 2009). At the same time, this removal led to 92-95% reduction of reactive phosphorus (orthophosphate).

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Materials and methods

The wastewater from two species will be used in the trials: Atlantic salmon (*Salmo salar*) and European sea bass (*Dicentrarchus labrax*). The trials will take place in the facilities of AquaBioTech Group and use a recirculating aquaculture system.

As wastewater is leaving the drum-filter with the use of back-wash pump, it will be collected in a tank (125L). The sludge tank will be connected, through a pipe, with the filter bag (secured in a frame). The filter bag will provide wastewater for each sample to a graduated cylinder, with the initial temperature recorded. To ensure the validity of the results, all water samples will come from the same cycle of backwashing every time.

Collected sludge will be treated with the solutions of flocculants, provided by DERYPOL S.A., in a stirrer/flocculation tester. The flocs will be allowed to settle for 15 minutes. For all screening and flocculation tests, turbidity and reactive phosphorus will be measured. Turbidity will be used as an indicator of suspended solids and reactive phosphorus for phosphorus content. Another important factor that is useful in wastewater treatment is the speed of settling of the flocculated particles, these analyses will also be carried out.

Apart from the quality of the filtered water either with pre-treatment using flocculant or not, an assessment will be carried out for the retained (inside the filter bags) solids composition and define what is their nutrient content. Solids will be collected after settling, without and with filter bags. The samples will be sent to an external lab, where the analysis for the content of these solids will be identified. Any positive results could be further exploited, by upscaling into commercial systems.

Acknowledgement

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INFLUENCE OF ENVIRONMENTAL FACTORS AND PATHOGENS ON PREFATTENING STAGES OF CUPPED OYSTERS *Crassostrea gigas* RAISED IN THE MIDDLE ADRIATIC SEA

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In last decades, demand for bivalves has significantly increased almost worldwide. In the Adriatic Sea, offshore bivalve molluscs farming is almost entirely based on mussel farming although some companies started to diversify their production. The impact of environmental factors and host/pathogens interaction on growth performance and mortality of Cupped oysters (*Crassostrea gigas*) were analysed on different batches, during the years 2018 and 2019. Sea Surface Temperature and Chlorophyll-a measures were obtained from the database provided by Moderate Resolution Imaging Spectroradiometer (MODIS) instruments aboard NASA's Aqua satellite. Our results show a significant growth rate of the oysters reared in the Adriatic Sea, but an increase of mortality in summer months. Histological analysis does not show the presence of specific pathogens, recording only a strong positivity with immunohistochemistry for *Vibrio* spp. in summer 2018, but not in 2019. The research of OsHV-1 and *V. aestuarianus* shows negative results in all the time points, but we demonstrated that there was a combined effect of Sea Surface Temperature, Chlorophyll-a and *Vibrio* clade *splendidus* concentration on oysters' mortality. Despite the high mortality and the resulting economic loss during the summer months, oysters farming seems to be a feasible activity in the Adriatic Sea. To maximize production and the profitability of the farm, new locations for product finishing could be suggested based on the findings of this study.

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THE IMPORTANCE OF SUBSTRATE IN THE IMMUNE RESPONSE IN THE ATLANTIC BLUE CRAB (*Callinectes sapidus*) AFTER A BACTERIAL CHALLENGE WITH *Vibrio alginolyticus*

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Introduction

The Atlantic blue crab (*Callinectes sapidus*, Rathbun, 1896) is a species of decapod crustacean belonging to the family Portunidae. It is native to the coastal areas and estuaries of the west coast of the Atlantic Ocean, but in recent years has spread to the Mediterranean Sea (Falciai and Minervini, 1992). On the western Atlantic coast, it is a species of high commercial value. Its sometimes-excessive fishing has led to an 80% decline in stocks in recent years in places such as the Chesapeake Bay (US East Coast) (Lipcius and Stockhausen, 2002). This fact is leading to numerous studies and interested people in the profitability of large-scale farming of this species, which would provide relief to wild populations in most cases (Zmora et al., 2004). Decapod crustaceans are sentient beings, which not only respond to noxious stimuli, but are also capable of feeling pain, discomfort, and stress (de Souza, 2022). Given that the Atlantic blue crab inhabits sandy and muddy bottoms in which it tends to bury itself **(**Falciai and Minervini**,** 1992), we considered that the presence or absence of substrate in the facilities in which these animals can be cultured could be fundamental for the welfare of these animals, as well as contributing to a significant reduction in stress. Therefore, the aim of this study is to demonstrate how the use of substrate can improve the immune response of Atlantic blue crabs when exposed to a pathogenic bacterium.

Materials and methods

Twenty specimens of Atlantic blue crab (217,5 ± 142 g) were obtained from the fish market (San Pedro del Pinatar, Murcia, Spain) and carried to the Marine Facilities at University of Murcia (Spain). The crabs were randomly separated in two groups, each group (n=10) was introduced into a tank with individual compartments for a single crab each one but sharing water and filtration (rack system) with the aim of avoiding fights. One of the groups (control) had no substrate at the bottom of the tanks, while the other one had 7 cm of sterile sand. After 30 days of acclimatising, a sample of 2 mL of haemolymph were extracted as initial time (t=0). Then both groups were challenged by bath with *Vibrio alginolyticus* (35 min, 2 x 10^6 ufc mL^{-1}). After this, crabs were sampled after 24, 48, 72 and 96 hours. After each experimental time, 2 mL of haemolymph were extracted from the V right pereiopod and the total haemocyte count and the cell´s percentage populations were determined. In addition, the obtaining of haemocyte lysate supernatant was used to measure the total quantity of protein, as well as the phenoloxidase and the lysozyme activities were measured. The results were expressed as mean ± standard error of the mean. Data were analysed by One-way ANOVA (followed by Tukey tests) to determine differences between experimental groups. The level of significance used was $p < 0.05$ for all statistical analysis.

Results and discussion

The percentages of each cell population under basal conditions (time 0) showed that the percentage of granulocytes was the lowest in both groups, followed by the number of hyalinocytes and semigranulocytes. However, our results showed a change in the cell percentages of the animals challenged with *V. alginolyticus*. More specifically, a decrease in the percentage of hyaline cells was observed at 24, 48, 72 and 96 hours in the crabs maintained with substrate. In the experimental group without substrate, this decrease was also observed, although it occured at 72 and 96 h. In the case of semigranulocytes, no time-dependent variations were observed in the animals maintained without substrate, although in the crabs maintained in the presence of substrate a significant increase in the percentage of these cells at 24, 48 and 72 h was observed. Comparing between experimental groups, a higher proportion of hyalinocytes in the haemolymph of crabs maintained with substrate at 0 and 96 hours were observed compared to the crabs maintained without substrate. In the case of semigranulocytes, the

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percentage of this cell type was significantly higher in animals without substrate at 24 and 72 h compared to results observed in crabs maintained with substrate. However, no variations were detected in the number of granular cell population between both experimental groups. All these data indicate the great capacity for adaptation and plasticity that these crustaceans can present in the face of a biotic stress factor such as the challenge by pathogenic microorganism. This can be related to the data provided by Du et al. (2012) in the giant freshwater shrimp (*M. rosembergii*) challenged with Spiroplasma, where the cell populations varied over time. The quantity of proteins in the haemocyte lysate supernatant varied between both experimental groups at 0, 24 and 48 h. Regarding the time, protein concentration increased at 24, 48 and 72 hours in both experimental groups. These values could be since the pathogen caused a reaction that triggered intracellular antimicrobial protein synthesis at 24 hours, which remained active until 72 hours, at which time the protein synthesis reaction subsided. In relation to the immune-related enzymes, the lysozyme did not show any variation between experimental groups or experimental times. However, the phenoloxidase activity showed a great variability between animals, being undetected at 0 and 24 h in the crabs from both experimental groups. After 48 h of challenge, the phenoloxidase was detected although their activity was stronger at 72 h, being expressed in three crabs of the group without substrate and eight of the group maintained with substrate. Furthermore, the quantity of enzyme per animal was higher in the experimental group maintained with substrate. This fact could indicate that the phenoloxidase is not present in the haemolymph of animals not exposed to any pathogen, which could be released at 48 hours of challenge, being more efficient in the immune response in the case of animals maintained in presence of substrate.

Conclusions

The results obtained could contributed to the knowledge about the immune response and the requirements necessary to improve the animal welfare of brachyurans. Recently, Zhu et al. (2022) reported that the use of substrate significantly benefited South Korean blue crabs (*Portunus trituberculatus*), reducing their cannibalism, improving their condition inside the tanks, and resulting in behavioural patterns of interaction with the substrate. Therefore, we could conclude that the substrate may not only act as a factor contributing to animal welfare in animal behaviour, but also that this state may be reflected in a better immune response to certain pathogens that often cause huge losses to aquaculture farms based on decapod production.

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FIRST INSIGHTS FROM A SURVEY ON SHRIMP FARMING

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Introduction

According to the Animal Welfare Act, species-appropriate husbandry must be ensured in animals kept for commercial purposes. The animal owner must collect and evaluates suitable, species-related animal welfare indicators for his assessment (Franks et al., 2021). Although regulations do not yet apply to crustaceans, an expansion is expected shortly (E. Parliament, 2021; TierSchG A, 2000). The effect of chronic stress on crustaceans and its markers are still poorly analysed (Wuerz et al., 2023). The CrustaWohl project focuses on welfare to optimise whiteleg shrimp (*Litopaeneus vannamei*) farming in RAS systems. Chronic stress is aimed to be investigated, with approaches on health observation, histology, physiology, genomics and shrimp behaviour.

As the first step of the project, a survey for shrimp farmers and experts was originated, in order to calibrate the experiments and to acquire data from shrimp farming practices. Some insights from the provisory results are presented with the aim of enriching the archive.

Materials and methods

A questionnaire of 19 points has been generated, which comprises aquaculture system management, species biology and water chemistry fields. Literature review, OCEANLOOP project partners' consultation and the project guidelines have been taken into account in its preparation. The survey was advertised in the EUROSHRIMP newsletter at the following link: https://www.euroshrimp.net/crustawohl-survey/.

Shrimp farmers and experts are invited to fill in. The estimated duration is expected in 10-15 minutes. It is anonymous and observes GDPR rules.

Results

Multiple selections to the same question are allowed. At the moment, the number of replies is still limited, with only 9 participations. 55% of the interviewees utilize a RAS and 27% Biofloc as a system. Aquaponics and other systems are also reported. According to Figure 2, bad water quality, mineral deficiencies and high stocking densities are indicated as the most limiting factors for shrimp welfare. System technical issues follow with a 20%. The most common abnormal biological observations comprise shortened antennae, gills erosion, eye lesions and uropods redness, which are noticed by more than 70% of the shrimp experts (Fig. 1). "Shortened antennae" and "uropods redness" affects more often a larger rate (15-30%) of the population. In Figure 3, irregular swimming dominates as abnormal behaviour observed, while 30-40% of the voters describe, also, lethargy, surface swimming, strong escape behaviour in response to stimuli, cramped body and no feeding.

Discussion

More contributions are aimed to get new insights and more accurate results. Some limiting factors are reported for shrimp welfare. For this purpose, the CrustaWohl project addresses stocking densities and water chemistry analyses, which could provide helpful outputs. Deficiencies in minerals might be an interesting matter for future projects. Health observations and behaviour related to stress have been already described in decapods, with a degree of similarity with the ones reported by shrimp experts (Sellars et al., 2004; Pedrazzani et al., 2023). The visible perceptions are supposed to be associated with physiological chronic stress markers. The project comprises, also, a larger scale environment, to reach a higher degree of meaningfulness and practical utility for farmers.

Conclusions

This investigation shows the limitations to farmed shrimp welfare from the shrimp experts' perspective. Some interesting insights are highlighted to address CrustaWohl and future projects.

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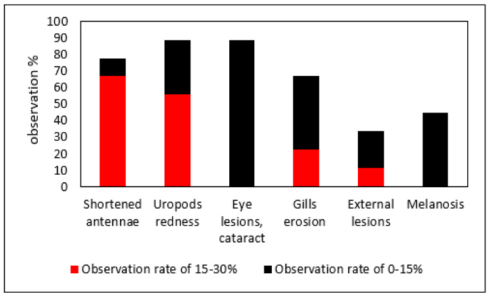
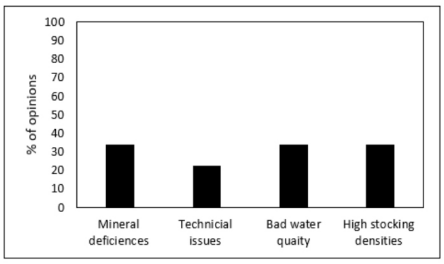
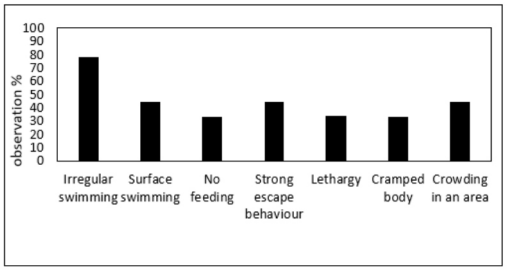


Fig 1. Frequency and rate observation of abnormal biological modifications (top-left).

Fig 2. Frequency of shrimp welfare limitations listed (bottom-left).

Fig 3. Frequency of abnormal behaviour observed (bottom-right).





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IMPACT OF OXYGEN LEVELS ON GILL INTEGRITY OF MEAGRE (*Argyrosomus regius*)

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Introduction

The employment of good water management practices is of extreme importance in aquaculture farming, including adequate dissolved oxygen levels. Sub-optimal oxygen levels impact respiration rate, stress and immune response, water quality, optimal fish growth, parasite occurrence, and fish survival. Some parasites, especially those that affect fish gills and skin, thrive in low-oxygen conditions (Schäfer N., et al, 2021; Jerônimo G. T., et al, 2022). By ensuring sufficient dissolved oxygen, fish farmers can mitigate the impact of parasites and support the overall health status and enhance productivity of their fish stocks (Akhter et al, 2021). The target of this work is to analyze the histopathological effects in the gills, at three different dissolved oxygen levels and evaluate the impact on overall fish performance.

Methods

The experiment was conducted at the Aquaculture Research Station (EPPO) facilities of IPMA (Olhão, Portugal), where meagre with mean body weight of 256.0 ± 1.8 g were distributed in 9 fiber-glass tanks (1.5m^3), with a density of 10.9 ± 0.1 kg m^{-3} per tank, in triplicates. Meagre were kept in natural temperature conditions, salinity 37 ppt, photoperiod 14L:10D and fed *ad libitum*. Fish were maintained with three different levels of dissolved oxygen: DO1 - low oxygen (2.5 - 3.0 mg/L); DO2 - medium oxygen (4.0 - 5.0 mg/L); DO3 - high oxygen (6.0 - 7.5 mg/L), for 4 weeks. At the end of the trial, gill samples were collected to evaluate the histopathological impact. The first gill arch was collected, fixed in formol (4%) and transferred to ethanol at 70%, after 24h. Then the tissue was processed using a standard histological technique (Martoja & Martoja-Pearson, 1970). Mounted slides were scanned with a Hamamatsu NanoZoomer C13140-01 and images were analyzed with the NDP.view2, Image viewing software. Histopathological changes were examined, using the semi-quantitatively analysis described by Mishra and Mohanty (2008).

Table 1. Gill histopathological analyses in *Argyrosomus regius* exposed to three different dissolved oxygen levels: DO1 - low level (2.5 - 3.0 mg/L); DO2 - medium oxygen levels (4.0 - 5.0 mg/L); and DO3 - high oxygen levels (6.0 - 7.5 mg/L).

Parameters	***DO1***	***DO2***	***DO3***
Infiltration of leucocytes	+	+	+
Hemorrhagic state	++	+	+
Epithelial edema	+	+	+
Telangiectasia	+	+	+
Lamellar aneurysm	+	+	+

–, None (0%); +, mild (< 10%); ++, moderate (10–50%); +++, severe (> 50%)

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Results and Discussion

In this of study, the histopathological lesions were used to determine the general health of fish and the overall condition of their aquatic environment. While some lesions may be permanent, others may be reverted under the correct water quality conditions (Nascimento et al., 2012). The results of the histopathological lesions in this study revealed that there were no notable variations between the different oxygen levels. The lesions observed, were infiltration of leucocytes, epithelial edema, telangiectasia and lamellar aneurysm, can be categorized as mild. However, the DO1 treatment presents a moderate hemorrhagic state compared to the other treatments (D2 and D3). These results can be explained by the fact that the fish attempt to increase the flow of blood over their gills to enhance the oxygen uptake. This increase in blood circulation can make gill blood vessels more fragile and sensitive to rupture, causing bleeding and hemorrhagic lesions. Hemorrhagic state can also be caused by other factors such as infections with ectoparasites, as described by Ribeiro et al (2023).

Acknowledgments

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CELLULAR RESPONSES OF RAINBOW TROUT (*Oncorhynchus mykiss)* ARTIFICIAL INTESTINAL PLATFORMS AFTER LONG-TERM EXPOSURE TO *IN VITRO* DIGESTED FEED

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Introduction

The search for alternative sustainable raw materials to improve current fish diets would be supported by reliable and predictive *in vitro* models. The H2020-FETOPEN project Fish-AI, aims to develop such an in vitro screening platform based on rainbow trout digestive enzymes and intestinal cells to evaluate nutritional and health values of novel aquafeeds. As part of this project, we used two different bicameral culture platforms to evaluate the effects of prolonged exposure to fish feed on intestinal health. We compared a simple system consisting only of an epithelial monolayer and a complex organotypic system that combined a stroma-like scaffold populated by rainbow trout (RT) fibroblasts with a functional epithelium.

Material and Methods

To generate the *in vitro* platforms, RT proximal and distal intestine epithelial cells (RTpiMI, RTdiMI) were seeded onto ThinCert (TC) inserts (Greiner BioOne, 0.4 µm pore size), a permeable PET membrane or onto the synthetic scaffolding Alvetex™ (Reprocell) (AV) previously populated with RTskin01, a fibroblast cell line derived from the trout dermis. Both were cultured at 20°C under ambient atmosphere. We used a diet rich in fish meal with crude protein and lipid level of 46 and 23% respectively. Feed pellet were digested *in vitro* with enzymes extracted from the RT gastric and intestinal segments of the digestive tract.

Barrier integrity and functionality were assessed through the measurement of transepithelial electrical resistance (TEER), apparent permeability (Papp) to 100 µg/mL 4 KDa FITC-Dextran (FD4) and *in situ* detection of the alanine aminopeptidase (ALP), a brush border enzyme. Once the models established an effective epithelial barrier, they were exposed for 21 days to increasing concentrations (6, 12, 25, 50%) of *in vitro* digested feed (IVD) diluted in Leibovitz L-15 medium supplemented with 10% FCS. Controls were run incubating cells with L-15 medium without IVD. Samples were fixed in 4% paraformaldehyde in PBS, processed for histological analysis and stained with hematoxylin and eosin (HE).

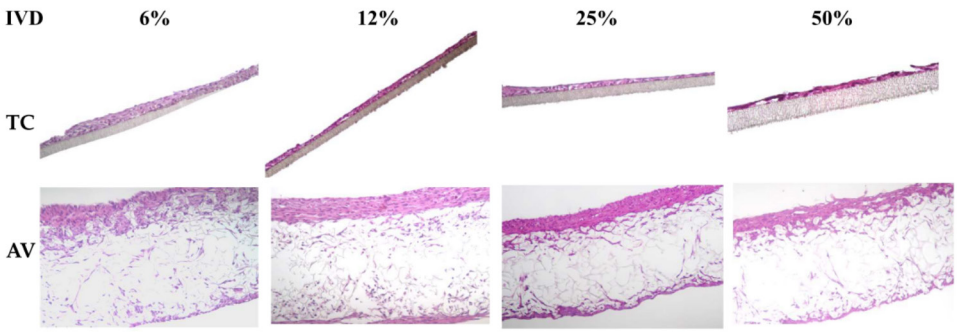

Fig. 1 Multilayered barriers formed by RT intestinal cells cultured on TC and AV after 21 days of exposure to different concentrations of in vitro digested feed (IVD).

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Results

During the 21 days of exposure to IVD, barrier integrity was conserved along the course of the experiment as indicated by the limited paracellular flux of FD4 compared to the respective inserts without cells. TEER values significantly increased from the baseline values in both cell lines cultured in both systems. The morphological analysis revealed that in all the combinations of IVD concentration, cell lines and culture platform, epithelial cells lost their monolayer arrangement and formed a multilayered barrier, with the only exception of RTpiMI exposed to 6% IVD cultured in AV, in which the cubic and polarized monolayer observed in the control was preserved. In AV, ALP activity significantly decreased in both cell lines exposed to 25 and 50% IVD affecting cellular functionality. This was not evident in the TC system, in which high IVD concentration did not reduce the enzymatic activity but rather increased the proteolytic activity in the RTdi-MI cell line.

Conclusion

High concentrations of IVD generated a mildly stressful environment in both platforms since long-term exposure led to increased proliferative activity at the expense of cellular differentiation. The fact that irritation occurred even with high levels of marine ingredients may be due to the lack of mucus-secreting cells that would provide the physiological protection for the epithelium. Interestingly, this reaction reminds our recent data, demonstrating that nutritional stress induces de-differentiation and proliferation of mature enterocytes in the RT proximal intestinal mucosa[1]. The fact that we observed a similar response *in vitro*, suggests that the data generated by the artificial intestinal platforms can be representative of *in vivo* trials. Since the cells grown on the Alvetex™ scaffold reacted with higher sensitivity to the exposure to the digesta this looks as the more reliable platform.

Acknowledgements

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GREEN AND RED MACROALGAE EXTRACTS INDUCE INNATE IMMUNE RESPONSES IN NILE TILAPIA AND RAINBOW TROUT IN VITRO

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Introduction

To keep up with an increasing demand for animal protein, the aquaculture sector has seen an increase in production over the past decades. The intensification of this sector has been accompanied by an increase in disease incidences. Besides prevention of diseases through vaccination and treatment of diseases by antibiotics, dietary supplementation with immunomodulators may provide an alternative route to maintain animal health in aquaculture. Among the better-known immunomodulatory feed additives are non-starch polysaccharides, with probably the best characterized compounds being β-glucans [as reviewed by: (Dawood et al., 2018; Petit and Wiegertjes, 2016)]. Another group of immunomodulatory substances are marine sulphated polysaccharides (MSPs) isolated from macroalgae. While MSP-rich extracts are gaining interest from the aquaculture sector as containing health-promoting compounds, their immunomodulatory effects are not always clearly defined.

Materials and methods

Green macroalgae (Ulva sp.) and red macroalgae (Solieria sp.) were processed to produce eight different crude extracts enriched for marine sulphated polysaccharides (MSP).

Antimicrobial activity of the MSP-rich extracts was investigated against 13 fish bacterial strains. Concentration of bacteria (CFU/mL) was determined and strains were diluted to 105 CFU/mL in Mueller Hinton Broth (MH broth). For all eight different MSP-rich extracts, extracts were first dissolved in ultra-pure water and sterilized by autoclaving, subsequently a two-times serial dilution was made from 12.5 mg/mL – 0.0061 mg mL−1. In a sterile culture plate, 100 µL bacterial suspension was combined with 100µL MSP solution dissolved in MH broth. Bacteria were incubated for 24h, or 72h for slow growing bacteria (i.e. *Aeromonas salmonicida, Aliivibrio salmonicida, Streptococcus agalactiae, Streptococcus iniae*). Each plate included a positive control with bacterial strain alone in MH broth, and two negative controls: only MSP extract at the different dilutions, and MH broth alone. Following incubation, optical density was measured at O.D. 600 nm and plates were photographed. Antimicrobial effects were determined as the lowest MSP extract concentration required to completely inhibit visible growth (O.D. 600 nm, or visually determined from photographs) of tested microorganisms after 24h, or 72h of incubation. Three replicates were made for each microorganism.

Nile tilapia (*Oreochromis niloticus*) were reared at 28 ± 2 °C temperature with a 12-12h light-dark cycle. The fish were fed a commercial diet twice per day. Rainbow trout (*Oncorhynchus mykiss*) were reared at 14 ± 0.5 °C temperature with a 12-12h light-dark cycle. The fish were fed a trout specific research diet twice per day.

Fish were killed with 0.3 g/L tricaine methanesulphonate in aquarium water buffered with 0.6 g L–1 sodium bicarbonate (Nile tilapia) or 2-phenoxyethanol (1 mL L-1) in aquarium water (Rainbow trout) and bled via the caudal vein. Head kidney was removed aseptically and total head kidney leukocytes (HKLs) were separated on a Percoll density gradient (51%), as previously described for Nile tilapia (Kumar-Velmurugan et al., 2012), and for Rainbow trout (Chettri et al., 2011).

Production of ROS was determined by a real-time luminol-based luminescence assay, as previously described with minor modifications (Petit et al., 2021). Cells were stimulated with one of the following: RPMI cell culture medium (control), zymosan (tlrl-zyd, 50 µg mL–1,) or one of the extracts at a concentration of 250, 500, 750, 1000 and 1500 µg mL–1. Chemiluminescence emission was measured in real time at 27˚C (Nile tilapia) or at 19°C (Rainbow trout) and expressed as area under the curve, as previously described (Petit et al., 2019). Fold changes were calculated as the area under the curve of stimulated HKLs relative to unstimulated HKLs (treated with RPMI).

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HKLs were seeded at a density of 4.5 x 106 per well in a 24-wells plates and stimulated with RPMI, zymosan (tlrl-zyd, 50 mg/ml,) or one of the extracts at a concentration of 500 µg mL-1 and incubated at 27°C (Nile tilapia) or at 19°C (Rainbow trout). At 3 and 6 hours post stimulation, cells were lysed in RLT lysis buffer and stored at -80˚C until RNA isolation.

Total RNA from cell lysate in RLT lysis buffer was isolated and stored at –80°C. Prior to cDNA synthesis, total RNA was treated with DNase I, Amplification Grade, and cDNA was synthesized using random primers (300 ng) and Superscript III First-Strand Synthesis for RT-PCR. cDNA samples were diluted in nuclease-free water prior to real-time quantitative PCR (RT-qPCR) analysis.

Gene expression was measured with RT-qPCR using ABsolute qPCR SYBR Green Mix in a Rotor-Gene Q, and fluorescence data were analysed using Rotor-Gene Analysis software version 2.3.5. The relative expression ratio (R) of each sample was calculated according to the Pfaffl method (Pfaffl, 2001) based on the take-off deviation of sample versus each of the PBS controls and normalized relative to elongation factor 1α (elf1α) as reference gene.

Statistical analysis was performed in IBM SPSS statistical data editor version 26. Data presented as fold changes were transformed with natural logarithm, prior to statistical analyses. Subsequently, transformed data was tested for normality by using a Q-Q plot and performing a Shapiro-Wilk test. Data was then analysed using a repeated measures linear mixed model followed by a Bonferroni post hoc test. All values are means and expressed with their standard deviation (SD), and data were considered significant for $p<0.05$.

Results

While all extracts showed direct anti-bacterial effects to some degree, two red algal extracts in particular, had high activity against several pathogenic fish bacteria in vitro. Stimulation of head kidney leukocytes (HKLs) in vitro with MSP-rich extracts showed fish species specific differences. In Nile tilapia, HKLs showed a dose dependent reactive oxygen species (ROS) production following stimulation with Ulva-derived extracts, while stimulation with Solieria-derived extracts did not induce ROS production. In Rainbow trout, HKLs showed relatively high reactive ROS potential and Solieria-derived extracts could induce significant ROS production, albeit without clear dose dependent responses. Gene expression of in vitro stimulated HKLs showed a clear induction of most cytokines measured (il1b, il10, tnfa, ifng, il12p40). Although cytokine gene expressions were more prominent in Nile tilapia than in Rainbow trout, immunomodulatory effects of both, Ulva- and Soleria-derived extracts appeared evident. HKL might sense the presence of MSP via unknown pattern recognition receptors with known downstream signaling pathways. For genes associated with Toll-like Receptor (TLR) signaling, regulation of irak1 in Nile tilapia was evident. For genes associated with C-type Lectin Receptor (CLR) signaling, regulation of both card9 and bcl10 was found, again in Nile tilapia, confirming earlier observed fish species specific differences. Possibly, immunomodulatory effects of MSP could be regulated by CLR-mediated signaling, at least in Nile tilapia, for which most if not all red algae and green algae extracts induced changes in gene expression.

Conclusion

Overall, induction of ROS production and gene expression read-outs suggest immunomodulatory effects of MSP-rich extracts derived from green algae (Ulva sp.) and red algae (Solieria sp.), at least in vitro. The observed effects suggest clear fish species specific differences between the effects of MSP-rich extracts.

MICRO- AND MACROALGAE AS FUNCTIONAL INGREDIENTS FOR GILTHEAD SEABREAM (*Sparus aurata*) INTESTINE RECOVERY

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Introduction

Gut health is crucial for aquaculture and a proper balance in intestine homeostasis and permeability has implications for fish feed efficiency, performance and health status. Over the last decades, innovation in aquaculture feed formulation has been directed towards functional feeds. Currently, macro- and microalgae aroused interest in their application as functional ingredients due to the biotherapeutic role they can play to enhance fish intestinal health, including modulation of the immune system, antioxidant status and gut integrity. Therefore, the objective of this study was to assess the effects of dietary inclusion of the microalgae *Phaeodactylum tricornutum* and/or the macroalgae *Gracilaria gracilis* on the gilthead seabream (*Sparus aurata*) intestine recovery after provoking an insult based on the administration of soy saponins. Physiological and genomic responses to macro-and microalgae inclusion on intestine homeostasis recovery and gut health were evaluated through the integration of data on plasma metabolic enzymes, and anterior intestine histology and gene expression analysis.

Material and methods

A control (CTRL), commercial-like diet, was formulated for gilthead seabream juveniles. Based on the CTRL formulation, three experimental diets were formulated by supplementation with microalgae (*P. tricornutum*; PHA) or macroalgae (*G. gracilis*; GRA) at 2.5%, or a blend of micro- and macroalgae at 5% (50:50; BLEND). The experiment was conducted at the Ramalhete Station of the Centre of Marine Sciences of Algarve (CCMAR, Faro, Portugal). Gilthead seabream juveniles with a mean body weight of ~176 g were distributed into 15 flat-bottom 500 L tanks under natural photoperiod conditions.

To study the intestine recovery after an insult in gilthead seabream juveniles and the recovery through nutrition, five dietary treatments were evaluated: a positive control (PCTRL), where fish were assisted-fed with two empty gelatine capsules, and for the remaining treatments (NCTRL, PHA, GRA, and BLEND), fish were assisted-fed with two gelatine capsules filled with soy saponins (850 mg saponins). The assisted feeding procedure was performed after 24 h of fasting, and the capsules were gently inserted into the anesthetised fish's stomach and pushed into the oesophagus (as tested preliminarly to avoid injury) in a 10 sec time-frame. Once recovered, the fish was transferred to the respective tank. After a period of 72 h without feeding, fish were fed their respective experimental diet for 20 days. Gilthead seabream from the positive (PCTRL) and negative control (NCTRL) were fed the CTRL diet during the experiment. Fish from PHA, GRA and BLEND treatments were fed the PHA diet containing microalgae, the GRA diet containing macroalgae, or the BLEND diet with the blend of micro- and macroalgae, respectively. The experimental diets were randomly assigned to triplicate tanks. Fish were fed by hand to apparent satiety. Water average temperature during the experiment was 15.2 ± 0.9 °C.
At the end of the trial, fish were fasted for 24 h. Fish from each replicate tank were bulk-weighed and counted. Three fish from each tank were euthanized. Blood was collected from three fish from each tank (n = 9 per treatment) and centrifuged. The collected plasma was used to study the enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). The anterior intestine (AI) of each of these fish was dissected and approximately 1 cm was sampled for histological analysis. For gene expression, ~25 mg of the AI of each fish was used to analyse the gene expression of twelve genes related to antioxidant, immune system and epithelium permeability responses. Results from five genes are still under analysis.

Results and Discussion

The activity of ALT and AST was not affected by the treatments ($p>0.05$). The ratio ALT:AST that indicates possible liver damage and the ratio AST:ALT that identify a possible disorder in other organs were also not affected by the treatments. On the other hand, fish fed the BLEND diet presented a significant ($p<0.05$) lower level of ALP. Lower ALP levels could in some cases indicate a lower anti-inflammatory response.

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Histological features of the AI showed a significant ($p<0.05$) increase in the number of mucosa cells in fish fed the NCTRL, PHA, GRA and BLEND diets, suggesting an intestinal recovery response. Fish fed PHA and GRA diets presented a significant ($p<0.05$) increase in mucosa vacuolation. A higher amount of cell vacuolation could be interpreted as a sign of intestine disruption.

Results from gene expression shed some light on intestine recovery responses in gilthead seabream juveniles. The expression of tight junction protein (*tjp*) gene was significantly ($p<0.05$) higher in fish from NCTRL, PHA, GRA and BLEND. Occludin (*ocl*) expression was significantly higher in fish fed PHA, GRA and BLEND. The upregulation of *tjp* and *ocl* may imply a protective effect on the fish intestinal epithelial barrier. Catalase (*cat*) gene expression was significantly ($p<0.05$) higher in fish fed NCTRL, PHA, GRA and BLEND diets. There was a significant ($p<0.05$) upregulation of the glutathione peroxidase (*gpx*) expression in fish fed PHA diet. The upregulation of *cat* and *gpx* could be an indicator of oxidative stress response and a compensatory mechanism for reducing oxidative stress. The levels of the immunoglobulin M (*igm*) gene expression were significantly ($p<0.05$) upregulated in fish from PHA. Tumor necrosis factor alpha (*tnf-α*) activity was significantly ($p<0.05$) higher in NCTRL, PHA, GRA and BLEND. Upregulation of *igm* and *tnf-α* expression may indicate an activation of the immune response. Fish fed BLEND showed a significant ($p<0.05$) upregulation of the proliferating cell nuclear antigen (*pcna*). *Pcna* upregulation indicates possible cell proliferation and epithelial regeneration in the intestine.

In conclusion, these results may indicate a positive regulation of both PHA and BLEND diets in gilthead seabream gut health. PHA and BLEND diets may be used as functional diets to preserve intestinal homeostasis and accelerate the healing process acting as immunostimulants activating the immune system and alleviating cellular oxidative stress.

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HYDROXYTYROSOL-RICH EXTRACT FROM OLIVE JUICE AS AN ADDITIVE IN GILTHEAD SEA BREAM FED A HIGH FAT DIET AND WITH FOOD RESTRICTION: DIGESTIVE ENZYME ACTIVITY

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Introduction

Increasing inclusion of plant-based products and highly lipid commercial diets due to the elevated cost of fish meal highlights the need of the sustainable development of aquaculture through the formulation of functional diets. In this sense, olive (*Olea europaea*) juice extract incorporation in aquafeeds has been found to enhance immune properties, gut health and functionality, and somatic growth (Gisbert et al., 2017; Rong et al., 2020), possibly due to the presence of bioactive compounds such as phenols, mainly hydroxytyrosol, which has anti-obesogenic properties (Lutfi et al., 2017).

The aim of this study was to evaluate the digestive process in gilthead sea bream (*Sparus aurata*) fed a high-fat diet, administered at two rations, *ad libitum* or restricted, and to test the effects of an olive juice extract rich in hydroxytyrosol as an additive.

Materials and methods

Gilthead sea bream juveniles (± 80.8 g) were acclimated to the animal facilities of the Faculty of Biology at the University of Barcelona (Spain). An experimental high-fat diet (24% lipids, 47% protein and 23 MJ/kg of digestible energy) was formulated and produced by Skretting ARC (Stavanger, Norway). The diet was produced in the absence (HF) or presence (HF-HT) of an olive hydroxytyrosol-rich extract (HIDROX®, 1.66%) provided by Oliphenol LLC. (Hayward, CA, USA). To establish two different conditions in terms of energy intake, the diets were administered at *ad libitum* (1.8% of body weight (BW) in the morning and 1.2% in the afternoon), or at restricted ration (1.8% of BW only in the morning, named R-HF and R-HF-HT groups). After 8 weeks of growth, samples of pyloric caeca (PC) and proximal intestine (PI) were obtained from nine animals per condition and time, just before feeding in the morning and at 5h post-feeding. Samples were directly frozen in liquid nitrogen and stored at −80 ºC for later analysis. Intestinal pH was measured, and after sample homogenization, the activity of digestive enzymes (alkaline protease, α-amylase, and lipase) and the trypsin/chymotrypsin ratio were analyzed.

Results and Discussion

No significant differences were found in growth between the HF and HF-HT groups, whereas food restriction significantly diminished it (-22.1%) (Balbuena-Pecino et al., 2023). Regarding digestive enzyme activities (Figure 1), higher levels were found in PC before feeding, as pancreatic enzymes can be released in this intestinal region prior to food intake to improve the digestive process. Conversely, 5h after feeding, the highest digestive activity was found in PI, where the digestion was taking place. When comparing HF and R-HF groups, in PC, a significant reduction in **α**-amylase activity was observed in PC, both anticipatory and 5h post-feeding, and lipase anticipatory activity was also down-regulated. However, both groups showed similar enzyme activities in PI. HYDROX addition to the high-fat diet tended to up-regulate the anticipatory activity of α-amylase in PC, and alkaline protease, α-amylase, and lipase activities in PI. In addition, it also increased the digestive activity at 5h post-feeding in the PC for all the enzymes studied. Finally, R-HF-HT fish showed the highest PC lipase anticipatory activity and an intermediate **α**-amylase activity between R-HF and HF-HT fish in both intestinal segments before feeding, as well as at 5h post-feeding in PC. Additionally, both restricted ration groups presented similar alkaline protease activity in PI before feeding.

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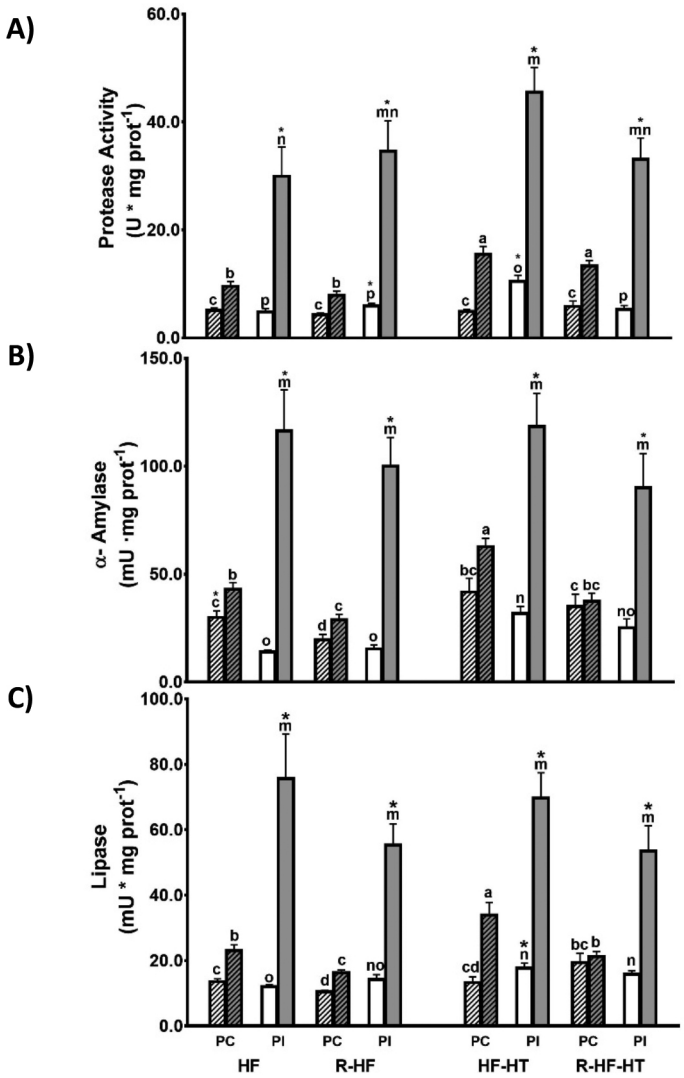

Figure 1. A) Alkaline protease, **B)** α -Amylase and **C)** Lipase activities in PC (bars with lines) and PI (smooth bars) just before feeding time in the morning (white bars) and 5h post-feeding (grey bars). Values are presented as mean ± SEM (n=9). Significant differences between experimental conditions are shown by letters (a-d for PC and m-p for PI), and between intestinal regions with *.

Conclusion

Food restriction promoted an optimization of the digestive function in fish, leading to relatively good growth performance despite a reduction in food intake. The observed growth reduction was less than what would be expected with a 40% food restriction. On the other hand, the effect of HT needs further studies as the observed increase in anticipatory activities suggests in general an adjustment of the digestive function to optimize the use of the given diet.

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NEW PRODUCTION SYSTEMS, NEW OPINIONS: EXPLORING PUBLIC PERCEPTION OF EMERGING AQUACULTURE SYSTEMS

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Introduction

Salmon (*Salmo salar*) aquaculture is an industry that has raised controversy and debate in Norway, Canada and Scotland (Flaherty et al. 2019; Osmundsen and Olsen 2017). The discourse surrounding the aquaculture industry includes a wide array of environmental and socio-economic matters (Weitzman and Bailey 2019), which can influence and shape public acceptance and trust in the industry. In all the aforementioned countries, open net pens sited along the coastline has been the primary production method of salmon. This production method is associated with several environmental risks, and despite being a relatively cost-efficient method, these challenges have hampered production growth. As a result, fish farmers are exploring new technological avenues that can reduce the environmental impact and utilize new production sites. Exposed, closed floating cages and land-based aquaculture are among the new production methods that are being developed and used by fish farmers. However, there might be potential drawbacks that can draw negative attention. On the other hand, there could also be advantages that could increase willingness to pay (WTP), trust and the reputation of the industry.

This study will explore public perceptions of new aquaculture production systems, and the public's willingness to pay for price premiums. The research question for this study is: what, according to the public, are the most important advantages and drawbacks of different production systems, and are consumers willing to pay for salmon produced using these production systems?

Methods

To investigate public perceptions of new production systems and willingness to pay for salmon produced using these systems, we are conducting an experimental panel survey in the salmon-producing countries of Norway, Scotland, and Canada. The survey is in the process of being fielded by the international, online polling company, YouGov.

We first examine how the public views conventional, land-based, closed floating, and offshore aquaculture by asking them to rank a series of real-world advantages and drawbacks to each production system. We next investigate their willingness to pay for salmon farmed using new production systems through a choice experiment where price is manipulated. After receiving information about the benefits and drawbacks of the production system (i.e., land-based, closed floating, or offshore), respondents are asked if they would rather buy salmon farmed using conventional methods or salmon farmed using the new production system. In Panel 1, the price for salmon farmed using the new production system is higher than the price for salmon farmed using the conventional system. In Panel 2, the price for salmon farmed using the new production system is the same as the price for salmon farmed using the conventional system. The survey design also includes general questions regarding attitudes towards conventional aquaculture, and what the respondents perceive to be the most negative environmental externalities from conventional aquaculture.

Once the results are ascertained (in June 2023), they will be analysed using SPSS.

Results

As of May-June 2023, the survey is in the process of being fielded, and the key results will be presented at the conference.

We hypothesise that respondents will favour new production methods over conventional methods, but not at an increased price.

Furthermore, we hypothesize that perceptions of conventional aquaculture will vary between the salmon producing countries; with Canadian respondents being more negative towards conventional aquaculture than Norwegian and Scottish respondents.

We also expect to see differences in the level of public support for the new production systems across countries. In Canada, we expect production methods that reduce the release of organic matter to receive the most public support whereas in Norway and Scotland we expect production methods that reduce lice and increase fish welfare to receive most public support.

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BLACK SOLDIER FLY (*Hermetia illucens*) PREPUPE MEAL DID NOT AFFECT INDIVIDUAL AND GROUP EXPLORATIVE SWIMMING TRAITS IN EUROPEAN SEABASS (*Dicentrarhus labrax*): AN ETHOLOGICAL STUDY ON FISH WELFARE

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Introduction

Given the growing importance of aquaculture to supply human demands for protein, it is necessary to optimize the farming techniques to obtain a product of excellent quality and concurrently reduce the impact on the environment (FAO, 2022). In this regards, new alternative foods that reduce the amount of organic pollution have been proposed. Among these, a replacement food has recently been developed with a substitution of fish proteins using black soldier fly (*Hermetia illucens*) full-fat prepupae enriched with spirulina (Zarantoniello et al., 2022). Although this insect-based meal has been demonstrated to reduce organic pollution of wasting feed in the environment by converting low-value organic waste into high-value ingredients (Barragan-Fonseca, 2017), its impact on animal welfare, and indirectly to the quality of product, has marginally been investigated.

Behavioral changes may raise because of stressful situation, such as recurring manipulation and wrong feeding regimes. Underfeeding, a state of lesser caloric intake assumed by individual, increased individual competitiveness and high stress levels with, consequently, reduce growth and survival rate. Although the physiological mechanisms of stress have been widely documented (4), a characterization of the behavioural indicators, especially for applying them to the farming context, is still demanding. Recently, it has been proposed a set of Operational Welfare Indicators (OWIs), which offer an unbiased assessment of the welfare state that can be practical implemented to the farm reality (5). Here, we applied this ethological approached to examine the behavioural response to a novel situation, i.e., open field test, of the European seabass (*Dicentrarchus labrax*) fed with different regimes of insect-based meal.

Materials and methods

The feeding treatment was conducted at Mj Energy srl Società Agricola (Treia, Macerata, Italy). After a first acclimation period, European seabass juveniles were randomly divided and assigned to one of the treatments. We administered two experimental diets characterized by 3 and 20% of fish meal replacement with full-fat spirulina-enriched black soldier fly prepupae meal. A group of control was feed with diet characterized by 0% of spirulina-enriched black soldier fly mean. Seabass juveniles undergone a 90-day feeding regime in aquaponic systems equipped with mechanical, biological, and UV filtration.

After the treatment, we performed the behavioural tests. Firstly, we observed individual fish exploration activities in an open field test, a standardize behavioural procedure for measuring personality and explorative activity in animal research. Twelve-fish per each experimental diet condition was individually collected and placed into a 75×75×30cm square-shape white arena, which was positioned into a larger tank connected to a circulating aquaponic system that provided constantly fresh water. Individual behaviour was recorded for 20-min. A second subsample of 60 fish (15 per each treatment) was observed in 4-fish group in the same experimental conditions 20-min.

Experimental recordings were then analysed with EthoVision XT software (ver. 11.5, Noldus Information Technology, Wageningen, The Netherlands). We extracted different individual and collective variable to investigate whether diet regime affected fish behavioural response to the novel situation. As individual variables, we measured distance covered, time spent in the immobility state, time exploring the center of the arena, mean turn angle of swimming activities (6). As group variables, we measured two categories of explorative behaviour (7): swimming activity (i.e., distance covered, mean acceleration, and time of immobility state), and spatial exploration traits (i.e., inter-distance from the centroid of the group, distance between subjects, and total perimeter of group). We additionally measured the ventilation frequency at the first 2-minutes and at the final 2-miutet for each individual in both behavioural tests as an indicator of the metabolic stress (8).

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Results

By using several behavioural OWIs, no significant differences emerged among treated groups from the individual and collective group experiment when dealing with a novel environment, indicating a positive outcome for applying spirulina-enriched black soldier fly prepupae meal in aquaponic systems for fish rearing.

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BENEFICIAL EFFECTS OF A MARINE *Bacillus* MULTI-STRAIN CONSORTIUM ENCAPSULATED IN ALGAE ON GROWTH PERFORMANCE, DIGESTIVE AND IMMUNITY GENE EXPRESSIONS, VIBRIO RESISTANCE, MICROBIOTA MODULATION AND TRANSCRIPTOME PROFILING OF WHITE SHRIMP *Penaeus vannamei*

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Introduction

Aquaculture production is increasingly looking towards innovative solutions to further improve gut health and production sustainability. Marine probiotics have the potential to support the control of infections and to improve zootechnical performances, while minimizing the environmental impact. We investigated the probiotic potential of *Bacillus* multi-strain consortium extracted from the marine environment and encapsulated in algae on white shrimps, *Penaeus vannamei*.

Materials and methods

20-day and 60-day feeding trials were conducted respectively on healthy *Penaeus vannamei* PL10 (n=3500, triplicate) and PL25 (n=350, triplicate) to evaluate the effects on growth and health parameters of a marine probiotic consortium (MPC) made of four marine *Bacillus* strains encapsulated in algae when used as dietary supplement.

Results

At the end of the 60-day trial, the treatment with MPC at 0.1% significantly improved growth performance (final body weight, weight gain, specific growth rate) and significantly decreased feed conversion ratio of White Shrimp *P. vannamei* ($P < 0.05$). Supplementation with MPC at 0.1% also significantly decreased the occurrence of total *Vibrio spp.* count in shrimp's hepatopancreas under normal conditions ($P < 0.05$). Overall, MPC significantly enhanced the mRNA expressions of (1) digestive genes in hepatopancreas, such as Trypsin, α-Amylase, Triacylglycerol lipase and Chymotrypsin BII; (2) immune-related genes, such as proPO, crustin genes in hepatopancreas and Dual Oxidase, mucin-like peritrophin, Penaeidin-3α in intestine genes ($P < 0.05$)), especially with MPC at 0.25%. Under challenge conditions, MPC significantly increased disease resistance of shrimp larvae against *V. parahaemolyticus* (VpAHPND) ($P < 0.05$).

The results of high-throughput sequencing showed a significant improvement in White Shrimps digestive tract bacterial communities when supplemented with probiotics. Moreover, the MPC enhanced the hepatopancreas gene pathway related to metabolisms of endocrine, immune and digestive systems. In addition, the beneficial effects observed in intestine gene pathway were mainly related to translation, transport catabolism, and signal transduction.

Conclusion

This study demonstrated the ability of the marine *Bacillus* spp. consortium encapsulated in algae to improve White Shrimp growth performance, digestive tract & immune status and disease resistance during both larval and grow-out phases.

EFFECT OF SULPHATED POLYSACCHARIDES DERIVED FROM BRINE WATER ON THE IMMUNE SYSTEM

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Introduction

Living organisms that live in marine environments produce sulphated polysaccharides (SP) that are highly soluble in water and remain as one of the most abundant compounds in brine water. These compounds have been described as presenting important biological activities, such as antioxidant (Wang et al., 2019) immunomodulatory and anti-inflammatory (Geng et al., 2018) properties. Since SP represent a large part of organic material present in sea salt polymeric material (Silva et al., 2015) we have investigated its activity on the immune system, using zebrafish (*Danio rerio*) as a model. The immunostimulant properties of SPs derived from salt pan brine water have been studied by Nunes et al., 2019, referring the possible use as functional ingredients to be incorporated functional food, for increasing the well-being of fish produced in aquaculture or for research models in laboratory systems. Zebrafish is an established animal model in research, that has been validated for studies in ecotoxicology, immune resistance and for testing ingredients for nutrition in aquaculture (Lawrence, 2007; Ulloa et al., 2014). Our objective was to test the effect of dietary delivery of SP from brine water in the resistance of zebrafish to *Aeromonas hydrophila* infection.

Materials and methods

Two experimental diets supplemented with 10 and 100 mg/kg with sulphated polysaccharides (SP10, SP100), were used to feed zebrafish during 40 days, starting from 15 days post fertilization. Larvae were reared in standard conditions until day 15 and groups of 100 larvae were transferred into 3 L tanks, in triplicates for the feeding trial. Larvae were fed 3x per day with each of the dietary treatments: control diet; SP10 continuous; SP10i 1x/week; SP100 continuous; SP100i 1x/week.

Lethal bacterial concentrations (LD50), were established in a preliminary trial with two different bacterial concentrations (10^6 and 10^7 CFU.mL^{-1}) added to 1L tanks and with three different exposure times.

After the dietary trial, 15 zebrafish juveniles from each replicate were stressed by caudal fin amputation and placed in a 1L tank containing 5.1x10^7 CFU.mL^{-1} of *A. Hydrophila* and exposed for 4, 7 and 24 hours. After the bath, the fish were placed 1L tanks with clean water, and mortality was recorded for three days.

Samples were collected for gene expression analysis of immune markers, immediately before exposure and during the 3 days after exposure to *A. hydrophila*.

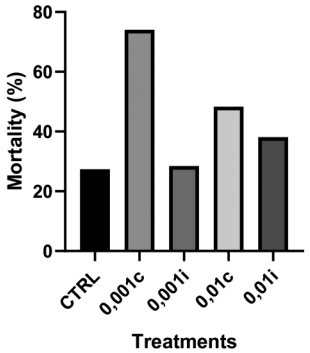

Figure1- Cumulative mortality after exposure of SP tretaed zebrafish juveniles to *A. hydrophyla* by bath.

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Results

Cumulative mortality upon exposure to *Aeromonas hydrophila* was significantly higher in zebrafish fed continuously with the diets supplemented with 10 and 100 mg/kg of sulphated polysaccharides from salt pan brine water. The groups that were fed intermittently, only once a week add mortalities similar to the control group.

Gene expression analysis revealed a strong response immediately after exposure followed by a downregulation after 24 hours of exposure.

Discussion

No beneficial effects were observed by the dietary supply of sulphated polysaccharides isolated from salt pan brine water in zebrafish larvae. However, the expression of immune marker genes revealed that the immune response was exacerbated in fish fed with the experimental diets compared to control group.

Further research is necessary to establish the dietary doses necessary to modulate immune response and optimization of the dietary protocols to be used.

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FEEDING HABITS OF THE YELLOW PERCH (*Perca flavescens)* IN RECTANGULAR AND ROUND TANKS

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Introduction

Yellow perch, Perca flavescens (Michill, 1814), is one of the most commercially important species of the Percidae family, most abundant in the inland waters of the northern regions of the North American continent. However, the reduction in the wild catch due to overfishing (Saoud et al, 2004), and the devastation of the natural habitat (Clapp and Dettmers, 2004) has resulted in an increased interest in research of the biology of this species, and the development of appropriate culture technologies. The slow growth rate ascribed to yellow perch usually evidenced in open pond culture conditions. Controlled conditions in a closed recirculation rearing system should prevent most of the problems which are common in outdoor cultivation systems and provide for optimal growth of yellow perch (Westers and Weeks, 2002). Perch proved to be very timid (compared to sea bass, trout and tilapia grown in the same culture systems) reacting to any activity above and inside the tanks. It was important to provide optimal tank design and hydrodynamic water flow characteristics while not disturbing the fish, so that they could maintain efficient positioning while feeding. The aim of the study was to observe feeding and behavior of yellow perch in two different tank designs, rectangular and round, and quantify the effect of these conditions on their growth.

Materials and Methods

15 g yellow perch fingerlings were stocked in two round and two rectangular 12000-liter tanks at maximum density of 50 kg/m^3. The final size was set at 160 g. As the fish grew, they were transferred to more tanks of the same design to maintain a constant stocking density. The round tanks had a tangential water inlet below the water surface, and a central effluent port, thus creating a circular flow. The "crossflow" rectangular tanks had a series of water inlets at one side, and drains on the other side of the bottom creating a tubular cross tank current. Fish were raised at constant temperature of 24-25°C. Dissolved oxygen level was maintained at saturation using oxygen injection. Calcium hardness was maintained between 80-150 mg/l using calcium chloride, and pH was maintained between 7.2 and 7.4 with the addition of sodium bicarbonate. Chlorides were adjusted to 300 - 400 ppm using sodium chloride and calcium chloride. During the entire experimental period perch were fed continuously with commercial slow sinking pelletized feed ("Ziegler Brothers", PA), contained 45% of crude protein and 12% fat, using automatic belt feeders. Fish were fed 5% of the body weight per day in the first month, 4% in the second, 3% in the third and fourth, 2.5% in the fifth and sixth month, and 1.5% in the last four months of the growing period (Jug-Dujakovic and Van Gorder, 2002). A sample of 50 fish was taken every month from the tanks to obtain a measure of average weight. Dead fish were counted every day to calculate the survival at the end of the experiment. The analysis of variance followed by the SNK multiple comparison procedure was used to test variations in growth rate.

Table 1. Mean values of weight of yellow perch, with standard deviations, at the beginning of the experiment, and after 8 months of cultivation, in round and rectangular tanks. Values with different lower-case subscript are mutually statistically different (ANOVA, $p < 0.05$)

Tank design	Replication	Initial weight	Final weight
Rectangular	1	16.42±1.8	170.2±14.4^1
	Mean value		**160.9±13.3^a**
	2		158.3±16.9^1
Round	1	16.27±1.1	181.3±16.2^2
	Mean value		**176.1±16.7^b**
	2		173.4±15.9^2

(Continued on next page)

Results and Discussion

When not feeding, the fish would swim indiscriminately, not always orienting against the flow of the water, a posture which was more pronounced in the rectangular tanks. In round tanks the administered pelleted feed would follow the current while slowly sinking, and the fish would position themselves to consume the moving food pellets. This pattern was less noticeable with crossflow tanks. Instead of maintaining perpendicular position to the water flow direction, fish would swim more randomly. More feed would reach the bottom of the tank and be removed uneaten.

At the beginning of the study, the 120-day old fish stocks had an average wet weight ranging between 14.7 and 18.1g. Eight months later, at the end of the experiment, the mean weight of fish was 176.1 g in the round tanks, and 160.1g in the rectangular tanks (table 1).

The difference of 16 grams of growth between tank designs represents an approximately 10% weight difference, or expressed in time, a month of growth under comparative rearing conditions. The grow-out phase of yellow perch (10g to 150g) in recirculating systems has been successfully demonstrated by Jug-Dujakovic and Van Gorder (2002). Effects of tank wall color and positive up-welling water flow on growth and survival of Eurasian perch larvae (Perca fluviatilis) was recorded by Jentoft (2006).

Conclusions

The circular flow of the water in round tanks elicited a natural swimming and schooling behavior with a common orientation by the fish populations (into the flow). This positioning and swimming behavior, and the accompanying effective feeding behavior resulted in increased feed conversion efficiencies, and improved overall growth and survival characteristics compared to those of populations cultured within cross-flow raceways.

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OPPORTUNITIES FOR INTENSIFICATION OF GRASS CARP, *Ctenopharyngodon idella* (Valenciennes, 1844) AQUACUULTURE IN EUROPE

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Status of grass carp aquaculture

In last two decades the climate changes and overfishing resulted in the intensificated research on introduction of new species in aquaculture, or the possibilities of increased cultivation of herbivorous and more climate resilient fish species that are already cultivated. In terms of sustainability, herbivorous species that tolerate high water temperatures are the best candidates. Such an example of a species that is already cultivated, but still has a great potential for intensification of farming in Europe is grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), a naturally active and fast-growing species. This species tolerates a wide range of temperatures, from 0° to 38°C, salinities up to 10 ppt and oxygen levels down to 0.5 ppm (Frimodt, 1995). Wu et al. (2023) found that juvenile grass carp exposed to different dissolved oxygen concentrations over prolonged time period showed different susceptibility and adaptation mechanisms. Low dissolved oxygen concentration greatly improves blood gas transport capacity of grass carp, while high dissolved oxygen concentration slightly improves aerobic respiration level. Its main feed are higher aquatic plants and submerged grasses, but it also takes detritus, insects and other invertebrates (Frimodt, 1995).

Grass carp was introduced in Europe in the 20th century for aquatic weed control and aquaculture purposes. Today, it is grown as a food fish in polyculture in carp ponds (Frimodt, 1995; Woynarovich et al., 2010), but it is also of special interest for sport fishing (Siyi et al., 2022). In its homeland of China, it is currently the most important freshwater aquaculture species. In this country, it is mostly farmed in monoculture, with yearly production of 5.76 million tons in 2021, what was 18.08% of China's total freshwater aquaculture production (FAMA, 2022). According to EUMOFA (2021) the main grass carp producers in Europe are Poland, Czechia, Hungary and Croatia, with the 1.761 tonnes. Its European production remained fairly stable, while the amount of all other farmed Asian carp species decreased by 60% over the ten-year period from 2008 to 2018. Although grass carp is one of the world's most important aquaculture species, it is considered as a pest outside of its native range due to negative impact on the aquatic, water quality and biodiversity (Dibble and Kovalenko, 2009). With regard to legislation related to non-native species in Europe, its cultivation is allowed in most countries in waters where population has already been established. Exceptionally, all non-native species could be farmed, but their spread into open waters must be prevented. One possible solution could be its farming in the closed RAS systems with the complete control of output water. Despite the mentioned facts affect the development of grass carp production in Europe, the results of numerous researches conducted in recent years can justify the intensification of its production, either in mono- or polyculture.

Production intensification possibilities

High stocking density in polyculture, may cause high total ammonia and phosphorus concentrations, and consequently, algal blooms (Dibble and Kovalenko, 2009). Li et al. (2019) showed that the use of artificial substrata could be one of the technological solutions to increase pond farming density of grass carp by increasing the bacteria that participate in nitrogen and phosphorus cycles. Except in the pond culture, grass carp growth was tested in different technological systems, such as in-pond tank culture system for high-intensive fish production and in RAS. In-pond partitioned aquaculture system that was used in last two decades for intensification of the production of the pond farmed species, and for reducing the use of land, showed that intensive high stocking density had a negative impact on the growth performance and muscle quality of grass carp (Lu et al., 2022). Opposite to the in-pond system, survival and growth rates of the juvenile grass carp overwintering in the RAS were significantly higher. At the same time, the RAS ensured production profit when compared to the loss in the in-pond system (Kristan et. al., 2018). Similarly, as for the common carp (Gavrilović et al., 2019), properly designed RAS, used for overwintering of juveniles in combination with pond farming during warm period could possibly provide the basis for the intensification of grass carp production with economically feasible results.

It could be concluded that technological improvement can be applied for the intensification of grass carp culture. Considering European market, parallelly with the production intensification, relevant marketing strategy that would include product diversification and development should be considered.

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Acknowledgement
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ASSESSMENT OF BODY SHAPE VARIATION USING ELLIPTIC FOURIER DESCRIPTORS AND THEIR GENETIC ESTIMATES IN THE FLATFISH SENEGALESE SOLE

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Introduction

Fish shape is highly relevant in aquaculture since it acts as a driver of consumer decision-making. Shape of the flatfish Senegalese sole is characterized by an elliptic and lanceolate midbody, long caudal and dorsal fin sizes and short jaws with a scarce number of reference landmarks. Hence, outline-based methods such as the Elliptic Fourier descriptors analysis (EFA) seem to be highly useful as predictor of these body curved shapes. Hence, EFA could be useful to assess shape variation and evaluate genetic components in sole. In this species, it is estimated that approximately 12-15% of farmed soles show external morphological alterations that impedes their commercialization (de Azevedo et *al.*, 2019). Guerrero et *al.*, 2021 reported moderate-high heritabilities for body ellipticity as inferred from standard length and body height, however, these traits do not capture complete whole-body variation. Thus, this study aims to evaluate whole-body variation using EFA. Phenotypic information was analyzed and compared with a new set of distance-based and ellipse fitting estimator traits. Genetic estimates for these traits were determined.

Materials and methods

A total of 2,271 soles from in eight evaluation batches (EB) were individually photographed at harvest, and the weight and length were *in situ* recorded. A piece of caudal fin (CF) was preserved for genotyping. Body contours were individually processed in Fiji 2.1.0/1.53c and images in binary format were imported into the package SHAPE to calculate the Elliptic Fourier descriptors (EFDs). A principal component analysis (PCA) of the EFDs was carried out to extract information for symmetrical (PCs) and asymmetrical (PCa) features of body shape. Coefficients of EFDs were calculated at ± two standard deviations and scores of PCs and PCa were used in subsequent genetic analysis.

Additionally, some well-established and new ellipse descriptors based on morphometric distance and ellipse fitting parameters were calculated: standard length (SL), maximum body height (MBH) to caudal peduncle height (CPH) ratio, ellipticity (ELL; (SL-MBH)/(SL + MBH)), whole-body perimeter (PER), the aspect ratio (AR; SL/MBH) as distance-based trait and the aspect ratio from the fitted ellipse (ARe), and solidity (SOL). ANOVA analysis were carried out to test statistical differences associated with gender, EB and the presence of amoebic nodules (AN). Genetic parameters were estimated by REML as implemented in the package WOMBAT with gender, EB and AN as fixed factor.

Results

PCA identified four significant PCs for EFDs that explained 94.0% of shape symmetric variation and 68.6% of the total variation, and four PCa that explained 90.7% of asymmetric variation and only 24.5% of total variation. PC1s and PC1a represented 52.8 and 58.7% of symmetric and asymmetric variation, respectively.

Statistical analysis of PCs and PCa indicated that males were statistically more elliptic (lower PC1s and higher PC3s) with wider CFs (as revealed by lower PC2s and higher PC3s) than females. Also, males tended to have obtuse angles in the CF (PC3a) with the head orientated toward the abdominal cavity (PC4a). Statistical differences in AN were highly influence by fish weight. Significant differences among EBs were found for all components. Statistical analysis of ellipse descriptors indicated that females were 12.7% heavier and 2.2% longer than males. Moreover, males were 2.4% more elliptic than females (AR, ELL and ARe) with a 1.6% lower MBH/CPH, but no differences in PER.

Heritability estimates were very high for PC1s (0.702), moderate for PC3s and PC4a (0.390-0.400) and low for PC2s, PC4s and PC1a-PC3a (0.039-0.168). Heritabilities for MBH/CPH, ELL, ARe and AR were high or very high (0.513-0.804) and moderate for SOL (0.273) and PER (0.432). Genetic correlations were very high between ELL, ARe and AR (≥ 0.90) and between PER, weight and SL (≥ 0.93). ELL, ARe and AR were strongly correlated with PC1s and PC4a (≥ -0.873 and ≥ 0.705, respectively). ELL and AR showed a high genetic correlation with PC3s (≥ 0.574). SOL was highly and negatively correlated with PC2s (0.916) and PC3a had the highest genetic correlation with ARe (-0.583).

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Discussion

Shape quality is very important during fish commercialization since consumers preferably buy products whose external appearance resemble wild fish (Calanche et *al.*, 2020). Senegalese sole's lanceolate body shape is considered a high-quality shape standard and major attention had been paid to mid-body ellipticity as the main trait to select for shape quality (Guerrero-Cozar et *al*., 2021). In our shape model, 20 harmonics were sufficient to capture main symmetric and asymmetric variation through the tested population. This study demonstrated that EFA and ellipse fitting estimators are effective and powerful traits to assess shape quality in Senegalese sole.

Captured variation of EFD was primarily related to the adjustment of whole and midbody to an ellipse and CF variation, and secondarily to head orientation. Very high heritabilities for body ellipse fitting traits indicated a high potential to improve it via selective breeding and suitable to be included with growth traits (negatively correlated) to avoid an excess of roundness bodies after some selection rounds. Moderate-high heritabilities were also found for traits related to head and CF peduncle (SOL or MBH/CPH). The latter could be relevant to select specifically CF functionalities and fish swimming capabilities. Heritabilities for CF-related traits were low, however, due to the influence of CF on consumer's perception and its use as a heath indicator, indirect selection using other correlated traits would be more efficient in genetic gain.

All the information here provided is highly useful not only for designing breeding programs but also for the improvement of operational methods to preserve shape quality and monitor health and welfare in the aquaculture industry.

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BEHAVIOURAL-BASED INDICATORS FOR DETECTING SATIATION LEVELS IN MARINE CAGES

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Introduction

Efficiency and cost in aquaculture depend largely on feeding, making it crucial to optimize feeding strategies to reduce feed loss and improve fish health and welfare. One way to achieve this is by monitoring individual and group swimming patterns, especially in response to external factors like feeding. Intelligent feeding control that utilizes behavioral changes and growth status is gaining attention as a useful tool for improving husbandry practices. Efforts are underway to identify behavioral indicators that can detect satiation levels and regulate feeding for different species. In this study, we investigated two behavioral-based indicators, speed and the feeding behavioral index (a newly defined metric), across different feeding scenarios for E. seabass. Our findings suggest that fish exhibit distinct behavior patterns in response to various feeding situations, and both the speed and feeding behavior index can identify threshold values that correspond to satiation levels, facilitating controlled feeding.

Materials and methods

A group of E. seabass fish of 300 g body weight was reared in a circular polyester cage (40 m diameter, 9 m depth) located at the pilot scale netpen cage farm of HCMR at Souda bay, Crete (certified as an aquaculture facility from the national veterinary authority; code GR94FISH0001). A submerged network camera (Fyssalis V3.1) capturing at 10 fps was used for monitoring and video recording during daylight hours. The camera was positioned at 4 m depth using a gyroscopic gimbal stabilizer to ensure it pointed upwards. We trained YOLOv5 (a machine learning model for object detection) to detect fish and adapted Deepsort (a model for tracking people) to track fish individually (using OPENCV/Python) and extract their speed and direction. In addition, we used computer vision techniques that detect changes in the crowding behavior of the fish, and we called the parameter feeding behavior index. To detect behavioral changes as response to satiation levels we modified the feeding schedule of the fish by varying three crucial feeding parameters: the feeding frequency (once, twice and three times a day), the feeding time (morning, afternoon and evening) and the feeding quantity (normal, reduced, overfeeding and no feeding). The experimental trial lasted from June 2022 until April 2023.

Results

Our preliminary results show that the feeding behavioral index shows different qualitative and quantitative evolution across the three different feeding quantities. The excitation step at the start of the feeding is significantly larger when fish are underfed than when overfed or fed normally (Figure 1). In addition, the duration of the excitation is longer. The speed on the other hand shows a significantly different qualitative behavior (Figure 2). There is a change in activity relative to the start of the feeding. When feeding is normal, fish show symmetry in the activity pattern relative to start of the feeding. They gradually increase their activity before feeding and gradually decrease it after feeding. In contrast, during reduced feeding fish appear to show increased activity before feeding, while during overfeeding periods, fish show more increased activity as response to the start of the feeding.

Conclusion

Our results indicate that fish show distinct patterns of behavior under different feeding situations, and both the speed and feeding behavior index can be used to detect threshold values that correspond to satiation levels and facilitate controlled feeding in marine cages. Under reduced feeding, fish show an early and prolonged excitation before feeding and a slow relaxation to the baseline activity after feeding times indicating strong anticipation for feeding and increased appetite. The sudden increase in the feeding behavior index also contains important information about the fish appetite, as larger activation steps in the feeding index suggest lower satiation levels. Last, the duration of excitation is also an important parameter that can reveal the satiation levels of the fish, suggesting that fish with higher satiation levels show longer excitation times in their feeding behavior index. Further studies are required to help us understand the contribution of other factors such as the human presence, or the internal circadian rhythm to the variation of the activity.

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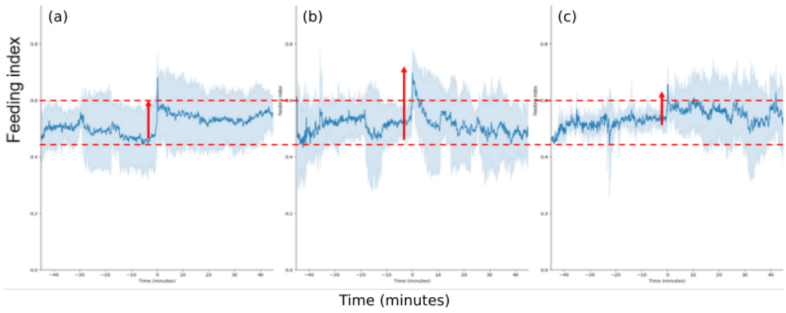

Figure 1. Feeding behavioral index versus time for the three different feeding quantities, normal (a), reduced (b) and overfeeding (c). Feeding starts at time = 0 mins.

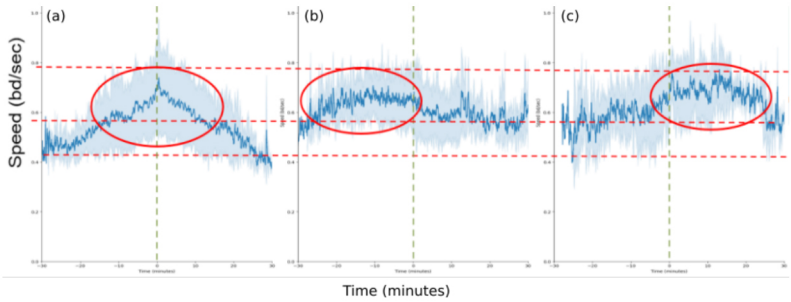

Figure 2. Speed versus time for the three different feeding quantities, normal (a), reduced (b) and overfeeding (c). Dashed vertical line shows the start of the feeding. Feeding starts at time = 0 mins.

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INTROGRESSIVE HYBRIDIZATION LEVELS OF TILAPIINES SPECIES IN LAKE VICTORIABASIN,KENYAINFERREDFROMMICROSATELLITEANDMITOCHONDRIAL DNA GENOTYPING BASED ON NEXT- GENERATION SEQUENCING

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Despite their high abundance and species richness, tilapiines have been compromised by various factors especially overfishing, climate change, and un-controlled fish translocations. Fish translocations have negatively impacted native tilapiine populations through competition, predation, hybridization, and introgression compromising their genetic integrity. The hybridization levels of different tilapiines in the Lake Victoria basin remains an understudied aspect relatively. The study utilized nuclear microsatellite and mitochondrial DNA (mtDNA) genetic markers to investigate hybridization signals and compare the genetic diversity of different tilapiines in Lake Victoria, Kenya, using next-generation sequencing. Low levels of hybridization from *Oreochromis niloticus* into other *Oreochromis* species were detected by Bayesian clustering analysis and principal coordinate analysis (PCoA). The results contribute to the need for conservation measures of these fish species.

AQUACULTURE AND CONSERVATION - WHERE TWO STURGEON WORLDS MEET

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Introduction

Worldwide, several sturgeon species are facing the threat of becoming extinct in the wild, and in Europe some are only surviving because of the success of ongoing recovery programs that include last resort measures such as *ex situ* conservation. *Ex situ* conservation involves captive rearing and propagation under controlled conditions and can serve as a stop gap measure while other recovery actions are implemented. It is critical that these programs preserve the different components of genetic diversity, on the one hand the differentiation between populations, which could reflect adaptations to different environmental conditions, on the other hand, the genetic diversity within populations which represents their adaptive potential. This attention to the genetic integrity will increase the chances of long-term population persistence even after the cessation of *ex situ* measures.

Discussion

In many cases, small population sizes or *ex situ* programs that were initiated long after population declines began result in difficulties to meet genetic diversity goals. Accordingly, programs must ensure an approach that maximizes genetic outcomes, through the integration of all suitable resources. While Recommendation 22 of the Vienna Declaration on Global Sturgeon Conservation by global sturgeon experts (Rosenthal et al., eds. 2018) stated that "Commercial farms, culturing sturgeons for consumer markets, may be important partners in conservation programs to bridge the time-window until the required public infrastructure for *ex situ* conservation is in place..." it has become that, despite numerous biodiversity programs and funding tools, the implementation or long-term maintenance of such public facilities in Europe has not been feasible in most cases. It is therefore mandatory to search for practical alternatives for species recovery.

Several sturgeon species are in the unique condition that while being threatened in the wild, they are raised successfully and economically in large quantities in private companies. This opens opportunities to explore private-public partnerships for the benefit of these species at risk. In this presentation we will outline prerequisites to implement such collaborations as well as the limitations. This will include the importance of maintaining genetic differentiation and diversity of subpopulations; as well as the separation of commercial and conservation rearing facilities to prevent disease transfer and to meet the criteria for fitness for release of juveniles.

Outlook

In the Conservation Session, it is anticipated to build on the discussion that has started at the EAS 22 in Rimini in 2022. In order to guide the discussion to a target oriented output, it is foreseen to focus on the following questions in the round table discussion:

- Which processes would lend themselves for engagement of private farms?
- What could be the benefits for the farmers?
- What could be benefits for the conservation program?
- What could increase the profitability of collaboration?
- How could a joint strategy look like?

Reference

Rosenthal, H., Gessner, J. Bronzi, P. 2018. Vienna Declaration on Global Sturgeon Conservation, WWF and WSCS, Vienna and Neu Wulmstorf, 16 pp.

ANALYTICALPLATFORMFORMONITORINGNITROGENSPECIESINRECIRCULATING AQUACULTURE SYSTEMS

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Recirculating aquaculture systems (RAS) represent a new way to farm fish in land-based aquaculture, in which the water is filtered and cleaned for recirculating through the fish culture tanks. However, the current RAS facilities still lack a fit-for-purpose digital control of the water chemical parameters to adjust/tailor the treatment trains and quickly identify problems related to the quality of the recirculated water [2].

In this work, a novel analytical platform for monitoring of chemical species in RAS is presented, namely nitrate and nitrite ions to better understand if the treatment of recirculated water is effective and adequate. The platform combines the powerful features of electroanalytical sensors with technological advances in terms of microfluidics and electronics to provide the control of water quality. It is divided into different modules, namely the sample processing and detection module which are controlled by an electronic module (Figure 1).

The sample processing module enables the collection of the water and the performance of pre-treatment steps to overcome interfering matrix effects, such as solid particles, salinity, and pH. For this purpose, the sample filtration is followed by two serial microfluidic chambers fabricated in poly(methyl methacrylate) (PMMA). The first includes a cation-exchange membrane and a pair of electrodes (Ag as working and Ag/AgCl as reference/counter electrodes) in which an anodic potential is applied to oxidize the Ag and remove the chloride ions by the formation of AgCl at the surface of the working electrode. The applied anodic potential, silver thickness, sample volume, and reusability are under optimization to achieve the best efficiency of the desalination cell. The second chamber enables the mixing of the desalinated sample plug with an acidic media to reach the required pH for analysis.

The detection module comprises solid-contact ion-selective electrodes for nitrate and nitrite ion as well as a custom-made Ag/AgCl reference electrode, both integrated into a PMMA microfluidic cell. Commercial carbon screen-printed electrodes modified with graphene oxide were used to prepare the sensors by drop-casting the corresponding polymeric ion-selective membrane on top (Figure 2). The proposed potentiometric sensors were evaluated in terms of analytical response, selectivity, robustness, and durability. Nitrate-sensors showed a sensitivity of 52.0 mV/decade within the linear range of 0.2-612 mg/L and a limit of detection of 0.1 mg/L at 0.1 M phosphate buffer background (pH 5.0). A fast response time (<20s), good reproducibility (RSD<1.4%), potential stability (0.3 mV/h), and durability of four weeks are some of the remarkable properties. Likewise, the nitrite-selective electrodes provided a sensitivity of 45.4 mV/decade over the linear range from 0.05 to 454 mg/L and a limit of detection of 0.04 mg/L within the same conditions. A response time <50s,

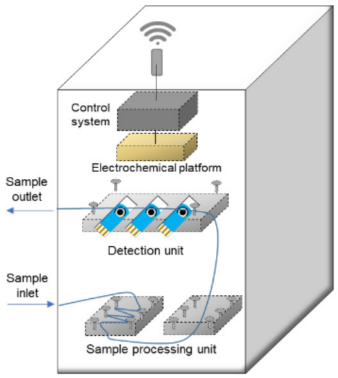

Figure 1. Scheme of the portable analytical platform. Pumps, valves, and electrical connections are not represented.

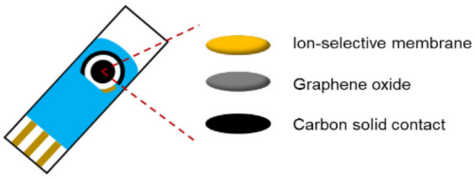

Figure 2. Miniaturized sensors.

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excellent reproducibility (RSD<0.4%), potential stability of 2.0 mV/h but a shorter lifespan of about a week were observed, mainly attributed to the leaching of the sensing element from the polymeric membrane. Additionally, the proposed nitrogen sensors showed great selectivity to the target species against common interfering ions present in seawater. Cations do not impose any interference due to the perm-selectivity of potentiometric sensors while the most interfering anions were iodide and perchlorate, however their common low levels in water are not a cause of concern.

The applicability of nitrate sensors was assessed, first, by the analysis of different natural water samples. Appropriate recovery percentages (88-108%) and an excellent agreement with a commercial nitrate probe (difference<8.5%) in the analysis of freshwaters confirmed the great reliability of the proposed sensors. Nevertheless, the presence of chloride at high levels in seawater worsened the accuracy, justifying the use of the aforementioned cell for the decrease in salinity.

Future work envisage the tailoring of the proposed analytical platform for on-site monitoring of chemical parameters in RAS. This innovative technology brings major advantages when compared to conventional techniques such as cost-effectivity, autonomous performance, and improved spatial/temporal analytical resolution, offering new opportunities to the aquaculture sector.

Acknowledgments
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HOW DO DIFFERENT RAS PROTOCOLS AFFECT ENERGY STATUS, INTESTINAL APPETITE REGULATION, AND PEPTIDE TRANSPORT IN ATLANTIC SALMON (POST-) SMOLTS?

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Introduction

Mortality at sea transfer is one of the major culprits for economic loss in the Norwegian salmon industry, reinforcing the tendency towards shortening the rearing time in open net pens. Recirculating Aquaculture Systems (RAS) have increased significantly in the last 10 years, RAS is expected to surpass flow-through systems in the next 5 years. Current production protocols favor high temperatures, the use of continuous light, and increased salinity to accelerate growth, where production of larger post-smolts (500-600 g) is becoming a common practice. Even though RAS is one of the most common production systems, there is a lack of standardized protocols, and the biological requirements of the fish are not always considered. Thus, optimizing the rearing strategies in RAS is crucial for producing robust (post-) smolts with better performance after seawater transfer. This study aims to understand the implications of different RAS protocols, in terms of photoperiod, salinity, and producing large post-smolts, on plasma metabolites related to energy status and expression of genes codifying the gut signals regulating the appetite and food intake and peptide transporters in Atlantic Salmon (*Salmo salar*).

Materials and methods

Atlantic salmon (113 g) was reared in RAS at 12 °C and different photoperiod regimes simulating no winter (NW, constant light 24L/0D), early winter (EW, standard winter signal 12L/12D and spring signal 24L/0D), late winter (LW, delayed winter signal of 12L/12D, normal spring signal of 24L/0D), and late long winter (LLW, prolonged winter signal of 12L/12D and standard spring signal 24L/0D); two salinities (fresh- (FW) and brackish (BW) water); and two sizes at seawater transfer (300 and 800g). Fish were sampled before each transfer size. Pentra 400 was used to measure the metabolites related to energy status (glucose, triglycerides, cholesterol, and total protein) in the plasma. Expression of genes coding for gut appetite regulator hormones (cholecystokinin; *cck*, and neuropeptide Y; *pyy*) and peptide transporters (PepT1; *slc15a1a*, *slc15a1b*, and PepT2; *slc15a2a*, *slc15a2b*) were quantified in the anterior and posterior intestine, using the $\Delta\Delta C_T$ method. Data were subjected to Two-way ANOVA, followed by Tukey ($p < 0.05$).

Results

Photoperiod and salinity significantly affected the metabolites in 300 g fish, with NW showing generally higher levels compared to EW and LW. On the other hand, neither photoperiod nor salinity had a significant effect on the metabolites in 800 g fish, except for triglycerides (Fig. 1). Expression of *pyy* was unaffected by the addressed parameters (data not shown). We are currently analyzing the *cck* results that will be presented at the conference. At 300 g, the photoperiod regime was the only factor significantly affecting almost all peptide transporters. At 800 g, on the other hand, only *slc15a2a* was affected by the photoperiod. Expression of *slc15a1* showed an approximately opposite trend to *slc15a2*; the former was upregulated in NW and EW, while the latter was in LW (Fig. 2).

Discussion and conclusion

Higher variations in the metabolite levels in 300 g fish might be because they have recently gone through the energy-demanding process of smoltification and are still adjusting their energy budget. While at 800 g, they have had more time to acclimatize to the environmental conditions. In agreement with this, the highest triglyceride levels were observed in NW and EW. This might suggest triglycerides as a more sensitive indicator of energy status in larger smolts. While plasma provides a good idea of the amount of ready-to-be-used energetic substances, it would be interesting to study the hepatic triglycerides and glycogen reservoirs to obtain a completer picture.

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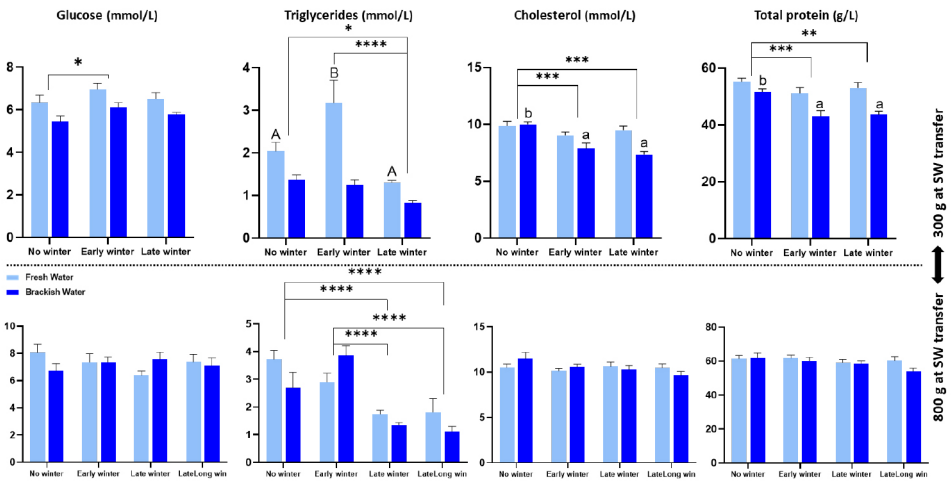

Fig. 1. Metabolic status in the plasma of Atlantic salmon in RAS under different photoperiod regimes, water salinities, and two sizes at sea transfer. Asterisks indicate significant differences among photoperiod regimes. Upper- and lower-case denote significant differences within FW and BW, respectively.

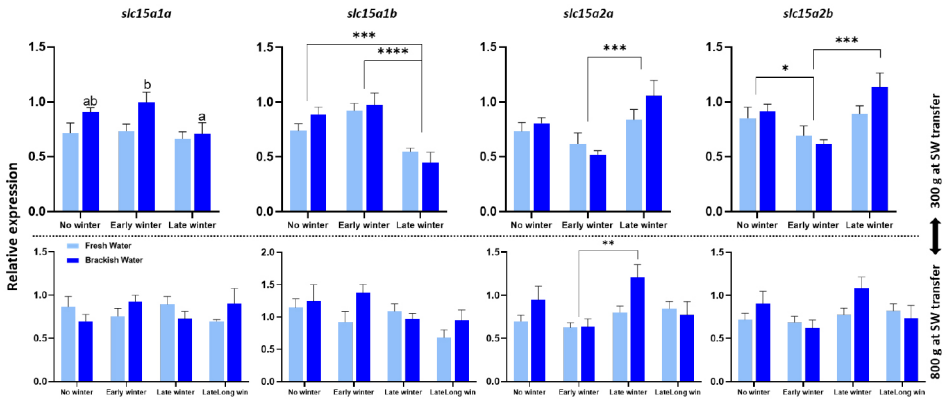

Fig. 2. Relative expression of genes codifying peptide transporters in the anterior intestine of Atlantic salmon. The rest of the legend is as in Fig. 1.

The opposite trends in the expression of members of PepT1 and 2 may point to different physiological roles (nutrient absorption and/or molecule sensing) of these groups, differentially responding to environmental conditions (Gomes et al., 2020). Short-term starvation of Atlantic led to the downregulation of *slc15a1* and *b,* while the upregulation of *slc15a2a* and *b* (Del Vecchio et al., 2021). Similarly, in our study, LW with the lowest energetic substances in the plasma, showed the lowest expression levels for *slc15a1b*, and the highest for *slc15a2a* and *b*.

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EFFECT OF FISHMEAL REPLACEMENT WITH SINGLE CELL PROTEINS *Candida* sp. AND *Nannochloropsis* sp. ON RAINBOW TROUT *Oncorhynchus mykiss* OSMOREGULATORY AND STRESS RESPONSES

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Introduction

The continued expansion of the aquaculture industry requires a sustainable supply of nutrients for aquafeeds. Single-cell protein (SCP) results from the culture of monocellular microorganisms such as yeast, bacteria, or microalgae. SCP are a promising avenue for ingredients in aquafeeds due to their high protein content and suitability for circularity through valorising side stream from agro-industrial activities (Sharif et al., 2021). Although growth and digestibility are important when assessing the suitability of novel alternative feed ingredients, comparatively understudied physiological aspects (stress resilience, health and welfare) are equally critical. This study assessed the effect of fishmeal (FM) replacement by yeast (*Candida utilis*) and microalgae (*Microchloropsis gaditana*) on stress and osmoregulatory responses in rainbow trout.

Materials and methods

Rainbow trout (initial body weigh approx. 185 g) were allocated between 4 isonitrogenous and isoenergetic diet treatments (in triplicate tanks) in freshwater RAS: *Diet 1)* control, 15 % FM and 40 mg. kg^{-1} astaxanthin, *Diet 2)* 5 % yeast (33 % of FM replacement) and 40 mg. kg^{-1} astaxanthin, *Diet 3)* 5 % microalgae and 40 mg. kg^{-1} astaxanthin, *Diet 4)* 5 % microalgae and 20 mg. kg^{-1} astaxanthin. During the experiment (57 days) water temperature, stocking density, and photoperiod were 14.8 °C, 29.4 kg. m^{-3}, and 12:12 L/D. At the end of the trial, 18 fish from each group (N=6 per tank) were sampled, from which 9 were immediately euthantised by anesthesia overdose (pre-stress) and the other 9 were subjected to acute stress challenge (ACT, i.e., 15 minutes of crowding at 300 kg. m^{-3}, followed by 45 minutes of recovery before euthanasia). Plasma levels of cortisol, using ELISA, ions (e.g., sodium, magnesium, and chloride) and metabolites related to the energy status (e.g., lactic acid) were measured using Pentra 400. Expression of genes involved in stress response (mineralocorticoid receptor; *mr*, glucocorticoid receptors; *gr1* and *gr2*) and neural plasticity (brain-derived neurotrophic factor; *bdnf*, neurogenic differentiation factor; *neurod*) were measured in the telencephalon, using RT-. Data were subjected to One-way ANOVA followed by Tukey to compare among diets, and to Student's t-test to compare between the two stress states ($p < 0.05$).

Results

Fish final average body weight was around 490 g. Diets incorporating SCPs exhibiting a reduction in SGR ranging from 5 % to 7.8 % along with an elevation in FCR by 2.3% to 4.7 %. However, Diet 4 was associated with low cortisol levels following the ACT, effects not seen for any of the other treatments. Additionally, the telencephalic expression of cortisol receptors in the pre-stress state *mr*, *gr1* (data not shown), and *gr2* were the lowest in Diet 4, while the expression of these markers was significantly higher following ACT. Regarding gene markers related to telencephalic neural plasticity (*bdnf* and *neurod*), although no significant differences were observed among treatments, Diet 4 again was the only group with significantly higher expression after ACT (Fig. 1).

The plasma ion levels of sodium were similarly more elevated following the ACT. However, in the case of magnesium and chloride, this elevation was statistically significant only in the case of Diet 4. Also, plasma levels of these ions and lactic acid were consecutively higher in Diet 4 at a pre-stress state, while magnesium showed reduced levels in response to the ACT (Fig 2).

Discussion and conclusion

Reduced levels of astaxanthin in Diet 4 were associated with changes in filet coloration (data not shown), as well as disturbances to the fish stress response during the ACT. Although cortisol responses were less clearly affected, downregulation of cortisol receptor genes (*mr* and *gr*) suggests cortisol induced overstimulation as a preservation mechanism to neural integrity (Vindas et al., 2017). Likewise, upregulation in neural plasticity markers (*bdnf* and *neurod*) in stressed ACT fish from Diet 4 suggests a larger effort needed to cope with the challenge and consistent with the presence of mild chronic

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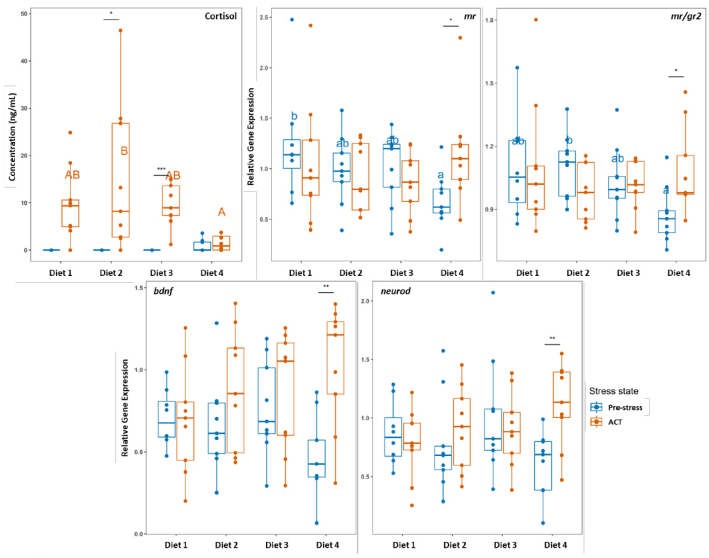

Fig. 1. Plasma cortisol levels and expression of genes related to stress (*mr* and *mr/gr2*) and neural plasticity (*bdnf* and *neurod*). Lower- and upper-case letters denote significant differences among diets for pre-stress and ACT, while asterisks indicate significant differences between the two stress states within each diet (*, **, ***, **** denote $p < 0.05$, 0.01, 0.001, and 0.0001, respectively).

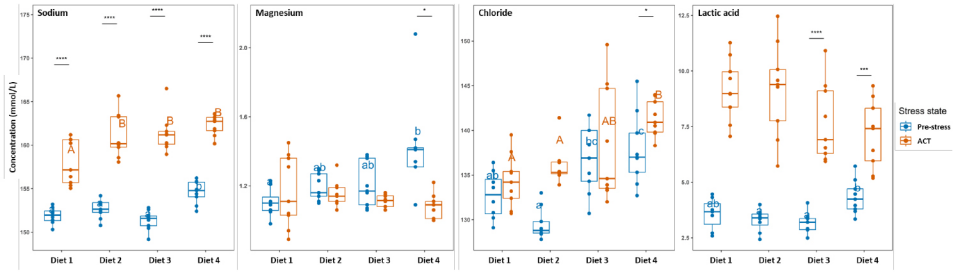

Fig. 2. Plasma levels of ions (sodium, magnesium, and chloride) and metabolites related to energy status (lactic acid, glucose, and creatinine enzyme). The rest of the legend is as in Fig. 1.

stress. This was consistent with the higher plasma ion and lactic acid concentrations observed in basal fish prior to the ACT that suggested elevated osmoregulatory and metabolic challenges even before the ACT. This is consistent with fish on Diet 4 that had received reduced astaxanthin levels being subjected to regulatory disturbances in physiology and having a hindered ability to cope with added stressors.

These results indicate that SCPs is a suitable dietary substitute for FM in trout without negatively impacting fish health and welfare, provided dietary requirements for astaxanthin are sustained, as the carotenoids naturally present in the *N. gaditana* do not seem to adequately fulfil the fish requirements. Besides coloration, astaxanthin is known to have anti-inflammatory and stress-reducing properties consistent with the poorer responses seen in fish exposed to Diet 4 (Lim et al., 2018).

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Acknowledgments

Project funded by EU Horizon 2020 - iFishIENCi (818036). Most analysis performed in the FBA high throughput state of the art lab (SacLab), NORCE, Bergen, Norway.

AQUA-FAANG FUNCTIONAL ANNOTATION OF SALMONID GENOMES

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Introduction

Genomic functional annotation goes beyond genes to describe regulatory functions of the genome that control genes and underpin genotype-to-phenotype determination. Such information is crucial for strategic improvement of farmed animals through genomic selection. Aquatic species however have limited functional annotations available to date. There is international initiative through the 'Functional Annotation of Animals Genomes' (FAANG) project (Giuffra et al. 2019) to deliver quality functional annotations of farmed species. The AQUA-FAANG project (https://www.aqua-faang.eu/) extends this initiative to commercially important fish species for aquaculture, including the salmonid fish Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). This project produced multi-omic assay data for several species for integration to deliver functional annotations of their genomes. Here we describe the standardized bioinformatic workflows implemented to produce from nearly a thousand matched RNA-Seq, ATAC-Seq, and ChIP-Seq assays, functional annotations of Atlantic salmon and rainbow trout genomes. We showcase the quality of assay data and resulting annotations, and how this data may be publicly accessed.

Methods

Multi-omic assay data for Atlantic salmon and rainbow trout was generated through standard protocols development by AQUA-FAANG. This included five stages of embryogenesis and a common panel of tissues (brain, gill, liver, muscle, ovary, and testis) from males and females at sexually immature and mature stages. This produced datasets for mRNA-Seq, ATAC-Seq, and ChIP-Seq (H3K27ac, H3K4me1, H3K4me3, H3K27me3 histone marks). Primary analysis of sequencing data utilized nf-core pipelines (Ewels et al 2020); rnaseq, atacseq, and chipseq pipelines respectively, to align read data to reference genomes. Custom blacklist regions from ChIP-Seq input controls removed signal artifacts from downstream analysis. We used ChromHMM (Ernst and Kellis, 2012) to model combinations of signals of ATAC and ChIP marks to segment the genomes into annotations of chromatin states, for each sample type and species.

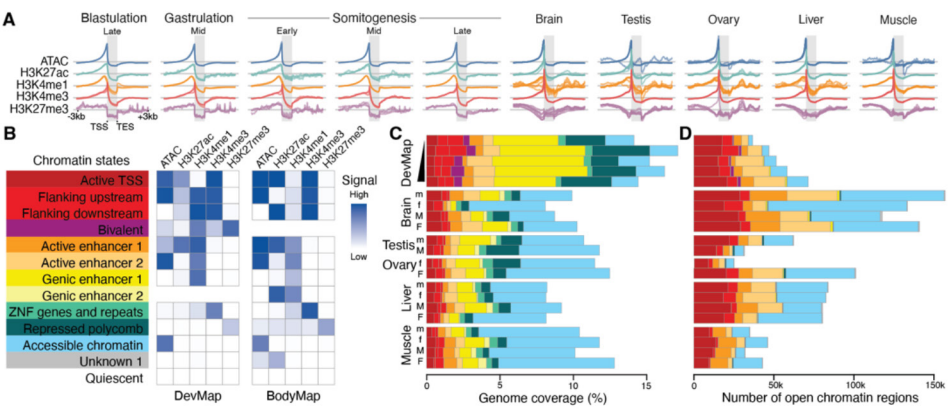

Figure 1. Overview of salmonid functional annotation.

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Results

Quality analysis of assay data showed the success of the protocols. Once such metric was the enrichment of ATAC and ChIP mark reads, showing active regulation of genes, enriched around the transcription start sites (TSS) of genes, with the exception of the gene repressor mark H3K27me3 (Figure 1A). We assigned distinct functional labels to the chromatin states we annotated (Figure 1B), including active and bivalent promoters, active, primed and poised enhancers, polycomb repressed regions, and unmarked open chromatin. Functional labeling of states was based on their combination of ATAC and ChIP mark signals, as well as other statistics such as coverage of genomic features, compared to literature on functional features. We observed some overall patterns in regulatory regions across the genome. We report on the genomic coverage of different states for different sample types (Figure 1C). Developmental stages had more promoter and enhancer activity that adult tissues. Genomic coverage of regulatory elements was similar across different tissues. Looking at distinct numbers of reproducible ATAC peaks of open chromatin, and the chromatin states of those regions (Figure 1D), we saw progressively more active regulatory regions across early development. Brain contained the most active regulatory regions of any tissue. We saw distinct different in mature and immature ovaries and testis.

(**A**) Distribution of reads from assays around all gene regions in the Atlantic salmon genome. Each sample is plotted per developmental stage or tissue. The range is 3kb upstream of gene transcription start site (TSS) to 3kb downstream of transcription end site (TES). All gene regions are normalized to the same length. The height of lines show the relative abundance of reads in the range, scaled per sample for a mean of zero. (**B**) Chromatin states annotated from modeling assay data. Two models for Atlantic salmon developmental stages (DevMap) and tissues (BodyMap) show 12 states with associated intensity of assay signal from high (blue) to low (white), and the associated label of chromatin state to the left. (**C**) Proportion of the Atlantic salmon genome covered by each chromatin state for developmental stages and tissues, excluding states unknown and quiescent. Tissues are separated into immature/mature male (m/M) and female (f/F) samples. (**D**) The number of robust, reproducible open chromatin regions from ATAC-Seq and their underlying chromatin states for the same conditions as (C).

Discussion

We present the functional annotation data to aid future efforts into precision breeding of desired trails of these salmonids for aquaculture. The protocols and workflows of the annotations will also be published to aid additional similar functional annotation of other important species. The functional annotations presented here were further analysed by will be showcased by co-authors through other oral and posters presentations at this conference.

Acknowledgements

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AQUATOR - THE BUSINESS ACCELERATOR FOR THE BLUE BIOECONOMY

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The potential of the sustainable use of aquatic resources is far from exhausted in Germany. Germany has a high level of education, high purchasing power and a well-developed awareness of the environment and sustainability. Nevertheless, the degree of selfsufficiency for aquatic products in Germany is only 25%. A lot of research is done here in the field of aquaculture and product development from aquatic resources. The challenge, however, is that the research results find their way into products and services of our everyday lives.

The AQUATOR is a business accelerator aiming at developing, accompanying and supporting entrepreneurial commitment. The AQUATOR was launched as one of eight sub-projects in the BMBF-funded innovation space “Blue Bioeconomy”. The team consists of different experts from a total of six universities, institutions and companies. We provide tailor-made advice and services in various subject areas, such as aquaculture, algae expertise, environmental balances, toxicology and socio-economic aspects. We are well connected to the authorities and a large, trust-based network; thus, we are able to overcome hurdles more quickly and create synergies.

After just two years, we are supporting more than 10 startups and companies of all kinds. For example, we offer access to scientific partners and to specific biomass, conduct scientific tests at laboratories with corresponding toxicological analyzes, organize access to relevant authorities, and support with scientific expertise and access to funding for the development of new products based on residual materials.

Our vision is a large number of companies in the field of the blue bioeconomy that support each other and are both economically and ecologically sustainable. The national bioeconomy strategy, launched in 2020, has six main goals. The AQUATOR contributes to each of these goals by supporting start-ups and companies. The focus of the AQUATOR is currently in northern Germany. In the long term, however, we want to support the entire industry as a central player and expand both nationally and internationally. In doing so, we rely on targeted and broad networking in order to be able to deal with the unique challenges of the aquatic bioeconomy.

The AQUATOR is currently a project funded by the BMBF. We are in a phase of restructuring and consolidation towards a suitable company form in order to be able to function more independently of project funds in the long term.

INTEGRATING SALICORNIA AND FLATHEAD GREY MULLET FARMING IN MARINE AQUAPONIC SYSTEMS

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Introduction

Nowadays, the principle of sustainability is widely used by practically all the economic sectors and most of activities presume to be sustainable. Sustainability is not a final condition nor an achievable goal; sustainability is a way ahead and a way of economic and social development. Under this scenario, sustainable strategies for improving blue food economies are essential to design a new approach for transitioning towards more responsible, comprehensive, exploitable, and favourably positive impact-generating food production systems. Among them, aquaponics presents an innovation in traditional aquaculture systems by combining fish farming and hydroponics (the soilless cultivation of plants). Potential advantages of aquaponics include enhanced sustainability, less resource consumption, and fewer environmental impacts compared to conventional aquaculture and agriculture practices (Baganz et al., 2022). The objective of this study was to evaluate the farming of *M. cephalus* in an aquaponic marine coupled system and compared to a conventional recirculating aquaculture system (RAS) in terms productivity, farming costs, fish performance and operational welfare indicators (OWIs). This approach will double the sustainability of aquaculture farming practices by testing a low-trophic fish species like *M. cephalus* in an aquaponic system in order to improve the sustainability of this practice by using the nutrient waste from the fish culture system as a nutrient source for growing the halophyte *Salicornia spp*.

Materials and Methods

Two different trials were conducted in this study. In a first one, the assay was set-up for testing the viability of growing salicornia in combination with a low trophic species under seawater conditions (35 ppt) in a coupled aquaponic system. We used three independent aquaponic systems in which we grew-up 100 flathead grey mullet fish (50-52 g in BW) per tank at an initial stocking rate of 3.5 kg/m^3 that was connected to the plant unit that contained wild seedlings of Salicornia spp. in 6.4 m^2 (141-145 of plants per unit). The second trial consisted of rearing flathead mullet in conventional RAS unit. For this purpose, 381 fish (30-50 g in BW) were evenly distributed among the three experimental tanks connected to the RAS unit (127 fish per tank; initial stocked biomass = 2.5 kg/m^3).

In all trials, fish were fed the same commercial diet (Nutra MP, Skretting; 55% crude proteins, 17.5% crude lipids). Fish were fed a feed ration of 2.5% of the stocked biomass using automatic belt feeders. In both trials, water quality parameters (temperature, oxygen, salinity) were measured daily, whereas nitrogenous compounds in water (ammonia, nitrites, and nitrates) were measured twice a week by our technicians. At the end of the trial, all animals were measured in length and BW, the apparent feed conversion ratio was determined, and the aquaponics plant yield (APY, kg/m^2/year) was calculated taking into consideration the yield of salicornia per m^2 in each aquaponic unit and the duration of the experimental period. Operational welfare indicators (OWIs) related to skin and fin conditions were recorded and compared between both farming systems.

Results and Discussion

In aquaponic systems, the increase of stocked fish biomass grew from 3.5 kg to 10.7 kg per tank (9.8 - 12.2 kg/tank), growth that was coupled with an increase in the stock density, values that increased from 3.5 kg/m^3 to final stocking values that ranged from 6.5 to 8.1 kg/m3. This increase in biomass was a result of an increase in the average individual weight of flathead grey mullets that reached 183.7 to 195.3 g (SGR = 0.94-0.99% of increase in body weight per day). Mortality rates in all three aquaponic systems was lower than 5% (98.4± 1.5%). Regarding the yield of salicornia, plants in all systems grew very well with survival rates higher than 95%. The final yield (plant biomass) per aquaponic unit ranged from 43.1 to 50.1 kg (6.7-7.8 kg/m^2). The increase in plant biomass from the beginning to the end of the trial ranged from 290 to 346 times. Flathead grey mullet reared on RAS units grew linearly from 39.2 g to 143.6 and 145.1 g, depending on the tank considered (Figure 16). Values of SGR were similar among the three replicate tanks (1.1% BW/day) and apparent FCR

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values ranged from 2.1 to 2.3. Fish survival was 96.1 ± 3.1%. The analysis of the OWIs of flathead grey mullet reared in the coupled marine aquaponic system and RAS revealed that this species adapted very well to both farming systems since no moderate or severe lesions were observed in animals. Most of the alterations were considered as very mild (disarrangement of fins) or loss of few scales over the skin. The loss of scales may not be attributed to rearing conditions since this species is very sensitive to handling and generally, some scales are lost when fishing, sedating, and measuring fish.

The energy cost (kW) for running each aquaponic unit based on the consumption of the water pump (0.1 kW/h/unit) and air blower (0.3 kW/h/unit) for the duration of the trial (142 days) was estimated at 340.8 kW/unit for the water pump 1,022.4 kW/unit. In total, each aquaponic unit consumed 1,363.2 kW, which represented an energy cost per kilogram of salicornia of 0.029 ± 0.002 kW/kg. In contrast, the electric costs of the RAS unit composed of a water pump, air blower, UV lamps and heat exchanger was 1.0 kW/h, 2.5 times higher than that for the coupled aquaponic system.

Conclusion

Flathead grey mullet showed good adaptability to farming conditions in RAS and aquaponic systems, even though the species is more difficult to handle and acclimate to intensive rearing conditions than initially expected. Fish performed similarly in terms of growth performance, FCR and OWIs between both tested farming systems. Coupled aquaponic systems are a sustainable strategy for the combined production of fish and salicornia with a minimal use of water (<5% of water renewal), an efficient use of land and water, and a reduced cost in terms of electricity (aquaponics are 2.5 times cheaper in running costs than RAS units). Furthermore, growing plants coupled to fish rearing provides a sustainable economic profit to the fish farmer by producing a high quality fresh product to the consumer. Present results indicated that this technology might be applied to commercial, or community based urban food production, industrial scale production in rural areas, small scale farming in developing countries (backyard aquaculture systems) or as systems for education.

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Microchloropsis gaditana, *Schizochytrium* sp., *Phaeodactylum tricornutum*, AND *Tisochrysis lutea* AS n-3 PUFA SOURCES IN THE DIET OF JUVENILE GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

In recent years microalgal biomass has become one of the most promising sources of bioactive compounds in aquafeeds, especially in terms of lipids and fatty acids (Soto-Sánchez et al. 2023). Among the several species *Microchloropsis gaditana*, *Phaeodactylum tricornutum*, *Schizochytrium* sp. and *Tisochrysis lutea* are of great interest to aquaculture with the first two well known as source of EPA and the last rich in DHA. That being the case, a dietary combination of these four species could potentially substitute fish oil in fish diets satisfying the essential fatty acids requirements. Ergo, the aim of this study was to evaluate the fish oil substitution by different blends of these four microalgal species in the diet of Gilthead seabream (*Sparus aurata*).

Materials and Methods

Juvenile seabreams of 8.77±0.01 g initial mean weight were obtained from a commercial fish hatchery and distributed after an acclimatization period of 15 days in triplicate to 18 closed seawater circulation system tanks (125L) (27 individuals/tank, 3 reps/dietary group). The groups were fed six different isoenergetic (21 MJ/Kg), isonitrogenous (48% CP) and isolipidic (15.5%) diets that satisfied the EPA+DHA requirements of the species (>1.8% of diet). The control diet (C) contained 8% fish oil, 4% soybean oil and 25% fishmeal resembling a commercially available seabream diet. Four other diets were formulated replacing 50% of the dietary fish oil of the control diet by a blend of microalgae biomasses of the species: *Schizochytrium* sp. and *M. gaditana* (SM), *Schizochytrium* sp. and *P. tricornutum* (SP), *P. tricornutum* and *T. lutea* (PT) and *M. gaditana* and *T. lutea* (MT). A sixth diet was also used as a reference containing 12% of fish oil as the sole dietary oil (FO). The inclusion level of each microalgae contributed a certain amount of proteins in the diet and as such fishmeal protein was also subsequently substituted. Fish were hand-fed to apparent satiation twice a day for 11 weeks.

Table 1. Growth performance and feed utilization of *S. aurata* fed with the experimental diets.

Parameters / dietary groups	C	FO	SM	SP	PT	MT
Final weight (g/fish)	35.93±0.87^{a}	35.40±3.53^{a}	33.81±2.06^{ab}	30.83±2.86^{ab}	27.98±1.29^{b}	32.27±2.49^{ab}
Feed intake (g/fish)	33.39±0.80^{ab}	34.43±0.67^{a}	34.04±0.89^{a}	30.80±1.18^{bc}	29.17±1.10^{c}	33.10±1.29^{ab}
Weight gain (g/fish)	27.17±0.85^{a}	26.64±3.53^{a}	25.03±2.07^{ab}	22.06±2.86^{ab}	19.22±1.30^{b}	23.50±2.50^{ab}
SGR (%/day)	1.79±0.03^{a}	1.76±0.13^{a}	1.71±0.08^{ab}	1.59±0.12^{ab}	1.47±0.06^{b}	1.65±0.10^{ab}
FCR	1.23±0.01^{b}	1.31±0.16^{ab}	1.36±0.09^{ab}	1.41±0.14^{ab}	1.52±0.06^{a}	1.42±0.11^{ab}
Survival (%)	100.0±0.00	100.0±0.00	98.77±2.14	98.77±2.14	100.00±0.00	98.77±2.14
Hepatosomatic Index (%)	1.41±0.14	1.35±0.14	1.08±0.21	1.19±0.08	1.25±0.22	1.24±0.17
Viscerosomatic Index (%)	7.58±0.47	7.20±0.14	6.57±0.42	7.55±0.84	7.67±0.40	7.42±0.07
Condition factor	1.38±0.04	1.33±0.02	1.36±0.02	1.38±0.01	1.34±0.03	1.35±0.02

Note. Values represent means ± standard deviation of triplicates. Values within each row not sharing a common superscript letter are significantly different ($P < 0.05$).

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Results and Discussion

The SM, SP and MT groups of fish had similar final body weight, weight gain, SGR and FCR with both control groups (C, FO) (Table 1) indicating that dietary fish oil can be partially replaced by the blends of *M. gaditana* and *Schizochytrium* sp., *Schizochytrium* sp. and *P. tricornutum*, *M. gaditana* and *T. lutea* without impairing the growth of *S. aurata* nor feed efficiency. A growth retardation occurring in the PT-fed fish was due to their lower feed intake, which denotes a lower acceptability of either *P. tricornutum* or *T. lutea* by *S. aurata*. A lower feed intake was also obvious in the SP group but not in the MT group, implying that the inclusion of *P. tricornutum* was probably the responsible factor for this decrease.
Up to date, the strategy of mixing different microalgae species to balance dietary fatty acids and to replace dietary fish oil has been scarcely studied. The blend of *Microchloropsis sp.* and *Schizochytrium* sp. has been previously proven successful as a fish oil replacement for *S. aurata* (Karapanagiotidis et al. 2022) as well as for other species (Qiao et al. 2014; Seong et al., 2021., Sarker et al., 2020a). A blend of *Tisochrysis lutea* with *Tetraselmis suecica* successfully replaced 36% of dietary fish oil in *Dicentrarchus labrax* without adversely affecting fish growth performance (Cardinaletti et al. 2018). Sarker et al. (2020) using different combinations of *Microchloropsis* sp., *Isochrysis* sp., and *Schizochytrium* sp. in the diet of *Oncorhynchus mykiss* reported that *Schizochytrium* sp. and *Isochrysis* sp. are good candidates for DHA supplementation and that the latter is better than *Nannochloropsis* sp. as a substitute for fish oil.

The present study showed that blends of specific microalgae species is a promising strategy for further fish oil replacement in the diet of *S. aurata*, that in turn can promote a more sustainable aquaculture production. Certainly, the effectiveness of such dietary manipulation for increasing the n-3 fatty acids in fish tissues should be further investigated.

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EFFECT OF FISHMEAL REPLACEMENT BY *Chlorella vulgaris* ON GROWTH PERFORMANCE OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Aquaculture sector is on a continuous search for suitable and sustainable protein sources that could successfully replace fishmeal in aquafeeds. Microalgal biomass can serve this need due to their high protein contents and low environmental impact (Glencross et al. 2020). *Chlorella vulgaris* is the most common microalgae species that can be easily grown in outdoor facilities, but still has not received adequate attention in fish nutrition. Thus, the aim of this study was to evaluate the dietary fishmeal replacement by graded levels of a *C. vulgaris* meal on the growth performance and feed utilization of an important Mediterranean farmed species, the gilthead seabream (*Sparus aurata*).

Materials and Methods

A total number of 360 *S. aurata* juveniles of 2.6 g initial mean weight were stocked at 12 tanks (125L) in a closed seawater recirculation system. Fish in triplicate groups (30 fish/tank, 3 tanks/dietary group) were fed to satiety, twice a day, four isonitrogenous (46%) and isoenergetic (21 MJ/Kg) diets differing in the inclusion level of a *C. vulgaris* (CV) meal: CV0 diet contained fishmeal at an inclusion level of 200 g/Kg and zero levels of CV, while in the rest diets the CV meal was included at 48 g/Kg (CV48), 72 g/Kg (CV72) and 240 g/Kg (CV240) corresponding to 20%, 30% and 100%, respectively of fishmeal protein replacement. After 60 days of feeding, fish were weighted to measure growth and feed utilization parameters. Data were analysed using one-way ANOVA and differences between means were determined by Tukey's multiple-range test (P=0.05).

Results and Discussion

Survival was high and similar among the groups (Table 1). Fish fed the diet with the highest inclusion level of *C. vulgaris* meal (CV240) had significantly lower feed intake (g/fish) compared to the rest groups denoting a lower palatability of *C. vulgaris* meal compared to fishmeal. This in turn has led to a reduced (P<0.05) fish growth performance in terms of final weight, weight gain and specific growth rate (SGR) in this group. Additionally, the increased feed conversion ratio (FCR) and decreased protein efficiency ratio (PER) denoted that *C. vulgaris* protein was not efficiently metabolized when totally replaced dietary fishmeal. On the other hand, fish growth performance and feed utilization were not significantly impaired in CV48 and CV72 groups. These findings suggest that *C. vulgaris* meal can successfully replace a part of fishmeal protein if it is used in low inclusion levels up to 78 g/Kg in the diet. The later was the maximum tested level that corresponded to 30%

Table 1. Growth performance and feed utilization of *S. aurata* fed with the experimental diets.

Parameters / dietary groups	CV0	CV48	CV72	CV240
Survival (%)	100.0±0.0	96.7±3.3	96.7±3.3	95.6±3.8
Feed intake (g/fish)	12.18±0.57^{a}	12.70±0.30^{a}	11.89±0.13^{a}	10.31±0.78^{b}
Final weight (g/fish)	12.94±0.61^{a}	13.16±0.89^{a}	11.57±0.70^{ab}	9.48±1.21^{b}
Weight gain (g/fish)	10.35±0.61^{a}	10.56±0.89^{a}	8.97±0.70^{ab}	6.88±1.21^{b}
SGR (%/day)	2.68±0.08^{a}	2.70±0.11^{a}	2.49±0.10^{ab}	2.15±0.21^{b}
FCR	1.18±0.05^{b}	1.21±0.08^{b}	1.33±0.10^{ab}	1.52±0.16^{a}
PER	1.89±0.07^{a}	1.83±0.12^{a}	1.64±0.12^{ab}	1.47±0.16^{b}

Note. Values represent means ± standard deviation of triplicates. Values within each row not sharing a common superscript letter are significantly different (P < 0.05).

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fishmeal protein replacement. This agrees with the findings of our previous study with the same species (Karapanagiotidis et al. 2022) where 30% fishmeal protein replacement was achieved by *C. vulgaris* meal, but that level corresponded to a much higher inclusion of CV in the diet (190 g/Kg).

C. vulgaris meal has been successfully used to partially replace fishmeal protein (FM) in diets of other fish species such as *Sciaenops ocellatus* (9.4 g/Kg dietary inclusion replacing 5% of FM, Patterson and Gatlin 2013), *Oreochromis niloticus* (171 g/Kg replacing 50% of FM, Badwy et al. 2008) and of crustaceans such as *Macrobrachium rosenbergii* postlarvae (80 g/Kg replacing 24% of FM, Maliwat et al. 2017). A total FM replacement was achieved in *Litopenaeus vannamei* (389 g/Kg dietary inclusion, Pakravan et al. 2017), while CV meal had even a growth promoting effect by totally replacing FM in *Pontastacus leptodactylus* (410 g/Kg dietary inclusion, Safari et al. 2022) and in *C. auratus* (711 g/Kg dietary inclusion, Shi et al. 2016). The present findings could help the aquaculture of gilthead sea bream to further decrease the dietary fishmeal levels by *C. vulgaris* meal towards more sustainable aquafeeds.

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BIOFIA - BIOINDICATORS FOR THE FARMING, HEALTH, AND PRODUCT QUALITY OF FISH IN AQUACULTURE

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Introduction

The transformation of aquaculture into urban regions and the integration of highly efficient automated fish aquaculture systems into agricultural and industrially circulation systems is progressing rapidly. Thus, fish aquaculture is now an integral part of the blue bioeconomy. One problem with this development is the insufficient knowledge of fish physiology, behavior, and the species-specific demands, which is required to improve animal health and welfare in new farming systems and increase harvesting gains. Research priorities of the project are therefore the monitoring of fish and the development of new animal-based welfare parameters to assess and improve fish performance and product quality. The analyses of species-specific molecular indicators (Rebl et al., 2020), including stress hormones (Seibel et al., 2021), and digital measurement technologies newly developed in the project allow the assessment of stress and health of salmonids in different rearing phases. They thus contribute to improve husbandry conditions in farming facilities and to optimize aquaculture processes. The project explores the welfare of Atlantic salmon during the rearing process using molecular methods and AI-based monitoring. The analyses conducted provide baseline data on the effect of dietary additions of microalgae of the genera Arthrospira, Chlorella, Schizochytrium, and Tetraselmis on animal welfare and product quality of the fish under the influence of stressors such as a salinity change from 12‰ to 32‰ or after a common sanitization of the farm water with peracetic acid. The research is closely related to the development of novel microalgae feeds (Michl et al., 2019; Microganic GmbH), detection of microbial patterns and their nitrification efficiency in recirculating systems (Hüpeden et al., 2020), parasitological and bacteriological studies (Unger & Palm, 2016), and testing the product quality of fish as human food (Molkentin et al., 2015).

Results

We used a set of putative biological markers testing the welfare of salmon (Muilekom et al., submitted; Müller et al., submitted). At the molecular level, we detected no negative effects after feeding any of the above microalgae diets (Müller et al., submitted, and 2023). Furthermore, we developed two AI-based neural networks (object-detection and keypoint-detection) for AI based fish monitoring (MonitorFish GmbH). No parasites affecting the results were detected in the fish. Our data show that the turnover rates of ammonia and nitrite oxidizing bacteria were sufficient to prevent accumulation of toxic ammonia and nitrite (Malinowski et al., 2023) and no notable negative impact of diets and stressors on the microbial patterns in recirculating systems, nor on tested fish fillet quality could be observed. With the Schizochytrium diet (SL 14%), the fish fillet quality improved. The synergistic summary and provision of all subprojects on the design of salmon-specific molecular biochips together with all other technologies used, can contribute to fish-based biological monitoring and thus to the testing of optimal farming conditions in aquaculture.

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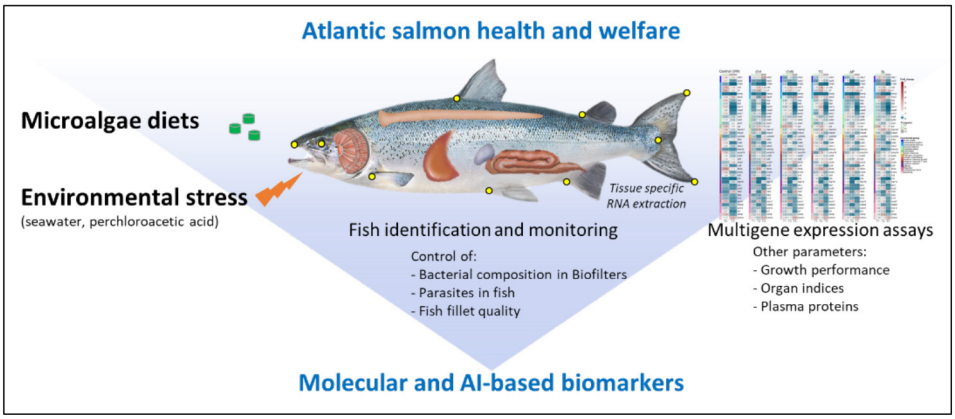

Fig. 1 Illustration of the comprehensive monitoring approach of the project.

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THE IMPACT OF HEMP SEED OIL (*CANNABIS SATIVA* L.) ON GROWTH AND HEALTH OF GILTHEAD SEABREAM

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Introduction

Hemp seed oil (HSO), a derivative with notable health benefits and high nutritional value has gained global attention for its positive effects. Extensive research has explored incorporating *Cannabis sativa* in livestock diets (Fallahi et al., 2022). This study investigates HSO's potential as a growth and health enhancer in marine fish like gilthead seabream, a significant European species. The aim of the present study is to showcase the aquaculture industry's beneficial utilization of medicinal plants, as a growth and health enhancer in marine fish like gilthead seabream, a significant Mediterranean species.

Materials and methods

280 gilthead seabreams (*Sparus aurata* L.) with an average weight of 4.91±0.05g were kept in 9 glass tanks (125L). Over 74 days, they were hand-fed to satiation. Diets were formulated with 535 g/kg crude protein and 21.3 MJ/kg energy, including 1% and 2% HSO-replaced soybean oil and control (CTRL) diets. After the trial, three fish per tank were euthanized (300 mg/L MS-222) for blood collection, cortisol levels, haematological parameters, and gut and liver histological examination. Body composition, fatty acid profile, and DNA damage (Comet Assay) in the liver, spleen, gut, and kidney were analyzed from three fish, while growth parameters were assessed.

Results

The inclusion of HSO in fish diets had limited impact on the examined parameters, except for ash content, which was notably lower in the CAN2% group compared to the control (Table 1). While total saturated fatty acids (SFA) remained unaffected, specific SFAs like 18:0, 20:0, and 22:0 increased in the CAN1% groups versus the control. Palmitic (16:0) and stearic acid (18:0) were the primary SFAs present, with myristic acid (14:0) in smaller amounts across all groups. Total monounsaturated fatty acids (MUFA) showed no significant change. Additionally, CAN1% groups displayed reduced levels of total n-6 polyunsaturated fatty acids (PUFA). Linoleic acid (LA, 18:2n-6) significantly increased in CAN2% groups compared to CAN1% groups. Arachidonic acid (ARA, 20:4n-6) exhibited higher levels in CAN1% compared to the control. Total n-3 PUFA remained unchanged in both CAN1% and CAN2% groups. Levels of a-linolenic acid (LNA, 18:3n-3) were significantly higher in CAN2% compared to the CAN1% group, while 20:5n-3 (EPA) and 22:6n-3 (DHA) were not significantly affected. The n-3/n-6 ratios significantly increased in the CAN1% group compared to the control. Intrahepatic fat content aligned with histopathological changes, revealing hepatocyte steatosis upon using *C. sativa* 2% as a feed additive. The liver tissue showed hepatocellular vacuolation due to lipid droplet accumulation, with no necrotic tissue or inflammatory cell infiltration observed. Cortisol levels and DNA damage in the liver, gut, kidney, and spleen remained unaffected by HSO supplementation in fish diets, as no significant differences were observed between the dietary groups and the control group.

Discussion

The study explored hemp seed oil's (HSO) impact on gilthead seabream. HSO had positive impacts on survival rates, growth, and food consumption, yet statistical significance compared to the control group was lacking. It's possible that longer experiments would reveal significant differences. Saoud et al. (2018) attributed reduced growth and higher FCR in tilapia fed with HSO to the potential presence of another cannabinoid like cannabidiol (CBD) in the seeds. HSO successfully replaced soybean oil in diets while maintaining fatty acid profiles promoting a beneficial effect as observed in sows (Vodolazska & Lauridsen, 2020) and hens (Raza et al., 2016). However, a 2% HSO addition led to hepatic steatosis and lipid accumulation. Haematological parameters stayed within normal ranges, suggesting minimal fish stress while proper concentrations of medicinal plant extracts are crucial to avoid potential toxicity within the food chain, even though these extracts enhance stress tolerance.

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Table 1. Growth performance, feed utilization, morphometric parameters, whole body proximate composition, and heamatological parameters of *S. aurata* fed diets with HSO at 1% and 2%.

	Parameters/dietary groups	CAN1%	CAN2%	CONTROL
Growth parameters	Survival (%)	94.44 ± 5.09	98.89 ± 1.92	95.55 ± 3.85
	Feed consumed (g $fish^{-1}$)	55.47 ± 5.13	53.62 ± 3.51	49.80 ± 2.74
	TL (cm)	14.72 ± 0.45	14.69 ± 0.25	14.27 ± 0.35
	IBW (g $fish^{-1}$)	4.89 ± 0.07	4.89 ± 0.07	4.94 ± 0.03
	FBW (g $fish^{-1}$)	49.47 ± 4.48	50.34 ± 2.53	45.58 ± 3.98
	WG (g $fish^{-1}$)	44.58 ± 4.44	45.45 ± 2.48	40.65 ± 4.01
	SGR (% day^{-1})	3.12 ± 0.12	3.15 ± 0.05	3.00 ± 0.13
	FCR	1.25 ± 0.07	1.18 ± 0.03	1.23 ± 0.06
	Lipid retention (%)	77.00 ± 3.45	78.78 ± 1.74	76.18 ± 5.28
	HSI (%)	1.97 ± 0.04	2.00 ± 0.04	1.96 ± 0.08
	CF	1.55 ± 0.01	1.59 ± 0.03	1.56 ± 0.02
Whole body composition	Moisture	64.70±0.40	64.38±0.64	65.33± 1.87
	Protein	49.64±1.24	49.65±0.17	49.72±0.68
	Lipid	37.79±1.29	36.94±1.39	37.17±0.57
	Ash	$10.51±0.37^{ab}$	$9.53±0.09^{a}$	$11.25±0.78^{b}$
	Gross energy (MJ kg^{-1})	25.20±0.28	26.26±0.23	25.57±0.87
Haematological parameters	Ht (%)	32.78±4.67	34.22±5.74	34.78±7.38
	RBC (10^6 /mm^3)	0.98±0.15	0.79±0.30	0.99± 0.17
	WBC (10^3 /mm^3)	65.83±1.45	43.31±2.21	52.94±3.50
	Lymphocytes (%)	60.40±2.59	61.11±1.58	59.21±5.78
	Granulocytes (%)	38.93±2.65	38.24±1.71	40.12±5.78
	Monocytes (%)	0.67±0.01	0.66±0.01	0.67±0.01

Data are presented as mean ± standard deviation (n = 3). Different letters within the same raw indicate a significant difference between diets (ANOVA; $P < 0.05$). Abbreviations: TL, final total length; IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; HSI, hepatosomatic index; CF, condition factor; Ht, haematocrit; RBC, red blood cells; WBC, white blood cells.

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IN VITRO TOOL TO EVALUATE MUCOSAL IMMUNITY OF EUROPEAN SEABASS

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Contextualisation

The increasing demand for European seabass (*Dicentrarchus labrax*) led to the intensification of farming practises, resulting in increased stress and disease outbreaks in aquaculture. The immune system of fish comprises a complex population of immune cells with functions that remain not fully understood. Specifically, there is an urgent need to address the knowledge gap concerning the intestinal immune system of fish. This is essential for the development of efficient tools aimed at enhancing fish health.

Advances in European seabass *in vitro* model research are being supported by two ongoing projects, *AquaCell* and *BASSinCELLS*, coordinated by Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) and Nord University. The multidisciplinary team is pursuing an innovative approach: creating of an *in vitro* intestinal model for E. seabass. This model will serve as a valuable tool for understanding cell population-specific mechanisms in disease states and within the context of immunonutrition, with an emphasis on the gut immune cells and their function.

***In vitro* intestinal model for European seabass**

Up to now, the researchers have achieved significant progress in developing protocols for isolating, culturing, and evaluating the phagocytic ability of immune cells from European seabass intestine. These findings hold the key to unlocking exciting and valuable research opportunities that will significantly benefit fundamental research on fish immunology and nutrition, contributing to the long term sustainability of the aquaculture sector.

European seabass *in vitro* models: A look into the future of aquaculture

These groundbreaking projects aim to achieve the following outcomes:

1) Establish a cutting-edge *in vitro* model to understand how the immune cell populations operate to shape the farmed fish immune function, particularly in the context of disease;
2) Reduce the need for animal-based *in vivo* experiments and associated costs;
3) Develop novel strategies to enhance fish robustness, leading to a reduced need for antibiotics, while simultaneously increasing commercial production efficiency and promoting sustainability within the aquaculture sector;
4) Investigate the potential of alternative feed formulations to modulate the immune system, thereby reducing the use of antibiotics and improving management practices in aquaculture.

Acknowledgements

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MICROPLASTIC POLUTION BUDGET ASSESSMENT OF DIFFERENT MULTI-TROPHIC AQUACULTURE SYSTEMS

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Introduction

There is continued pressure to intensify aquaculture production to fulfil market needs. The expansion of the industry and increased diversity of materials used to build and maintain open, and recirculating aquaculture systems (RAS) have paralleled the development of synthetic polymers over the last decades (Astudillo et al., 2009). Synthetic materials offer greater strength and durability than natural fibres for the construction of ropes, infrastructures, and pipes, whilst often also being less costly and easier to handle. All plastic material within an aquaculture site is maintained and controlled for chemical degradation, biofouling and corrosion, and regularly inspected to ensure strength and stability. Broken and fragmented equipment as well as debris released from intense use however are sources of plastic emission from aquaculture operations at a local and global level, whilst accurate estimations of their contribution remain unknown (Lusher et al., 2017).

Figure 1 - Large Volume Microplastics sampling device operating at Bertraghboy Bay sampling site.

The ASTRAL project (All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture) focuses on integrated multi-trophic aquaculture (IMTA) farming, aiming at defining, supporting, and promoting this type of sustainable aquaculture production across the Atlantic area. To estimate the environmental sustainability, the fingerprint of different IMTA production systems is needed. ASTRAL is looking at the challenges related to the release of microplastics from aquaculture operations in both open and recirculating systems as well as quantifying the marine derived sources of plastics potentially impacting the IMTA labs. A monitoring plan was designed and applied at selected IMTA labs, using a novel sampling equipment able to preconcentrate large volumes of seawater onto appropriate filtering membranes.

Materials and Methods

Three sites were selected within the present study: A) the coastal open multi-trophic aquaculture facility located in Port-a-Bhuiltin (Scotland) managed by the Scottish Association for Marine Science. Being productive throughout the year, the farm aims at adding shellfish (native oysters, king scallops) to traditional seaweed production; B) the coastal open multi-trophic aquaculture facility located in Bertraghboy Bay (Ireland), managed by the Marine Institute. The farm aims at introducing new species combinations and C) The onshore partially (50 %) recirculating multi-trophic aquaculture facility "Buffeljags Abalone" located near the city of Cape Town, South Africa and managed by Viking Aquaculture. The farm aims at culturing a sub-tropical sea urchin species in temperate seas as well as integrating aquaculture of urchins with Ulva spp. and abalone. The occurrence of plastic fragments in different environmental compartments potentially affected by aquaculture production activities such as seawater and marine sediments was investigated at increasing distances from the open systems aquaculture facilities. The sampling grid was defined in relation to the site's location, and the direction and intensity of the main surface and sea bottom currents present at site. Three sampling sites were selected from each of the open systems. In the partial RAS system, sampling sites were selected in the water inlet, outlet, as well as in some selected areas inside the recirculating system (abalone & Ulva raceways). For the seawater collection a Compact Large Volume Microplastics sampling device was developed. A large volume of seawater is conveyed by a compact stainless steel pump unit to a cascade of two stainless steel filters of 10 and 300 μm mesh size where particles (including micron-sized plastic fragments) are trapped. After each sampling, filters can be easily released from the small portable filter holder unit (fig.1) and accommodated on pre-cleaned glass petri dishes of appropriate size, sealed, kept in cold and dark conditions prior to the sample preparation and analysis steps. The sediments were collected by means of a Van Veen grab device. The first 0 to 5 cm sediments layer was collected for analysis. The extraction and purification method for all samples included a combination of a multistep enzymatic-strong alkali-oriented incubation followed by density-based separation to extract plastic fragments from digestates. Extracted samples were analysed first by μ-FTIR (Fourier Transform Infrared) microscopy and finally by GCMS-pyrolysis technique.

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Results and Discussion

The evaluation of the seawater samples and sediments using a vibrational microscopy-oriented technique shows the occurrence of 27 different polymer types. Among them the most recurring ones were polyethylene (PE), polystyrene (PS), polypropylene (PP), ethyl vinyl acetate (EVA), polycarbonate (PC), polyurethane (PU), (polyvinyl chloride) PVC and polyamide (PA66). Furthermore, the same samples were analyzed by pyrolysis mass spectrometry. The mass-based analysis back confirmed the occurrence of these polymers and further detected the occurrence of styrene butadiene rubber as a proxy of Tyre and road wear particles. The observed fragments were mapped against the IMTA lab inventory of polymers used in their daily operation. The sites targeted as reference in the study showed a similarly representative pool of polymers but with different accumulation levels. The preliminary results point out a complex distribution of polymers which hamper the interpretation of the source of microplastics in the aquatic environment. The ongoing work will contribute towards an improved understanding of the complexity, introduction, and potential emission of synthetic polymers in open and recirculating IMTA systems that will contribute towards the improved sustainability of modern aquaculture systems.

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HEMP PROTEIN POWDER FEASIBILITY AS FEED COMPONENT FOR EURASIAN PERCH *Perca fluviatilis* L.

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Introduction

The cultivation of industrial hemp (*Cannabis sativa* L.) is a rapidly growing branch of agriculture globally with an expected annual growth rate of 16.9 % by 2030 (Polaris Market Research 2021). Hemp cultivation area has increased by 75% between 2015 and 2019 in EU and probably will rise further due to hemp environmental benefits like high carbon storage, prevention of soil erosion, increase of biodiversity and low need for pesticides. Hemp fiber is used in textile industry, construction, biofuels production and many new innovative applications. Hemp seeds are source of nutrition for both human and animals. The attempt to use of hemp seed in striped bass feed has given promising results (Sample 2022). The aim of this study was to test feasibility of hemp protein powder (HPP) as an ingredient of extruded feed for Eurasian perch *Perca fluviatilis* L.

Materials and methods

Experimental feeds. Four extruded feeds containing 0% (control group), 10% (HP10), 20%(HP20) and 30%(HP30) of HPP (50% of crude protein; commercially available diet supplement for human) were prepared. Additionally the feed containing 30% of HPP and phytase addition (2000 IU per kg) has been extruded.

Fish and the experiment design. Eurasian perch from pond culture of National Inland Fisheries Research Institute in Olsztyn, Station in Żabieniec was used for test. Fish of 68.1 g ± 2.7 mean body weight (N=500) were equally distributed to ten fiberglass tanks (0.3 m^3) working in RAS. Tanks were randomly assigned to experimental groups. Each group was present in duplicate. Fish were fed experimental feed appropriate for given group using belt feeders for approximately 10 hours a day for 10 weeks. Then, fish sample (n=15) from each tank was taken for body measurements. Sampled fish were euthanized and dissected. All the viscera and separately the liver were weighed. The remaining fish were weighed to determine the final total wet weight. Growth indicators (SGR, FCR, PER, VSI, HIS) were calculated based on collected data.

Statistical analysis. The analysis was done using Statistica 13 software (Statsoft, USA). Shapiro-Wilk test and Levene's test were used to assess data normality and variance homogeneity respectively. As SGR, FCR and PER data revealed lack of variance homogeneity, The Kruskall-Wallis ANOVA was used to test difference significance. For remained data, ANOVA procedure and Tuckey's post hoc test were applied.

Results

The growth of fish was very similar in all experimental groups. No significant differences were found in final body length and weight and specific growth rate. The mean body length varied between 16.7 cm and 17.0 cm and body weight between 92.8 g and 100.3 g in control and HP30F, respectively. Specific growth rate varied from 0.39 to 0.59 in HP30 and HP30F, respectively. The lowest mortality was noticed in HP30F group (1%) and the highest one in the control group (7%).

Hemp protein content did not influence significantly the feed conversion ratio (FCR)(varied between 2.00 and 3.31) and protein efficiency ratio (PER)(varied between 0.54 and 1.08) although the differences seems rather high. The only significant difference (ANOVA $p<0.05$) was found in the liver weight. HSI was significantly higher in the control and HP10 groups (2.44% and 2.19%, respectively) when compared to remaining groups (1.56% - 1.76%). Generally it has been noticed that results were slightly better with rising hemp powder addition up to 20%. The worst results were achieved in HP30 group (SGR 0.39, FCR 3.31, PER 0.54), however the best results were achieved in HP30F group (SGR 0.59, FCR2.0, PER 1.08).

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Conclusion

Hemp protein powder addition to feed up to 30% does not influence on the growth of Eurasian perch. However, results obtained for HP30 group suggest that higher content of HPP can limit feed conversion and fish growth. The positive effect of phytase is rather unexpected result as it is considered that hemp seed do not contain phytic acid. HPP addition did not cause increased mortality during the experiment and HSI level was significantly lower in groups fed higher levels of HPP. Presented results a quite promising, however more intensive study is needed especially to explain the role of phytase.

Acknowledgment

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BOOSTING FISH GUT PERFORMANCE WITH ALGAE BIOREFINERY PRODUCTS – AN *EX VIVO* APPROACH

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Introduction

Algae are rich in bioactive compounds with a strong nutraceutical potential for human and animal health. However, the utilization of these compounds in aquafeeds has been limited by the bioavailability of these compounds and the limited capacity of most species to digest and extract them from intact algae.

Biorefinery arrives as a valuable set of tools that process algae in different manners, hopefully unleashing their potential as functional boosters in aquafeeds. The application of mechanical and chemical processes to break the cells and its compounds, extractions of soluble compounds to enhance and/or isolate their characteristics, and separation methods such as membrane filtration to separate soluble fractions from residual biomasses are only some of the strategies that have been applied to pave the way for bringing functional algae-derived ingredients to aquaculture nutrition.

The aim of this study was to assess the functional potential of two microalgae, *Nannochloropsis oceanica* and *Phaeodactylum tricornutum* broken and hydrolyzed biomasses and a macroalgae *Gracilaria gracilis* broken cells biomass in a Gilthead seabream (*Sparus aurata*) intestinal explant model.

Material and Methods

Broken cells of *Nannochloropsis oceanica* and *Phaeodactylum tricornutum* were obtained by high-pressure homogenization (HPH), whereas *Gracilaria gracilis* was obtained by bead milling. Microalgae broken cells were further processed by different enzymatic hydrolysis. The resulting products were freeze-dried and applied in two different dosages in an intestinal explant model, under previously standardized conditions. The intestinal response to the biorefinery-derived ingredients was evaluated through the transcriptional modulation of genes encoding for proteins related to innate immune response (e.g., *cox2*, *IgM*), antioxidant response (e.g., *cat*, *gpx*, *nrf2*) and to epithelial integrity and permeability (e.g., *tjp*, *cldn12*, *ocl*).

Results and Discussion

Ingredients derived from biorefinery process of *P. tricornutum* and *G. gracilis* presented the strongest functional potential. Here, *P. tricornutum* broken cells and hydrolyzed fractions triggered the stronger epithelial immune response and prompted a modulation of the tight junction complex. Interestingly, hydrolyzed *N. oceanica* had a boosting effect on the expression of genes related to epithelial integrity and permeability regulation, but not on immune-related ones, indicating that each algae species presents different potential and requires dedicated processing to unrestraint their bioactivity to be applied in aquafeeds.

Acknowledgement

This study was part of the Blue Bioeconomy Innovation Pact (Pacto da Bioeconomia Azul), project N. C644915664-00000026 funded by the Resilience and Recovery Plan (Plano de Recuperação e Resiliência) of the European Union and Portuguese Government, as well as the project MICROBOOST – PT-INNOVATION-0102 – funded by Iceland, Liechtenstein and Norway through the European Economic Area (EEA) grants. This study also received Portuguese national funds from FCT - Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020. B. Carvalho acknowledge Fundação para a Ciência e a Tecnologia (FCT) for grant PhD Fellowship 2022.12615.BDANA

EFFECT OF MEDICINAL PLANTS, YEAST AND *Bacillus licheniformis* ON THE GROWTH, SURVIVAL, IMMUNE RESPONSE AND DIGESTION OF SHRIMP (*Penaeus vannamei*) CHALLENGED WITH *Vibrio parahaemolyticus*

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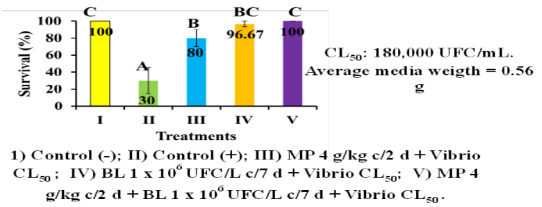
In shrimp farming viral diseases produce important economic losses. The use of prophylactic methods as natural additives wthout inmmune resistance and environmental problems were proben. Medicinal plants powder, yeast and *B. licheniformis* were used in the survival in the digestive and immune systems of *P. vannamei* challenged against *V. parahaemolyticus*.

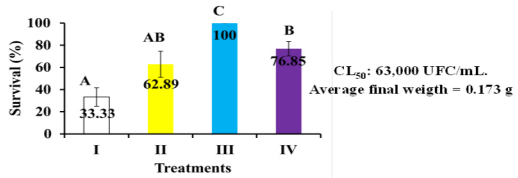
Four bioassays were carried.Postlarval white shrimp stage were used to analyze the digestive and immune-related genes expression by RT-qPCR.

The combination of MP in the food and *B. licheniformis* in the water (3 x 106 CFU / L) significantly improved the survival of *P. vannamei* challenged with *V. parahaemolyticus*. *B. licheniformis* inoculated in the water, did not alter the expression of the trypsin digestive gene.

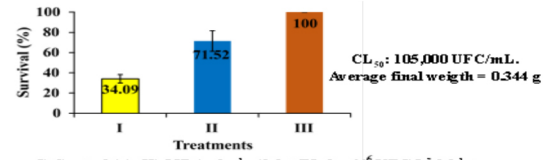
B. licheniformis in the water decreased the expression of the SOD gene (related to the immune system), which plays an important role as an antioxidant, decreasing the concentration of superoxide anion, the product of the phagocytosis process. *B. licheniformis* in the water did not alter the expression of the genes of the immune system penaeidine4 and lysozyme. The mixture of MP added in the feed and *B. licheniformis* in the water prevent the AHPND in *P. vannamei* cultivated in the laboratory.



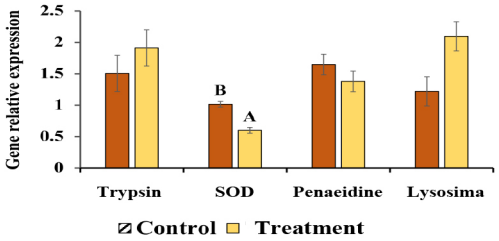
1) Control (-); II) Control (+); III) MP 4 g/kg c/2 d + Vibrio CL_{50}; IV) BL 1 x 10^6 UFC/L c/7 d + Vibrio CL_{50}; V) MP 4 g/kg c/2 d + BL 1 x 10^6 UFC/L c/7 d + Vibrio CL_{50}.



Control (+) ; II) Yeast 2 g kg^{-1} 2 d^{-1} + *Vibrio* CL_{50}; III) BL 3 x 10^6 UFC L^{-1} 3 d^{-1} + *Vibrio* CL_{50}; IV) DKT 3 g kg^{-1} 2 d^{-1} + *Vibrio* CL_{50}.



I) Control (+); II) MP 4 g kg^{-1} c/2 d + BL 2 x 10^6 UFC L^{-1} 3 d^{-1} + *Vibrio* CL_{50}; III) MP/2 d^{-1} + BL 3 x 10^6 UFC L^{-1} 3 d^{-1} + *Vibrio* CL_{50}.



BL 3 x 10^6 UFC L^{-1} 3 d^{-1}

PROSPECTS AND PITFALLS OF USING BRAIN FUNCTION TO ASSESS THE EFFECTIVENESS OF STUNNING IN FISH

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Background

Countries within the European Union (EU) often take pride in its comparatively stringent and comprehensive animal welfare legislations (https://api.worldanimalprotection.org/). In the EU, all animals should be protected from any unnecessary pain, suffering or injury (COUNCIL DIRECTIVE 98/58/EC). In addition, during killing and slaughter, all animals must be stunned before the method of killing is applied, and the animal must not regain consciousness before it dies (Article 4, EC No. 1099/2009). When the European Commission reviewed the welfare of its five key aquaculture species during slaughter in 2017, they identified serious shortcomings when it came to safeguarding the welfare during slaughter. For most species and in most countries, the methods used for stunning did not comply with humane killing. The report also highlighted that it essential to neurologically investigate (e.g. using electroencephalograms, EEGs) the humaneness of the stunning method in order to verify whether the fish immediately loses consciousness or not instead of relying solely on the visual verification of consciousness.

Material and methods

Recently, we have developed, validated, and implemented a non-invasive technique to measure and interpret EEG in fish (Bowman et al. 2019, Fig 1). With our technique, three silver chloride disc electrodes are fitted to a custom-made silicone suction cup. The electrodes are connected to three shielded wires that are threaded through the silicon cup and connected to an animal bioamplifier that fed the signal to a PowerLab system. A thin layer conductive EEG paste is applied in to each of the electrodes to ensure good contact between the skin of the fish and the electrodes. During EEG measurements, the cup is centered above the approximate location of the left optic lobe and secured using suction generated by the peristaltic pump. Using this technique, a fish can be judged to be unconscious and insensible if the EEG shows abolition of evoked electrical activity in the brain, *e.g.* visual evoked responses (VERs) and/or changes that are incompatible with consciousness (*i.e.* grand mal epilepsy, frequency shifts and/or prolonged quiescent periods on the EEG).

Results

Using this technique, we have successfully carried out a series of studies and identified several serious welfare hazards with the various stunning and slaughter procedures that are currently used (Bowman et al. 2019, 2020, Brijs et al. 2021 & Hjelmstedt et al. 2022). Our studies show that the technique can be used to investigate the effects of various stunning/killing methods, including electrical stunning, CO_2-narcosis, percussive stunning and chemical narcosis, on brain function (Bowman et al. 2019, 2020, Brijs et al. 2021 & Hjelmstedt et al. 2022). Furthermore our findings strongly support that it is critical to apply neurological investigations when the humaneness of the slaughter method is investigated rather than relying on visual verifications alone, as the latter is unreliable and underestimates the time it takes for a fish to become unconscious. In addition, our results indicated that some neurophysiological indicators of unconsciousness that traditionally have been used to validate electrical stunning procedures in birds and mammals might not be appropriate for fish. For example, our results clearly show that the presence of an epileptic-like seizure following an electrical stun does not guarantee a prolonged period where the fish is unresponsive to visual stimulation (*i.e.* absence of VERs). Instead, the fish can regain responsiveness within seconds after the seizure ends (Hjelmstedt et al. 2022).

Conclusion

These findings have severe welfare implications since many stunning methods thought to render fish unconscious may only cause paralysis, which means that fish are still sensible when exsanguinated and eviscerated. In addition, the loss of sensibility can be a gradual process, during which it is unclear when the fish is no longer susceptible to anthropogenic stress and, therefore, not capable to experience pain, distress, and anxiety. In such situations, it is difficult, even for a trained expert, to make an objective and evidence-based interpretation of an animal's level of consciousness. Collectively, these results highlights the necessity to carefully consider and evaluate how neurophysiological indicators of unconsciousness should be used to validating different stunning methods and to safeguard the welfare of farmed fish during slaughter.

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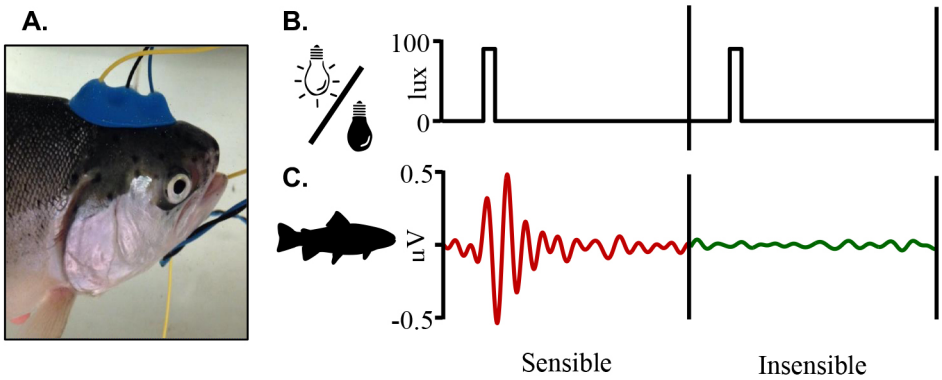

Figure 1. Non-invasive technique for determining loss of brain activity in rainbow trout. (A) the custom-made cup used to measure the electroencephalogram (EEG) in trout. (B) the light signal from the stroboscope light used to trigger a visually evoked response (VER) in the EEG. (C) VER's in a fully sensible fish (left panel) and following a percussive stun that lead to complete loss of VER's (right panel). Each segment represents the average of 120 stroboscope light pulses at 2 Hz.

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NEW INSIGHTS OF *Porphyra* CULTIVATION IN FRANCE: CASE STUDY IN NORMANDY (WEST COTENTIN)

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Introduction

Porphyra sp., also known as Nori, is a red alga and one of the most valuable edible seaweed in the world. The global Nori production mostly occurs in Asia (China, Japan and Korea) with farming of *Pyropia* species (phylogenetically close to *Porphyra* species) with 3 million tons for 2.7 million USD in 2018. In Europe, the production of Nori comes almost exclusively from harvesting of wild populations of *Porphyra* species, and so far, the commercial culture is performed by only one company located Portugal. In France, *Porphyra* production exclusively comes from wild hand-harvesting, especially in Brittany (30 tons in 2021).

With the increase of Nori demand, harvesting pressure on wild *Porphyra* populations increases as well. Therefore, it becomes essential to develop and optimize new cultivation methods of *Porphyra* in Europe, to promote local economy and suitability with already existing aquaculture activities.

The western coast of Normandy region has a great potential for *Porphyra* productions, wild populations grow on its rocky shores and have been noticed growing abundantly in many intertidal oyster farms as well. In this study, we explore the potential of two production methods of *Porphyra* species in intertidal oyster farms in the West Cotentin area:
- Natural seeding by natural recruitment of *Porphyra* on oyster pockets
- Artificial seeding and nursery cultivation of plantlets before transfer at sea

Materials and Methods

For natural seeding, empty oyster pockets (0.5m² each) have been placed in 3 and then 4 oyster farms monthly from November to March, and at 3 different bathymetric levels (high, middle and low intertidal) in order to identify the time and bathymetry that promote natural seeding. The biomass was harvested in June to avoid damages caused by summer heatwaves. The experiment was renewed three years: 2020-21 (3 locations), 2021-22 and 2022-23 (4 locations). In 2021-22 and 2022-23, other species of seaweeds colonizing oyster pockets were also harvested and weighted.

Artificial seeding was performed in 2021-22 by cultivating the filamentous phase (*Conchocelis*) of *Porphyra purpurea* in lab in order to obtain conchospores to seed oyster pockets in tanks. The plantlets were then kept in nursery cultivation ponds for 2 months before being transferred in oyster farms for open sea growth until harvesting in June 2022.

Results & Discussion

Natural seeding gave harvestable biomass values, especially during the year 2020-21 on pockets from the site located in low intertidal, and laid out in December and January. The mean dry biomass could reach 137g±80gDW/pocket (Fig.1). In 2021-22, the biomasses were very low for the 4 locations, but the maximum was still observed on oyster pockets laid out in December and January in low and middle intertidal (12.6±6.6gDW/pocket). The year 2021-22 was indeed unusually affected by a mild winter with sometimes brutal frost waves, and then precocious heatwaves in spring, compared to 2020-21. These climates factors may have affected both recruitment on pockets and growth of young plantlets. The year 2022-23 showed better results but the biomass was less important than in 2020-21. More biomass was harvested in oyster farms in the low intertidal, on pockets put in January (88.8±49.2gDW and 52.9±27.8gDW/pocket). Besides, one of the locations in low intertidal showed similar results for oyster pockets placed in November 2022. Biomass variations within each site still remains high (Fig.1). In terms of purity, the other seaweeds species growing among *Porphyra* were filamentous *Ulva* sp. (Fig.1). In farms were *Porphyra* biomasses were the most important, the proportion of *Ulva* biomass was the lowest on pockets put in January 2022 and January 2023 (<30%), which confirmed January as the best time for natural *Porphyra* seeding.

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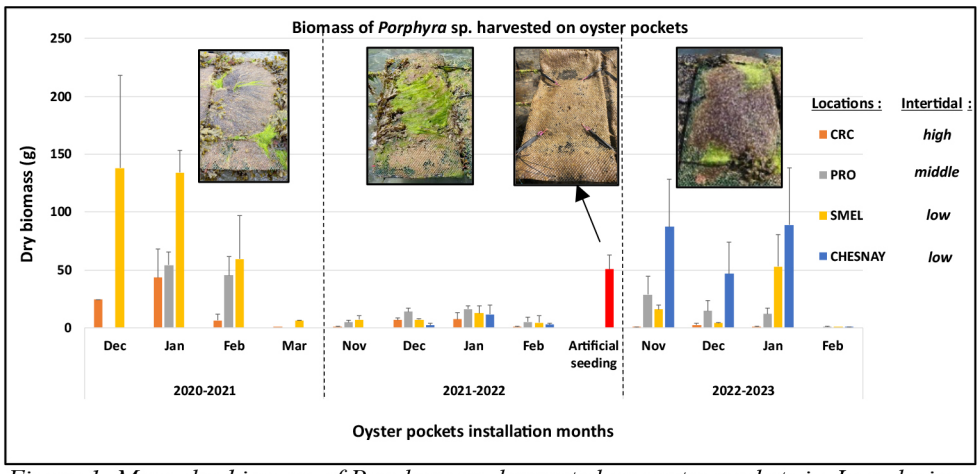

Figure 1. Mean dry biomass of Porphyra sp. harvested on oyster pockets in June during the three years of natural seeding in the four oyster farms and by artificial seeding in June 2022 (red bar). Errors bars stands for standard deviation. Pictures give a look of the harvested biomass in terms of purity and integrity on oyster pockets.

Artificial seeding worked as well. The seeded oyster pockets where *Porphyra* plantlets grew successfully gave 50.6g±12.1gDW/pocket. These values are much more important than those obtained by natural seeding the same year 2022, and the standard deviation is lower than those calculated for artificial seedings in 2020-21 and 2022-23 (Fig.1). Besides, no other undesirable species like *Ulva* sp. was noticed, the purity was 100% (Fig.1). Artificial seeding and nursery cultivation of plantlets had an effect on these results: the seeding was more homogenous, precocious stages of plantlets protected against climate issues, avoiding contaminations from other seaweed species like *Ulva* sp.

Conclusion

Both techniques showed interesting results for *Porphyra* cultivation. Natural seeding is easier and cheaper as it does not need all the nursery culture systems, only empty oyster pockets. The best time and bathymetry to put oyster pockets for *Porphyra* recruitment should be in winter, especially December and January, in the low and middle intertidal. It can give high biomasses of *Porphyra*, but this technique remains highly variable between years and within culture areas: vulnerability to climate events, undesirable species recruitment (*Ulva* sp.) and heterogeneity of conchospores fixation. Artificial seeding requires aquaculture and lab systems that can quickly have a significant financial cost. However, this cultivation method improves the homogeneity and quality of produced biomass.

MARITIME SPATIAL PLANNING WITHOUT AQUACULTURE-SPECIFIC LEGAL FRAMEWORK MAKES EU AQUACULTURE GROWTH TARGET UNATTAINABLE

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Introduction

Over a million tonnes of seafood is imported to the EU annually to meet the growing demand, while aquaculture production from most coastal EU States remains stagnant at zero (0) to 50 thousand metric tonnes annually. This is just under 26% of the 3.2 million metric tonnes produced by only 6 European countries: Norway, Spain, the UK, France, Greece, and Italy. This low level of aquaculture production has been largely attributed to a lack of access to space and water and bureaucratic legislation. This study aims to highlight that while MSP is an important tool for the futuristic growth and development of Aquaculture, the absence of aquaculture-specific legislation in the planning system is primarily responsible for the low productivity and must be addressed in tandem with the development of Maritime Spatial Planning if there is to be any significant growth in aquaculture. Many articles have pointed to the social and spatial hindrances and bureaucratic regulations and call on Maritime Spatial planning to provide a solution. However, a very important and necessary consideration is having a specific legal framework that clearly outlines how aquaculture is to be developed; the type of aquaculture that is supported, and the permit/licenses required. By examining the countries with the highest production of aquaculture, it can be observed that while they also have challenges with social acceptance, spatial conflicts, and bureaucratic obstacles, there is a legal framework in place that fosters aquaculture. It is therefore not a surprise that the countries with more aquaculture-specific regulations are the highest-producing countries. Consequently, it is recommended that countries seek to update or develop aquaculture-specific regulations in concert with their Maritime Spatial Plans in order to foster real growth. The absence of this is not making aquaculture growth a priority.

IMPACT OF BIOACTIVE COMPOUNDS DIETARY INCLUSION IN GILTHEAD SEA BREAM (*Sparus aurata*) AND SEA BASS (*Disentrarchus labrax*) FILLETS SENSORY QUALITY; PRELIMINARY RESULTS

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Introduction

Dietary inclusion of polyphenol-rich natural products for farmed fish has been subject to contemporary research (Ahmadifar, et al., 2020). However, there is very little knowledge on the effects of such supplementations in the sensory quality and storage stability of the produced fish fillets.

The present study aimed to examine the effect of the dietary supplementation of polyphenol-rich olive oil by-product compounds and bioactive *Spirulina* peptides, on the sensory quality of European sea bass and gilthead sea bream, freshly slaughtered or after 8 months of deep-freeze storage.

Materials and methods

European sea bass and gilthead sea bream of commercial weights (300 to 400g) having previously received one control (Ctrl) and one olive-oil pomace and Spirulina-supplemented (Sup) experimental diet were used. Diet characteristics and feeding protocols have been previously described (Fountoulaki et al., 2022). Fish were ice-slaughtered in accordance with the commercial practice. Fillets of both studied species and from both the dietary groups were then tested for their sensory properties, both as fresh (t0) or after 8-month (t8) of deep-freeze (-20^{0}C) storage. A trained panel of 12 panelists conducted the tests and a conventional descriptive analysis (DA) method with 24 descriptors for odour, taste, flavour and texture was used (Alexi et al., 2018). Principal Component Analysis (PCA) was used to explain the results statistically.

Results

In gilthead sea bream the two first principal components (F1 & F2) explain the 82.5% of the total variation (Fig 1) while in sea bass the 82.7%, respectively (Fig. 2). The panellists could distinguish between both the storage time and the dietary group the fillets belong. For both studied species F1 separates samples according to the storage time point. In gilthead sea bream F2 separates samples according to diet treatment, while in sea bass it is the third component (F3, explains 17.3% of the total data variation) that functions similarly.

In respect to the individual descriptors bream at t0 has a more intense fish oil odour compared to t8 ($p<0.1$). Bream Ctrl_t8 was found more chewy ($p<0.1$) compared to the rest of the samples. Bass Ctrl_t8 was also found more chewy ($p<0.1$) but also had a more intense grilled meat odor ($p<0.05$) compared to its counterpart at t0. Bass fed with Sup exhibited a higher odor intensity a t0 compared to t8 ($p<0.05$).

Conclusions

In the current study, it becomes evident that dietary inclusion of bioactive polyphenolic compounds, can have an effect in the sensory quality and stability of fish fillets. The complementary chemical evidence is still required to understand these differences.

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DIFFERENCES IN UTILISATION OF DIGESTIBLE MACRONUTRIENTS FOR ENERGY GAIN BETWEEN 30 AND 90 GRAM RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Whereas in the past feed formulation in aquaculture was predominantly based on digestible energy (DE) it is now shifting towards a net energy (NE) approach (Kaushik & Schrama, 2022). The main advantage of this approach is that it takes into account differences in the utilisation efficiency of digestible protein, digestible fat and digestible carbohydrates and therefore leads to a more accurate feed evaluation (Schrama et al., 2018; Groot et al., 2021). However, it has been observed that at least the composition of growth (protein versus fat gain) can influence the efficiency of digestible macronutrients for energy gain in such a NE approach (Groot et al., 2022). Since composition of growth also changes with increasing fish size (higher fat to protein gain) it was hypothesized that fish size could also influence the utilisation of digestible macronutrients for energy gain.

Materials & methods

Four different diets ranging in protein, fat and carbohydrates were fed to two size classes of rainbow trout, 30 and 90 gram, at three different feeding levels. Growth, feed intake, initial and final body composition were measured, from which energy retention was determined. Furthermore, faeces was collected for determining digestibility of protein, fat and carbohydrates. The design of this trial allowed for multiple regression analysis to determine the utilisation efficiency of the different digestible macronutrients for energy gain whilst separating the maintenance energy needs. By doing so, it was also possible to establish specific NE formulas for 30 and 90 gram rainbow trout.

Results

The results of this study showed first of all that both the utilisation efficiency of digestible nitrogen and energy intake for growth (k_{gDN} and k_{gDE}) were lower for the 90 gram fish (figure 1 & 2). However, it was only significant ($P<0.05$) for k_{gDE} which decreased from 79% to 72% in the 90 gram fish indicating that these fish converted energy less efficient into energy gain. Secondly, differences were also found between size classes in the estimated NE formulas and the corresponding partial efficiencies of the digestible macronutrients for NE ($k_{gNE,CP}$, $k_{gNE,Fat}$ and $k_{gNE,Carb}$). The lower k_{gDE} in the bigger fish seemed to be mainly related to a lower $k_{gNE,Fat}$ ($P<0.05$) even though $k_{gNE,Carb}$ did showed a tendency to increase ($P<0.1$). $k_{gNE,CP}$ on the other hand was not different ($P>0.05$) which corresponded with the lack of a size effect on k_{gDN}.

Conclusion

The results of the current study demonstrate that energy utilisation in rainbow trout is affected by fish size and should therefore be taken into account when moving to a NE approach in feed evaluation for this species.

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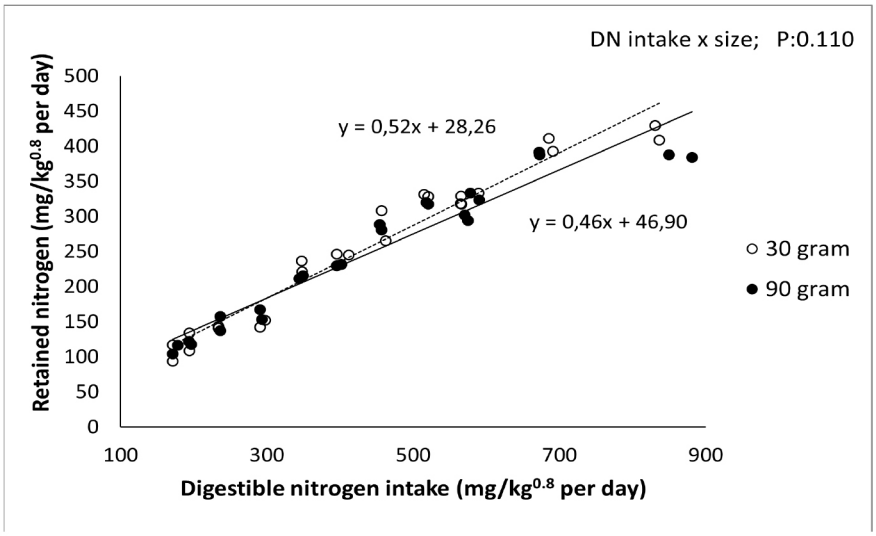

Figure 1. The relationship between retained nitrogen (mg/kg$^{0.8}$ per day) and digestible nitrogen intake (mg/kg$^{0.8}$ per day) for two size classes of rainbow trout (30 and 90 gram).

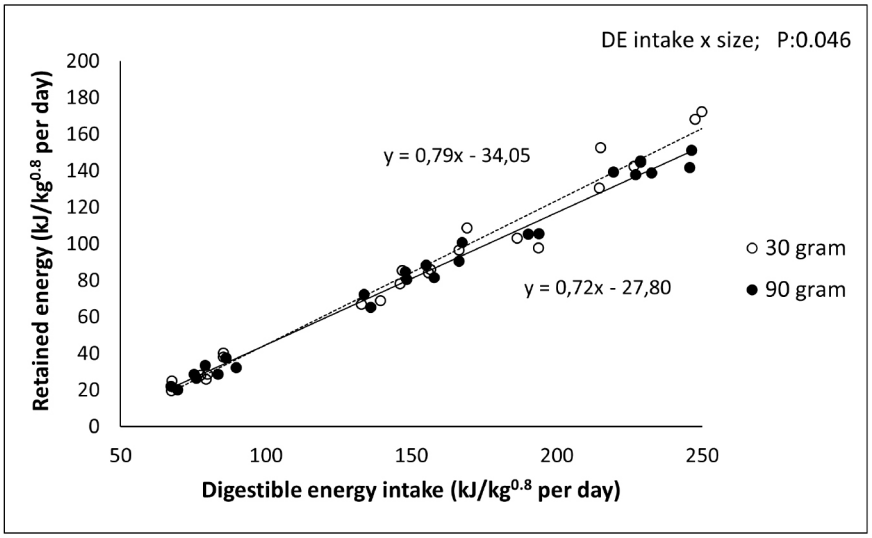

Figure 2. The relationship between retained energy (kJ/kg$^{0.8}$ per day) and digestible energy intake (kJ/kg$^{0.8}$ per day) for two size classes of rainbow trout (30 and 90 gram).

RESOURCE RECOVERY AND INCREASING PRODUCTIVITY IN A SUSTAINABLE INTENSIVE NEAR ZERO WASTE ASSIMILATION BASED RECIRCULATING AQUACULTURE SYSTEMS (AsRAS)

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Introduction

Food security, specifically in water-scarce regions, is a local and global aim which requires innovative solutions. Assimilation based recirculating aquaculture systems (AsRAS) such as aquaponics, algaponics and bacterioponics can be such a sustainable solution. All AsRAS involve the integration of conventional recirculating aquaculture system (RAS) with assimilation based reactor in a symbiotic arrangement. In aquaponics for example, fish excretions are assimilated as a nutrient source for vegetable production (Zhu et al., 2022), where in algaponics and bacterioponics it would be replaced by macro or micro algae and bacteria respectively. As a result, the assimilation of the fish waste by plants or bacteria treat the water, and enables its recirculation back to the fish tank. This practice allows for extremely high efficiency in the use of water and nutrients, greatly limits the discharge of pollutants and recovers or saves a lot of energy (Yogev and Gross, 2019).

Methods

In recent years we have developed three different AsRAS that are operated on fresh or brackishwater in multi-loops near-zero waste setups. The systems include separate loops for fish production (RAS) and for the assimilation organism growth which facilitate optimal conditions for each crop. In addition, in some systems two separate treatment loops are used to treat the solid waste (e.g. from fish and inedible plant bits) by anaerobic digestion, producing nutrient-rich supernatant which is good for plant growth and energy via biogas.

Results

In all tested systems fish stocking density reached up to 80 kg/m^3 with typical density of approximately 50 kg/m^3. Feed (45% protein content) was applied daily at 2% of body weight. Typical fish performance was observed with a survival rate >95% and feed conversion ratio ranged between 1.1-1.4. Significant energy saving was demonstrated; from >80% in the aquaponics system and 50% in the bacterioponics/biobloc based RAS. Carbon sequestration was 1.4 higher than the feed carbon in the aquaponics system, which reduced the carbon footprint of typical RAS by 64% where similar or even higher reduction is expected for the algaponics-based AsRAS. Moreoven in all of the studied AsRAS significant reduction of nitrous oxide was recorded when compared to traditional RAS.

These studies are among the first to demonstrate highly efficient AsRAS production with near-zero water and waste discharge and with significant energy recovery or savings. We postulate that AsRAS systems have a great potential to replace the traditional nitrification-denitrification based RAS.

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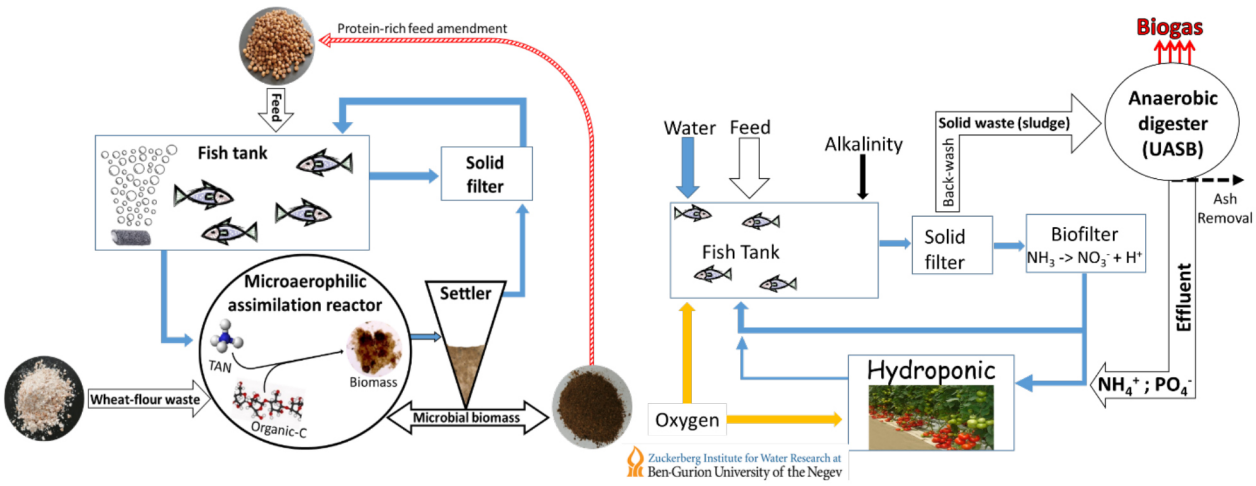

Fig. 1. Examples of near zero waste AsRAS (a) Bacterioponics and (b) Aquaponics

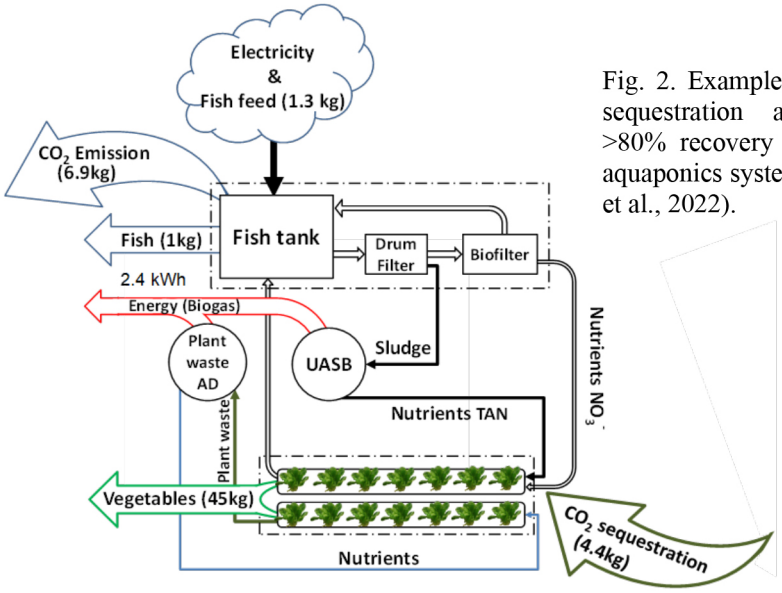

Fig. 2. Example of 64% carbon sequestration and significant, >80% recovery of energy from aquaponics system (After on Zhu et al., 2022).

RISKY COCKTAILS: MULTI-MYCOTOXIN CONTAMINATION OF AQUACULTURE FEED

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Introduction

Mycotoxins are notorious contaminants of animal feed known to exert toxic effects in animals, including aquatic species. Fungi that produce mycotoxins infest crop plants in the field and agricultural products during storage. Therefore, the inclusion of plant-based feed ingredients in aquaculture feedstuffs can result in the introduction of mycotoxins. As mycotoxins are thermostable molecules that resist conditions applied during the feed production process, mycotoxins end up in compound feed. During storage of compound feed for aquaculture in moist and warm conditions, additional mycotoxins may be produced.

Our knowledge of mycotoxin prevalence and impact in the aquaculture sector lags behind what is known about these issues in the terrestrial livestock industry. Therefore, in this study, we investigated mycotoxin occurrence in feedstuffs destined for aquaculture species. To this end, we analyzed mycotoxin concentrations in 273 samples of aquaculture feedstuffs collected in Asia, Africa, Europe and South America.

Materials and Methods

We collected 273 samples of aquaculture feed from different countries in Asia, Africa, Europe and South America and analyzed concentrations of 51 mycotoxins and less-investigated fungal metabolites ("emerging mycotoxins") using multi-analyte liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. Analytes included aflatoxins and other *Aspergillus* mycotoxins, *Fusarium* mycotoxins (e.g., deoxynivalenol, zearalenone, fumonisins), *Penicillium* toxins, ergot alkaloids and *Alternaria* toxins.

Results

In total, 46 mycotoxins and fungal metabolites were detected in aquaculture feed samples and 97% of the samples were contaminated with at least one mycotoxin/metabolite. We found that 36% of the samples were contaminated with the cancerogenic mycotoxin aflatoxin B1, and 5% of the samples exceeded the maximum level set for this mycotoxin in fish feed in the European Union (i.e., 10 µg/kg). Furthermore, *Fusarium* mycotoxins fumonisins, deoxynivalenol, and zearalenone were prevalent in aquaculture feed samples (detected in 63%, 59%, and 54%, respectively). In addition, less investigated fungal metabolites such as enniatin B1 (48%), beauvericin (38%), and alternariol (34%) were frequently detected. Co-contamination of aquaculture feed samples with multiple mycotoxins/metabolites was common. In total, 72% and 36% of the samples were co-contaminated with ≥5 and ≥10 mycotoxins/metabolites, respectively.

Discussion

Mycotoxins and other, less investigated fungal metabolites were almost ubiquitously present in aquaculture feedstuffs analyzed in this study. Mycotoxins are known to cause adverse effects in aquatic species such as reduced growth, impaired reproductive performance, increased mortality and immunosuppression. Furthermore, mycotoxins may accumulate in edible tissues. It is therefore necessary to closely monitor mycotoxin concentrations in aquaculture feed and take appropriate measures to minimize their negative impact.

Based on our results, co-contamination of feed with multiple mycotoxins is the rule rather than the exception. This finding is not surprising, as compound feed is a blend of a variety of raw materials, each of which has its own mycotoxin risk profile. Due to toxicological interactions, these "cocktails" of mycotoxins in feed can have stronger toxic effects than each individual mycotoxin would have on its own. Our findings underline the necessity to monitor the concentrations of multiple mycotoxins in aquaculture feed simultaneously and to investigate the combined toxicity of mycotoxins in aquaculture species.

BIOTECHNOLOGICAL TOOLS FOR THE RESEARCH AND CONTROL OF NODAVIRUS IN AQUACULTURE

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Introduction.
Nodavirus (NNV) is the most threatening marine virus in the Mediterranean area, and it is spreading in the number and type of viral isolates and host fish species, either wild or culture. In our research group we have been studying the immune response of fish against NNV for 2 decades, focusing on gilthead seabream and European sea bass models. While European sea bass is highly susceptible to some classical NNV genotypes the gilthead seabream acts as a resistant and reservoir species. However, their potential vertical transmission, the emergence of new natural NNV recombinants, producing high mortalities to traditionally-resistant species such as gilthead seabream, the climate change and the aquaculture diversification might increase the deleterious effects of NNV infection in the aquaculture sector. Based on this, we are now increasing our knowledge about fish-NNV interactions in the following topics:

1. Generation of biotechnological tools to study NNV and the fish immune response. We have developed several fish cell lines (from seabream, sea bass, European eel or Senegalese sole) susceptible to NNV, antibodies for localization studies and transcriptomic databases, useful as research and diagnostic tools. These tools allow us to increase their applications to other host, marine viruses or, even, other fields.

2. Deepening of the antiviral immune response of fish. We are identifying and characterizing, among others, the involvement of antimicrobial peptides (AMPs) and cell-mediated cytotoxicity as two of the most important antiviral immune mechanisms. Thus, we are able to analyze levels of antibodies and AMPs, or the adaptive cellular cytotoxic activity in fish, novel techniques that are routinely applied in our laboratory.

3. Effect of culture conditions and welfare on susceptibility to NNV. We are characterizing the lethality and presence of the virus in survivors and its success on vertical transmission under different culture conditions, size, or sex of different species susceptible to NNV. We have evidence that survival is more dependent on size than on culture conditions, but the presence of the virus in survivors is not.

4. Design and generation of treatments against NNV. We have developed several effective NNV vaccines in previous projects. We are currently developing the application of sea bass AMPs as antiviral treatments in aquaculture. So far, we have already shown that 4 AMPs derived from sea bass can be applied both as preventive and therapeutics of the disease produced by NNV in sea bass fingerlings.

5. Study of the impact of emerging contaminants on the antiviral immune response. We have evaluated the possible effect that some contaminants, mainly nanoplastics, may have on the immune response of fish and their resistance to NNV infections.

Conclusions.
In short, our group aims to investigate the fish health status and well-being with interests for the aquaculture sector. We mainly focus on the prevention and improvement of the resistance against nodavirus infections. To this end, we have generated various very interesting biotechnological tools that are exploited through material transfer agreements with research centers and companies, which clearly and decisively adhere to the objectives of the ThinkInAzul program.

Acknowledgments.
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INDUCED SWIMMING ACTIVITY MODULATED IMMUNE PARAMETERS IN THE SKIN MUCUS OF EUROPEAN EEL (*Anguilla anguilla*)

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Introduction

It is well known that exercise promotes health in mammals (Pascoe et al., 2014). However, in farmed fish, the physiological effects of swimming activity are often overlooked. Swimming is a fundamental behavioral element that contributes to fitness in most fish species. In fact, reduced swimming performance has been associated with poor disease resistance and welfare (Castro et al., 2013). The current study aimed to address the lack of knowledge on swimming physiology by investigating the effects of induced swimming activity on immune parameters in European eel (*Anguilla anguilla*), a commercially important species for aquaculture in Europe (FAO, 2023).

Materials and Methods

Sixteen European eels (total length: 39.9 ± 0.7 cm; body weight: 108.8 ± 6.1 g) were equally distributed between two treatment groups: swimming and non-swimming (control). In the swimming group, fish were placed individually in acrylic tubes (length: 67.0 cm; width: 8.5 cm) and induced to swim at 0.3 BL s^{-1} (n = 8) for 7 hours. In the control group, fish were also placed individually in acrylic tubes, but kept at minimal water flow (< 0.1 BL s^{-1}; n = 8). Immediately after the trial, fish were anaesthetised with MS-222 (0.1 g L^{-1}). The skin mucus was collected by carefully scraping the dorso-lateral surface of the fish with a cell scraper. The collected skin mucus was shaken vigorously, and centrifuged (2,000 × *g*, 10 min, 4 °C). Blood samples were collected immediately with heparinised syringes *via* the caudal puncture. The blood was centrifuged (10,000 × *g*, 5 min, 4 °C) to obtain plasma. Cortisol, glucose, and lactate levels were determined in plasma, while lysozyme, peroxidase, protease and antiprotease activities were measured in both plasma and skin mucus. Data were analyzed by unpaired t-test to determine differences between groups. The level of significance used was $P < 0.05$ for all statistical analysis.

Results and Discussion

Some studies have associated induced swimming, at moderate to high speeds, with an enhanced immune response in farmed fish (Liu et al., 2018; Hou et al., 2022). Although, the immune responses in fish plasma remained unchanged in the current study, the lysozyme, peroxidase and protease activities increased in the skin mucus in the swimming group. The skin mucus plays an important role in fish, as it acts as the first defence barrier, preventing pathogen attacks and contains several antimicrobial factors (Dash et al., 2018). In addition, fish skin mucus is an important factor in swimming performance as it reduces water resistance (Bernadsky et al., 1993). However, the possible effects of swimming activity on the structure and composition of skin mucus remain to be elucidated. The increased enzymatic activity observed in our study shows how skin mucus activity can be modulated by swimming. The European eel is a catadromous species, characterized by its endurance swimming performance, showing sustained and steady swimming during migration. While plasma glucose and lactate levels remained unchanged, cortisol levels were lower in the swimming group. Some studies have already shown that swimming can alleviate chronic stress by lowering circulating cortisol levels (McKenzie et al., 2021). Thus, swimming may contribute to optimize immune status in eel, as evidenced by the increased activity of immune-related enzymes in skin mucus.

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Conclusions

In this study, the results demonstrated that induced swimming caused an increase of immune-related enzymes in skin mucus. As migratory species, their efficient swimming abilities are essential to travel long distances and through different habitats, ensuring successful performances and development during migration. In aquaculture, understanding and optimizing swimming conditions is crucial for promoting growth and maintaining overall welfare, contributing to sustainable and profitable farming practices.

Acknowledgments

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EVALUATION OF THE BIOAVAILABILITY OF POLYPHENOLS FROM *Salvia lavandulifolia* VAHL. BY-PRODUCTS USING A GASTROINTESTINAL MODEL OF GILTHEAD SEABREAM (*Sparus aurata* L.)

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Introduction

In recent years, the aquaculture industry has focused on the use of agro-industrial by-products as a potential source of bioactive compounds, being some of the more interesting those produced after the distillation of aromatic plants. Although these residues present wide industrial applications as sources of polyphenols (Sánchez-Vioque et al., 2018), their potential use by the aquaculture industry its being only recently evaluated (Orso et al., 2022). However, optimal dosage of dietary polyphenols related to their biological responses requires an accurate estimation of their actual bioavailability, which is affected by a number of factors, such as the physical conditions used during feed preparation, the interactions with other components of the feed matrix and the biochemical transformations taking place within the gastrointestinal tract. One powerful tool frequently used to assess these aspects in humans and terrestrial animals are *in vitro* digestion models. In the case of aquatic organisms, models simulating the conditions existing within the gastrointestinal tract have been extensively used to assess bioavailability of key nutrients in wide range of feed ingredients, but to date they have been not applied in an equivalent form to study that of bioactive compounds (Martínez-Antequera et al., 2023). Considering this, the present study was aimed to evaluate the impact of different factors that may affect the digestive bioavailability of polyphenols present in the distillation by-products of *Salvia lavandulifolia* (Spanish sage) using an *in vitro* system simulating the digestive tract of gilthead seabream (*Sparus aurata*).

Materials and Methods

The sage polyphenolic extract was obtained from distillate dried leaves subjected to water extraction at 1:150 ratio (w/v). After filtering, the solution was lyophilized and the dried extract was added, at a proportion of 2.5%, into simplified feed matrix simulating a feed for seabream. Once prepared, different *in vitro* digestibility tests involving both stomach (acidic) and intestinal (alkaline) stages of digestion were carried out. For this purpose, membrane bioreactors similar to those described by Morales & Moyano (2010) were used. Each bioreactor consists of a digestion-leaching cell consisting of two chambers separated by semi-permeable membrane. Sea bream digestive enzyme extracts and substrates (feed matrix containing the plant extract) were added to the upper chamber and kept under continuous stirring by a magnetic stirrer. The products released during the reaction time that passed through the membrane into the lower chamber (digestates) were recovered in falcon tubes at different time intervals by a constant flow of the same alkaline buffer. These products were kept frozen (-80 °C) until extraction and subsequent analysis of the released for the qualitative and quantitative analysis of the polyphenolic profiles in both, the sage aqueous extract and the release phenolic compounds, a HPLC-DAD method previously described by Lozano-Perez et al. (2023) was applied.

Results and Discussion

A total of eleven compounds of different chemical nature were identified in the primary plant extract, which can be grouped into phenolic acids, flavonoids, and derivatives of these in their glycosylated forms. Among the identified compounds, rosmarinic acid and hesperidin stand out as the main phenolic and flavonoid compounds, representing a 52% and 23 % of the total identification, respectively. Other compounds corresponding to salvianic acid and luteolin-7-*O*-glucuronide were also identified reaching levels close to 10%. These results, at qualitative level, are in agreement with those reported by Lozano-Pérez et al. (2023) and Sánchez-Vioque et al., (2018). However, at quantitative level, differences in concentrations could be

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related to the source of the sage by-products and even to the extraction method applied. Regarding the polyphenolic profile of the products recovered after *in vitro* digestion, a total of six polyphenolic compounds were quantified and they presented a quite different bioavailability under the changing pH conditions of the gastrointestinal phases and also depending on total digestion times. Luteolin-7-O-glucoronide is the higher release rate flavonoid 14.9% while the phenolic acids reach around an 8-10%. The results evidenced that potential bioavailability of ingested polyphenols is greatly depending on their chemical nature and on the different factors involved in their digestive processing within the digestive tract of aquatic organisms (Martínez-Antequera et al., 2023).

Conclusions

The methodology carried out has made it possible to confirm that, the use of *in vitro* digestive modelling together with high pressure liquid chromatography, are adequate tools for determining the bioavailability of polyphenols of a sage extract, along with the characterization of the compounds released under different gastrointestinal conditions in the simulated digestive tract of gilthead seabream. Thus, as a first approach, *Salvia lavandulifolia* water soluble extract could be proposed as a bioavailable feed additive in *Sparus aurata* diet. Deeper studies need to be developed in order to confirm this statement.

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IMPACT OF THERMAL CHALLENGE ON IMMUNE SYSTEM IN ATLANTIC BLUE CRAB (*Callinectes sapidus*)

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Introduction

The recent expansion of Atlantic blue crab (*Callinectes sapidus*) has generated great concern in the Mediterranean Sea because is considered a non-indigenous species which would represent one of the greatest biological issues (Marchessaux *et al*., 2023). In the field of aquaculture, the crustacean segment, encompassing approximately 8.7% (equivalent to 10.5 million tons) of the overall aquaculture harvest, assumes considerable significance. Crustaceans only have an innate immune system, which constitutes the humoral and cellular defence against pathogens or different situations (Bouallegui, 2021). These defences are activated in a cooperative way to initiate effective defensive actions and can be classified according to two main responses: i) a humoral-mediated response involving humoral factors produced and released by the immune cells; and ii) a cell-mediated response that requires a direct action of the cell itself, such as phagocytosis, initiating the cell death pathway mechanisms. Several authors have been studied how environmental factors (temperature, pH, salinity, etc.) affect molecular parameters and immune-related enzymes (e.g., phagocytic activity, phenoloxidase, etc.). For instance, immune response to several stress challenges (e.g., salinity and temperature) have been studied in relation to how family Portunidae modulate their responses under pathogen challenges (Lei *et al*., 2022). However, the study of the immune response of the family Portunidae is still limited. Therefore, the aim of the present study was to assess the impact of an abrupt change in temperature on the immune response of Atlantic blue crab to detect potential biomarkers of animal welfare to improve their integration into aquaculture.

Materials and Methods

Twenty specimens of Atlantic blue crab (343.66 ± 162.94 g body weight) were obtained from the fish market (San Pedro del Pinatar, Murcia, Spain) and carried to the Marine Facilities at University of Murcia (Spain). The crabs were randomly separated in two groups, each group (n=10) was introduced into a tank with individual compartments for a single crab each one with containing 30 L of water and 7 cm of sterile sand sharing water and filtration (rack system). The racks systems were previously adjusted to 20 ºC (unchallenged) and 30 ºC (challenged) and the samples collected at 3, 6, 12, 24 and 48 hours after challenge. As time zero, haemolymph samples were collected 48 hours prior to thermal challenge. At each experimental time, 2 mL of haemolymph were extracted and the total haemocyte count and the cell´s percentage populations were determined. Haemocyte lysate supernatant (HLS) was used to measure the total quantity of protein and several immune-related enzymes activities. Data were analysed by One-way ANOVA ($p < 0.05$).

Results and Discussion

The cells numbers in the haemolymph did no show variations between the experimental groups. However, the number of haemocytes increased at 30 min in giant river prawn *(Macrobrachium rosenbergii*) exposed to 34ºC for 2 h (Chang *et al*., 2015). The percentages of hyaline and semigranular cells showed no change between groups. Contrarily, the values of hyaline cells varied between experimental groups at 30 and 60 min in giant crayfish (*M. rosenbergii*) (Chang *et al*., 2015), although at 120 min the levels did not show variations in line with our findings. In the case of granular cells, the numbers decreased in the challenged group at 12 h of trial. Chang et al. reported differences in this type of cells at 30 and 60 min, however, is difficult to extrapolate these results since their experimental time was only 2 h. The amount of protein present at intracellularly and extracellularly levels no showed variations between experimental groups. Contrarily, Matozzo *et al*. (2011) observed variations in plasma protein concentrations in Mediterranean green crab (*Carcinus aestuarii*) exposed to an increase of temperature to 30ºC (2ºC per day, 7 days). Our results showed no variations in phenoloxidase activity between experimental groups. These outcomes align with the results obtained in Mediterranean green crab (*C. aestuarii*) which were challenge with an increase of water temperature to 30ºC (2ºC per day, 7 days). (Matozzo *et al*., 2011). These results could suggest that a sudden and progressive rise in temperature does not cause variations in the phenoloxidase concentration in HLS. In our study, no variations in cell-free haemolymph lysozyme levels were observed between the experimental groups. However, a thermal shock in Pacific white shrimp (*L. vannameii*) showed variations in the lysozyme values at 3 and 6

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hours, as well as a decrease at 12 h compared to time 0 (Peregrino-Uriarte *et al*., 2012). In the case of esterase, our results no reported variations between the experimental groups. The modulation of esterase activity mediated by some type of stress in crabs has not been studied until now. Finally, the antiproteases are molecules capable of neutralizing excess proteases which lyse proteins in the animal. In our study, variations between both experimental groups at 24 h were observed. Therefore, the activity of antiprotease under thermal challenge could provoke a modulation of the protease activity in the cell-free haemolymph of Atlantic blue crab. This may be due to the protection that these molecules provide to the proteins that at high temperatures can be affected by the action of endogenous proteases.

Conclusions

This study aimed to investigate how a thermal challenge could modulate the immune system of the Atlantic blue crab to found potential biomarkers of animal welfare to improve their integration into aquaculture. However, we could emphasize the relevance and the need to deepen the related studies in the field of crustacean immunology to solve certain challenges and improve the animal welfare of this group of invertebrates in the field of aquaculture.

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ASSESSMENT OF HAEMATOLOGICAL PARAMETERS, BIOCHEMICAL METABOLITES, AND GENE EXPRESSION IN RAINBOW TROUT (*Oncorhynchus mykiss*) FED LEAD-DIET

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Introduction

The accumulation of heavy metals that are absorbed in living organisms throughout the food chain can confer a hazardous impact on human health. In the case of fish, they can accumulate heavy metals such as lead (Pb) from the water and diet (Alsop et al., 2016). According to literature, even low concentrations of metals due to synergistic action can be toxic to organisms and can reduce or eliminate species from an ecosystem due to adverse effects on the immune system, decreased fecundity or mortality (Butrimavičienė et al., 2021). Therefore, the aim of this study was to evaluate the effects of Pb-diet evaluating haematological and biochemical parameters in order to demonstrate defense pathways in response to toxicity. In addition, several genes involved in detoxification and inflammation processes were evaluated as well as the organo-somatic indices in rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Seventy-two specimens (160 g +/- 10 g average weight) of rainbow trout, obtained from a local hatchery (Cuenca, Ecuador), were randomly divided into six (three groups in duplicate) experimental group tanks (12 fish in each tank, 750 L pool). Three experimental groups were established as follows: i) Unexposed group (control) fed diet with 0 mg of lead kg^{-1}; ii) Exposed to 120 mg of lead kg^{-1}; and iii) Exposed to 240 mg of lead kg^{-1}. The animals were fed at a rate of 3% during 21 days. The organ somatic indices (OSI) for liver and spleen were calculated. The number of red blood cells (RBCs) and white blood cells (WBCs), as well as the determination of haematocrit (Ht), and haemoglobin (Hb) were evaluated. The quantification of Glucose (GLU), Cholesterol (CHO), Bilirubin Total (BILT), Direct Bilirubin (BILD), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) were determined. For gene expression, RNA extraction was performed using the SV Total RNA Isolation System and revers transcribed using ipsogen® RT Handbook QIAGEN. Total RNA was treated with DNase I to remove genomic DNA, Real-time PCR reactions were conducted in a 96-well plate using the Applied Biosystems 7500. A factorial ANOVA analysis was conducted to compare the impact of lead exposure in diet at 15 and 21 days.

Results and discussion

The values of OSI did no show variations in liver and spleen of exposed fish. Our results showed variations in the values of RBC, Ht, Hb, MCH, MCV, and MCHC which progressively decreased as the concentration of Pb increased. Our results agree with previous data in several fish species, the number of WBC was increased in blood of fish exposed to 120 and 240 mg of Pb kg^{-1} at 15 days. The levels found in the liver enzymes showed a disordered behavior compared to other studies. For instance, the ALT values showed decreased in correlation with the increase in Pb at 15 and 21 days of exposure. The values of AST increased, which is directly related to the increase in values of other biomarkers tested such as BILD, BILT and CHO, which increased as toxicity augmented to 120 and 240 mg of Pb kg^{-1} at 15 and 21 days. These results could suggest that these enzymes are non-specifically altered in the liver after short-term exposure, damaging the tissue which would elicits a pathogenic response in liver enzyme activity. Variations in CHO concentration could be due to the hazardous effects of Pb on the cell membrane. Thus, the cholesterol levels could be good indicators of environmental stress in fish as has been previously reported (Heydarnejad et al., 2013). Exposure to higher concentration of Pb resulted in an increase in GLU values compared to the unexposed group at 15 days. However, at 21 days of exposure, the levels of GLU decreased in these group. The values of GLU showed a similar behavior compared to previous studies in rainbow trout where the decline in GLU is most likely a result of experimentation and the exhaustion of energy stores (Ricketts et al., 2015). Thus, rainbow trout exposed to Pb showed a peculiar behavior in terms of transaminase enzymes, increasing AST/TGO values, and decreasing ALT/TGP values. This fact could suggest that these variations could be used to determine the levels of damage in liver tissue and cell functions. In this study, the expression of *mt-a, mt-b, cyp1a, cyp2k1, cyp2m1*,

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and *cyp3a27* genes liver were inversely correlated with the increase in Pb and the exposure time. In this sense, Klaassen et al. (1999) commented that this could be due to metal-induced damage to metal transcription factor (MTF-1) alleles, resulting in the constitutive silencing, of MT-I and MT-II gene expression. In this study, the expression of cytokines such as Interleukin 21, TNF-alpha, Interferon 1, and Interleukin 6 were analyzed in the spleen, which showed an inverse correlation with the increase in Pb concentration and exposure time. The results obtained are in line with the results obtained by Zhang et al. (2020), where the expression of *il-21* gene showed the ability to induce the expression of various cytokines, including *ifn-γ, il-10, il-6, tnf-α* and *il-1β*, indicating the pleiotropic effect of *il-21* gene in immune response.

Conclusions

In conclusion, this study revealed that exposure to Pb in diet had significant effects on the hematological, biochemical, and molecular parameters of rainbow trout. More specifically, Pb exposure led to alterations in the RBC, Hb, and Ht levels. The toxicity level was directly proportional to the increase in values of AST, BILD, BILT, CHO, and GLU, while a decrease was observed in the values of ALT, indicating liver damage. Furthermore, Pb exposure activated detoxification genes such as *mt-a, mt-b, cyp1a, cyp2k1, cyp2m1*, and *cyp3a27* in an inversely correlated manner. The inflammation-related genes, such as *il-a21, tnf-α, inf 1*, and *il-6*, showed both direct and inverse alterations in the spleen and head-kidney. Inflammatory processes were found to be severe after 15 and 21 days of exposure to this heavy metal. Our results suggest that testing protocols need to be established for rainbow trout to prevent contamination by heavy metals. This will contribute to promoting healthier food chains and restoring farms and hatcheries in the paradisiacal area of Cajas (Ecuador).

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COMPARATIVE REGULOMICS GIVES INSIGHTS INTO THE CONSERVATION AND EVOLUTION OF REGULATORY ELEMENTS FOLLOWING WHOLE GENOME DUPLICATION IN SALMONIDS

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Introduction

Whole genome duplication (WGD) is widespread in eukaryotes, and has been linked to phenotypic diversification during evolution. The common ancestor of salmonids underwent a lineage-specific WGD event ~100 million years ago and a large proportion of the genome is retained in duplicate, offering an ideal vertebrate system to understand the role of WGD in genome evolution. The huge commercial importance of these species to aquaculture further demands improved understanding of genome function and regulation, which is still poorly understood. In the current study, we make extensive use of the functional annotation data generated through the European AQUA-FAANG project, including 0.6 billion ATAC-Seq and 4 billion ChIP-Seq reads, to investigate duplicated regulatory elements in the genomes of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). The objective was to examine the conservation of regulatory element activity through ontogeny in both species.

Methods

Duplicate-aware whole genome alignments including Atlantic salmon and rainbow trout were generated with Cactus (Armstrong *et al*, 2020) to align the duplicated syntenic regions in both species. The genome was broken into syntenic blocks before alignment (Gundappa *et al.* 2022). Northern pike (*Esox lucius*) was included in the alignments as a closely related outgroup to the salmonid-specific WGD. High-confidence ATAC-Seq peaks (open chromatin regions) representing multiple stages of embryogenesis, and six adult tissues at two stages of sexual maturation, were overlapped with the Cactus alignments. The coupling of sequence and regulatory element conservation in open chromatin regions was established with respect to duplicated and orthologous regions.

Results

Our alignments captured a high proportion of duplicated and orthologous sequences across the genomes of Atlantic salmon, rainbow trout and northern pike, validating the robustness of our approach. After cross-referencing open chromatin regions with these alignments, we split the data into distinct categories according to their conservation between the two salmonid species (Fig. 1). The proportion of open chromatin regions overlapping both duplicated sequences retained from WGD increased across embryogenesis, being highest at the late somitogenesis stage, and was variable across adult tissue types, with brain showing the highest proportion (Fig. 1B). Reciprocally, we identified the lowest proportion of open chromatin regions in singleton sequences (i.e. where the other duplicated sequence was lost) at the equivalent stage of development and tissues (Fig. 1B).

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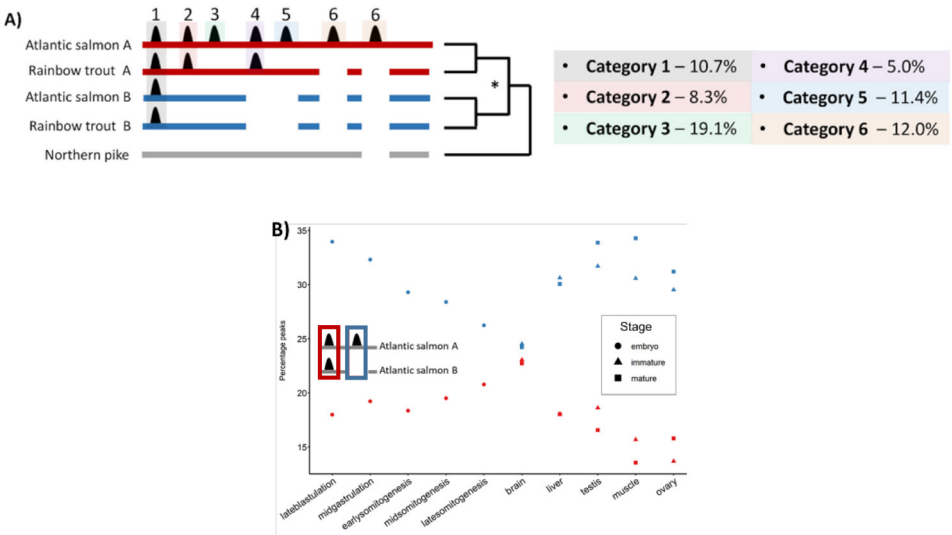

Fig 1. A. Illustration of genome alignment overlapping orthologous duplicated regions with six observed categories of open chromatin conservation. A and B represent duplicated regions retained from ssWGD B. Proportion of open chromatin regions in duplicated or singleton sequences of the Atlantic salmon genome according to different sample types.

Discussion

Our results validate the use of genome alignment to understand the dynamics of regulatory element activity across the duplicated genomes of salmonids. Our results are broadly consistent with the hourglass model of development (Duboule, 1994), suggesting highest evolutionary constraints on gene regulation during the phylotypic stage, a pattern previously observed across species, but not in relation to WGD. We are currently overlaying chromatin state annotations generated by ChromHMM to understand the co-evolution of specific regulatory element classes (e.g. promoters/enhancers) and duplicated gene expression across different stages and tissues in both salmonid species. In addition, we are linking the open chromatin regions to conserved non-coding elements of different evolutionary ages. The results of this work, by revealing conserved regulatory elements linked to salmonid phenotypes, will support the uptake of functional genomic information into salmonid genetics and selective breeding approaches supporting sustainable and profitable salmonid aquaculture.

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IMPACTS OF FEEDING REGIME VARIABILITY ON THE FILLET QUALITY AND HEALTH OF ATLANTIC SALMON (*Salmo salar l.*) FARMED IN NORTHERN NORWAY

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Introduction

A challenge in any fish farming is to ensure that the feeding (meals) of the fish is optimized to ensure optimal growth. The feeding strategies of Atlantic salmon are plastic and determined by physical factors such as day length, temperature, and season variations. Although different approaches can help obtain satisfactory results, drawing bombastic conclusions in feeding studies with salmon can be very difficult. There is a limited understanding of the best feeding strategies for better performance, health, and welfare of post-smolt Atlantic salmon reared in sea cages. The objective of the present study was to establish whether different feeding regimes would be more effective in understanding the best feeding practices in the sea, supporting salmon health.

We tested the hypothesis that diverse feeding regimes (feeding frequencies and percent overfeeding) in different seasons will affect fish growth and welfare, resource utilization, and quantity of feed waste. Our approach was to avoid underfeeding (which inhibits growth) and excessive overfeeding (which increases feed waste) to achieve best practices for the feeding process in Atlantic salmon.

Material and Methods

The feeding trial was conducted for 41 weeks at LetSea's R&D sea facility in Northern Norway between June 2022 and March 2023. Large smolts used for the trial were obtained from Kvarøy Smolt, Norway. At the start of the trial and with a mean weight of 700 g, the fish were randomly distributed amongst 12 marine net pens (5x5x5m; 125 m3) at an abundance of 150 fish per pen. The experiment was performed in triplicates, where all the cages were fed simultaneously with conventional industrial feed.

There were four study groups (3 replicate/group); fish of a particular group were fed with one of the following feeding strategies (FS): **FS1** (2 meals a day + 0% overfeeding), **FS2** (restrictive feeding; 1 meal a day + 0% overfeeding), **FS3** (1 meal a day + 10% overfeeding) and the control group **(CF)** consisted of 2 meals and 10% overfeeding, with the purpose of investigating production efficiency, health, and quality parameters. Fish were fed using automatic feeders (Betten, Norway).

At the end of the experiment, all fish were weighed, and welfare scoring was performed. Fat and color, melanin/pigmentation, gaping, and texture of the end product were compared between the groups. Furthermore, muscle histology and product quality samples were also collected. Fat and color analysis were performed using Near-infrared spectroscopy (NIR- DS2500) and digital Salmofan.

Results and conclusions

Our study shows that all four explored feeding strategies were well accepted by the fish, which means the fish were eating well, indicating good growth. We did not observe any significant differences) in weight gain, TGC, and feed efficiency (TGC values over 3 and FCR values below 1). Temperatures ranged between 11 and 9°C from late June and mid-November and between 7 and 4°C from late November to mid-February. Oxygen saturation was stable and sufficient with regard to season throughout the study. We did not observe any corresponding trend in the feeding responses with respect to changes in temperature and oxygen. Results of effects on the product quality will be presented at the Conference.

In summary, this study suggests a non-restricted feeding regime as an effective tool in obtaining a healthy Atlantic salmon of good quality and desirable growth during the 10 months period of a typical commercial production cycle. The study shows that a chosen feeding strategy can have an impact on several growth and quality parameters in Atlantic salmon. Further research is recommended in order to describe the mechanisms underlying these observations.

THE EFFECT OF DİFFERENT DRYING METHODS ON THE ANTIOXIDANT ACTIVITY, PHYCOCYANIN QUALITY AND DNA PROTECTION OF *SPIRULINA* (*Arthrospira platensis*)

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Introduction

Drying method can affect the amount of functional component of Spirulina. Spirulina culture, which was developed in different nutrient media prepared with geothermal water by producing Spirulina under greenhouse conditions with 420 L volume of geothermal water, was dried by different methods and analyzed.

Material and Methods

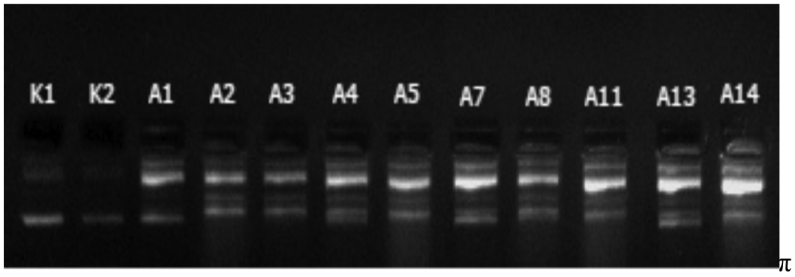
Wet biomass harvested at the end of the trial; It was dried in two different ways: spray drying (110°C) and conventional method in a fan oven (40°C). The resulting product was named "dry biomass" or "Spirulina flour". In the products obtained, phycocyanin, antioxidant capacity, anti-radical activity, flavonoid and phenolic component content and DNA protection were determined.

Results

The best DNA protective activity was obtained according to the band appearance of A13: 50% Pear Geothermal (40 °C Traditional Drying) and A14: 50% Pear Geothermal (105 °C Spray Drying), which represents 50% Pear groups. The bands expressing the DNA protection of the products are presented in Figure 1.

The phycocyanin quality of Spirulina dried in 2 different ways is presented in Table 1. Total antioxidant level, total oxidant level and anti-radical activity level are presented in Table 2.

The correlation between total antioxidant level, total oxidant level and anti-radical activity level and nutritional elements of water is presented in Table 3.



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Grups	TAS (mmol/L) 40 °C Dry	TAS (mmol/L) Spray Dry	TOS (mmol/L) 40 °C Dry	TOS (mmol/L) Spray Dry	IC50 40°C Dry	IC50 Spray Dry
Schlösser	1,9662	1,8352	0,021517	0,004363	0,616	0,522
%25 Geothermal	2,5209	1,588	0,005949	0,021021	1,074	0,435
%50 Geothermal	4,3333	3,921	0,006049	0,074368	0,001396	0,858
%75 Geothermal	3,2958	1,0636	0,005652	0,023699	0,658	1,162
%100 Geothermal	1,23359	1,543	0,006148	0,027268	0,491	0,714

OFF-THE-SHELF AI VISION TECHNOLOGY SUPPORTING SHRIMP BIOMASS ESTIMATION ACROSS THE ATLANTIC

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Introduction

The strict control of animal biomass and physico-chemical parameters within cultivation tanks plays a fundamental role in shrimp farming activities. Accurate biomass estimation is crucial for efficient feeding planning and reducing environmental impacts. Off-the-shelf AI solutions may be an important ally for biomass estimation within aquaculture applications. This can avoid time-consuming animal weighting activities, free up professionals for other activities and contribute to more sustainable and efficient production. This work presents a case study on the practical deployment and validation of an off-the-shelf AI vision solution for shrimp biomass estimation within the Atlantic Area. The main challenges and advantages are discussed from a user and technological perspective.

Materials and Methods

Description of the data Acquisition setup

The video footage is acquired inside the cultivation tanks with a high-definition wide-angle camera tied to two lighting units protected by a waterproofed acrylic cage (Figure 1). This device is fully immersed in the water and the camera is positioned at a distance of 10cm from a checkered background. The system has been designed to mitigate the impact of high turbidity on the image quality as follows: (a) it used a short distance between the camera and background, and (b) two lighting units to improve conditions.

Dataset Description

Sixteen video recordings were acquired at the IMTA lab, totalling 37 hours of video footage. We have extracted 9032 images of shrimps, out of which 6180 images were annotated with bounding boxes and pixel coordinates for a set of anatomical landmarks such as the shrimps' eyes and the root of their tails.

Description of the data processing architecture

Our solution's software architecture can be splitinto three modules. The first module consumes images and produces pixel coordinates of anatomical landmarks. It relies on a Region-based Convolutional Neural Network (R-CNN) as described by Kaiming He et al. (2017). The second module consumes the pixel coordinates of the detected landmarks to assess the average length of the shrimps by combining the distances between several pairs of landmarks. The third module consumes the estimated average size of the shrimps to assess their average weight. This module relies on a length/weight polynomial model fitted on ground truth data. Called sequentially, these three modules can give an estimate of the shrimp's average length and weight from a stream of images. The choice of this architecture has been driven by the fact that the shrimps are rarely entirely visible within video footage. Working with body segments allows us to extract size information even if the animals are partially masked. A user feedback session is conducted after the first set of technology deployments in a relevant environment.

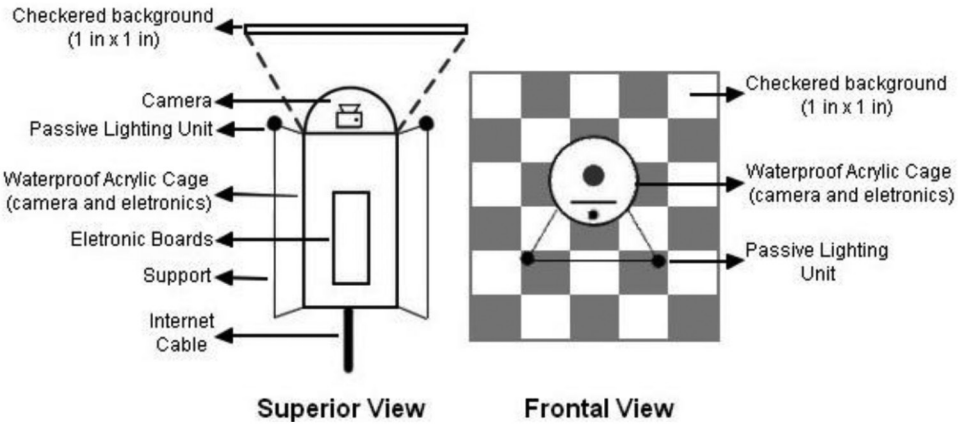

Fig. 1 - Setup for data acquisition

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Results and Discussions

The solution described above has been applied to estimate the animals' weight and size in a cultivation tank. The models provided relative errors of 8% for the length and 17% for the weight. Some experiments are ongoing to investigate how model performance is affected by environmental conditions and animal size. The user feedback meeting aimed to discuss technology usefulness and weaknesses to best consider the application needs during technology development. The main weakness is the lack of control over the number of animals passing in front of the camera. It prevents the exploitation of two-thirds of the resulting video footage. We discussed two possible approaches to circumvent this issue: (1) attaching the camera to a feeding plate usually used by the IMTA lab within the cultivation tank; (2) using a smaller tank custom-made to have optimal recording conditions (no turbidity, controlled lighting, 100% of the tank fitting in the camera's field of view). The second approach requires sampling shrimps from the cultivation tanks and temporarily moving them to the "data acquisition tank" to carry out the biomass estimation. The IMTA lab highlighted the usefulness of the AI vision technology for shrimp biomass estimation, even considering a more controlled data acquisition setup.

Conclusions

Our work highlights that computer vision and deep machine learning are useful tools to perform biomass estimation even when reliable data collection from cultivation tanks becomes challenging because of high level water turbidity.

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COMPUTER VISION-BASED URCHIN BIOMASS ESTIMATION: A CASE STUDY IN SOUTH AFRICA IMTA FARM

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Introduction

Measuring the total biomass of aquaculture production is a critical aspect of aquaculture management as it enables farmers to monitor the growth and development of their aquatic organisms, adjust feeding regimes for optimal growth, and plan harvest schedules (Chen et al., 2022). Although a common practice, manual assessment of biomass estimation at aquaculture farms is time-consuming and usually relies on weighing a sample percentage of animals which may be sensitive to physical disturbance (Khanjani, 2020). The present case study investigates a practical deployment of image-based computer vision systems to support urchin biomass estimation at an Integrated Multi Trophic Aquaculture (IMTA) farm in South Africa. It also discusses the main challenges, requirements and possible limitations to best support aquaculture applications.

The urchin Tripneustes Gratilla is not native to South Africa but is a commercially valuable species for the far Eastern export market. This is one of the new fast-growing, high-value species investigated at the ASTRAL South Africa IMTA laboratory at Viking Aquaculture's Buffeljags aquaculture farm on the south coast of the Western Cape. The urchins are grown in open baskets placed in large seawater tanks (Figure 1) with active aeration and water circulation to simulate the dynamic inter-tidal conditions in which urchins occur naturally. The prototype developed estimates the diameter of individual urchins in the images using computer vision. The advantages of a computer vision system for urchin diameter estimation include standardization of the process, reduction of skilled operators, and reduced stress on the animal compared to manual measurement procedures. It is a non-invasive tool aligned with animal welfare principles for successful harvests and profitability. The laboratory aims to estimate urchin diameter with up to 10% relative error.

Methodology

Towards the development of automated measurements of sea urchin size using computer vision sensing, images of the growing urchins are captured monthly. For the development and calibration of the computer vision algorithm, the urchins are photographed underwater in a rigid basket designed to reduce background unevenness in the mesh, therefore providing a consistent reference for calculating scale. Each photographed urchin is measured for its corresponding diameter and mass. In the development phase, measurements for training and validation require that the urchins are removed from the water to measure their diameter using calipers and are placed on a small scale to be weighed. The removal of the urchins from the water is performed only in the development phase of the algorithm, as the computer vision system intends to avoid physical disturbance of the animals in an operational setting. The computer vision algorithms were developed using the Python programming language. Figure 2 illustrates the image processing block diagram.

The image processing step allows the animal diameter to be estimated based on the scale information provided by the crop basket screens/mesh. The HSV color space represents the images. A boundary operation delimits the pixels representing an urchin and allows the removal of the image background. Blur and morphological operators (erosion and dilation) remove noise from the image and improve the representation of the animal. Next the HoughCircles algorithm detects the urchin outlines. Then another step of the algorithm is performed to calculate the circumference of the urchin's body without the spines. Figure 3 shows the step-by-step of the algorithm.

Results and Discussions

Table 1 presents the results obtained in the current version of the algorithm. The results were divided into two groups, one group comprises the images that do not have ulva over the urchins, and the other group has ulva over the urchins. Ulva is a type of sea lettuce used as food for the urchins and is therefore frequently present in their environment, often sticking to the urchin's spines. The dataset of the current version contains 33 images. The Ulva make processing the images more difficult, resulting in lower accuracy in the diameter estimation.

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Figure 2

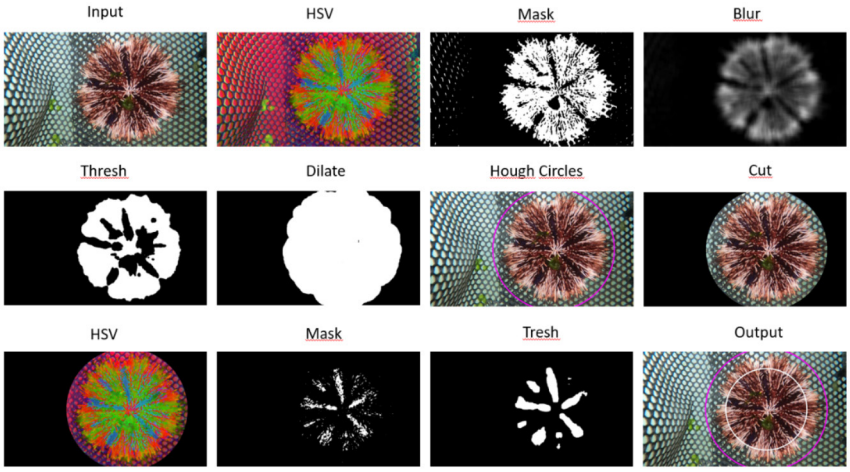

Figure 3

Table 1

	No ulva	With ulva
Error	9,9%	17,1%

So far, improvements are being developed for estimating the diameter of the urchins; after obtaining a better accuracy in this estimate, the next step is to improve the algorithm for estimating biomass, which will have as input parameter the calculated diameter. The technology supports urchin biomass estimation avoiding time-consuming and manual processes. It may also contribute to animal welfare through a non-invasive image-based system.

Acknowledgements

This work is part of the ASTRAL (All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture) project. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement Nº 863034.

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SUPPORTING AQUACULTURE APPLICATIONS WITH LOW-POWER DATA COMMUNICATION: A CASE OF STUDY ON MULTITROPHIC FARMING WITH LORAWAN TECHNOLOGY

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Introduction

Monitoring key physico-chemical parameters is critically important to support the expanding aquaculture industry that provides a sustainable source of seafood for people worldwide. Uncontrolled conditions may lead to severe impacts on animal health and growth rate, compromising entire cultivation systems. Although a common practice, manual assessment of physico-chemical parameters is disadvantageous to aquaculture applications in which delayed actions to control water quality account for most of animal loss cases (Lafont, 2019). Using LPWAN (Low Power Wide Area Networks) technologies may become an ally for monitoring physical-chemical parameters. This technology enables communication between radiofrequency (RF) devices with low energy consumption. Among the existing LPWAN networks, LoRa technology is a promising alternative for aquaculture farms. The LoRaWAN network layer comprises a complete solution for managing and integrating sensors into the end-user application. This work investigates the system issues associated with the practical deployment of an end-to-end cloud-based system (a) coupled with long-range low-power communication protocol (b) intended to monitor key physico-chemical parameters within an Integrated Multi Trophic Aquaculture (IMTA) farm in Brazil.

System overview

The deployed system aims to monitor key physico-chemical parameters - Dissolved Oxigen (DO), Temperature, Salinity, Conductivity, Turbidity and Ph - within a multitrophic cultivation tank from an IMTA farming facility located in Rio Grande – RS, Brazil. The system architecture (Figure 1) consists of a LoRaWAN network. The system end-node comprises four sensors, a microcontroller, and a LoRa radio with enabled LoRaWAN protocol. The AC powered end-node collects sensor measurements every 5 minutes and sends the data to a central gateway through LoRaWAN radio communication. The LoRaWAN gateway directs the received LoRaWAN packet (sensor readings) to an AI Data Analytics platform (named AIDAP), which is a cloud-based platform that integrates different sensors, allowing easy data access and visualisation for early alarm systems.

Deployment Results and Discussions

The system has been deployed in the IMTA LAB Greenhouse 5 (tank 1). The sensors were strategically positioned 1 m from the aeration tubes and 70 cm deep. The LoRaWAN gateway has been installed 35 meters away from the tank. Once operational, the end-to-end cloud-based system could presentthe collected data in real-time through the end-user application interface. Before the technology deployment, the IMTA lab farmers used to carry out manual physico-chemical measurements twice a day. The LoRaWAN system provided 240 sensor readings per day, representing a significant contribution to best support aquaculture applications. The technology deployment enabled the end user to early detect and mitigate parameter variations through an integrated cloud platform, for instance, DO levels as presented in Figure 2. The deployed system and user validation have been key assets in the farming facility in avoiding production loss and impacts on animal welfare. A series of user feedback meetings guided the technology design and optimisations to better meet IMTA's day-to-day needs. Upcoming optimisations include local data backup, self-powering capability and a customised alarm system.

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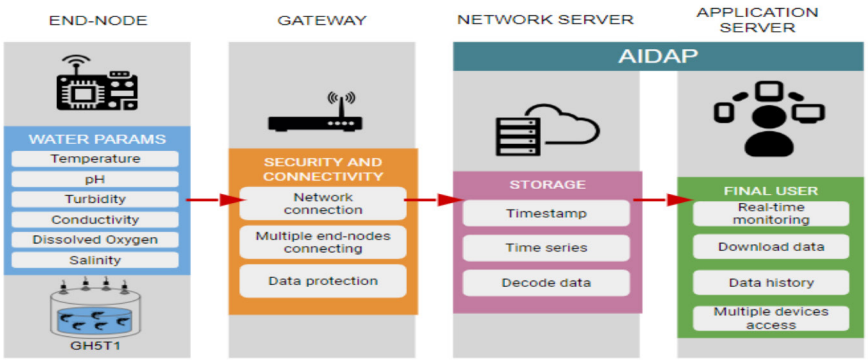

Fig. 1 - LoRaWAN network structure for the system.

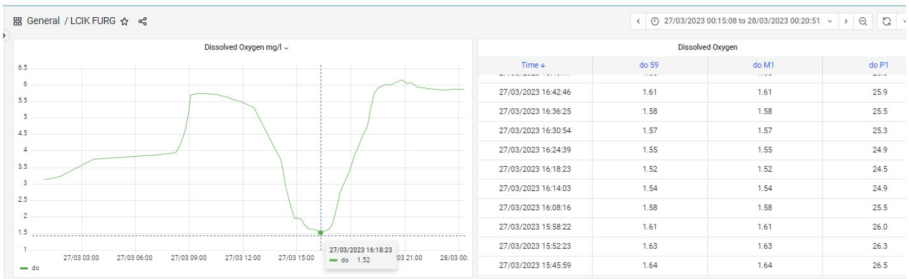

Fig. 2 - Dissolved Oxygen levels variations.

Conclusions

This case study addressed the feasibility of implementing a cloud-based end-to-end system and a LoRaWAN network architecture to support aquaculture day-to-day activities. The system monitors physicochemical parameters of the cultivation tanks in a representative IMTA facility in Brazil. The system delivered satisfactory results for quasi-real-time physico-chemical monitoring. It allowed early detection of parameter variations through a cloud-based platform. The immediate mitigation action played an important role in preventing impacts on aquaculture production and animal welfare. The end-to-end system optimised the data acquisition process, centralizing and standardizing the data collection. It also increased data acquisition frequency, best supporting aquaculture management. The user feedback provided valuable insights to guide technology optimization. A few system limitations could be established with this practical deployment, including areas of difficult access which can lead to data communication issues (e.g. packet loss). Such limitations will be addressed in future versions of the system.

Acknowledgements

This work is part of the ASTRAL (All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture) project. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement Nº 863034.

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GENETIC GAINS TAILORED TO YOUR NEEDS

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Selective Breeding is the process of improving one or more desirable traits of a cultured species through the selection of superior parents for the next generation. A breeding program is the implementation of a selective breeding strategy and the set of tools needed to deliver the desired outcomes. The approach selected should be designed to maximize the economic return by balancing input costs and with the expected genetic and economic gains for a commercial aquaculture producer.

In this talk we will discuss the general concepts and common strategies for breeding program management, from the simplest requiring the least amount of investment to the more complex with more investment required but greater genetic gains delivered. The aim of the talk is to provide aquaculture producers with the key elements to enable the informed assessment of the options for new or improved breeding program designs, and how they can tailor their program and genetic gains to their needs. The three general options for enhanced selective breeding management are: 1) Mass Selection managing diversity and inbreeding, 2) Family based selection and 3) Genomic Selection. These management strategies should be used to build upon a good genetic foundation. It is recommended to assess the genetic base at the beginning, or before changing the strategy, of any breeding program.

Key to assessing genetic diversity and to more sophisticated breeding strategies are genomic tools. The recent development of industry-wide, single nucleotide polymorphism (SNP) genotyping panels from 200 to 50,000 SNPs provides access to such tools at a very reasonable cost. When jumping from the basic to the complex, investment can be expected to increase with the need for genotyping, tagging equipment and supplies, more complex data collection, organization, and analyses, in addition to the training of personnel. While it is possible to change from a mass selection program directly to a genomic selection program, or to transition to a family-based plan on the journey to increased genetic progress. In all cases, training of personnel, and staged build-up of infrastructure and capabilities will be part of the process.

In summary, there are multiple options for enhanced selective breeding program management, each requiring different inputs and investment with varying potential returns and gains. Key to the selection of a genetic improvement design is the consideration of individual program's breeding goals, capacity, and available budget as well as the selection of the appropriate tools to support such a design.

EFFECTS OF TEMPERATURE, SALINITY AND DIET ON FATTY ACID COMPOSITION OF THE RAGWORM *Hediste diversicolor* (OF MÜLLER, 1776) (ANNELIDA: NEREIDAE)

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Introduction

There is an urgent need in food and feed production to change from linear systems towards circular, recycling based solutions. Many wastes or rest raw materials from linear systems are in fact valuable side streams containing precious compounds. Sludge from land-based aquaculture is such a side stream which comprise faeces and uneaten pellets. A second readily available side stream is the solid phase remaining after biogas production commonly known as "solid biogas digestate" (SBD). Both are nutrient- and energy rich and require handling at the production site, hence the question arises if these side streams can be utilized in a more sustainable way. The common ragworm *Hediste diversicolor* is an omnivorous, burrowing polychaete showing potential as an extractive species in IMTA systems. Both SBD and aquaculture sludge have proven to be suitable feeds for polychaetes, but growth rates can be lower than for worms fed high quality diets such as formulated fish feed (Wang et al., 2019a). It has recently been shown that this species has the capacity for endogenous production of omega-3 long-chain polyunsaturated fatty acids (Kabeya, et al., 2020), however it is yet not understood how environmental cues affects this ability. We conducted two sets of experiments to assess the combined effects of diet, temperature and salinity on total body fatty acid composition in *H. diversicolor* juveniles.

Materials and Methods

Polychaetes (*H. diversicolor*) were collected at low tide at the mud flat of Leangen Bay, Trondheim, Norway (63°26'24.5"N, 10°28'27.7"E). To investigate the effects of diet and temperature on fatty acid (FA) composition of *H. diversicolor*, worms were fed mixes of solid biogas digestate (SBD) and salmon aquaculture sludge (SS) along a 4-step feed gradient ranging from pure SBD to pure SS, and a 5-step temperature gradient ranging from 5.8 to 17.1 °C, for 15 days, using fish feed (FF) as a control. A second experiment was conducted to investigate the effects of salinity and temperature on the same variables. Here, the worms were fed the diet which yielded the highest growth rates in the first experiment (33:66 % SS:SBD) along 5-step salinity- and temperature gradients ranging from 5 to 40 ppt and 7.7 to 17.9 °C, respectively, for a duration of 28 days. In both experiments, worms were fed isonitrogenous diets equalling 30 % of the worms' total body nitrogen per day (Wang et al., 2019). Both experiments were conducted in a temperature

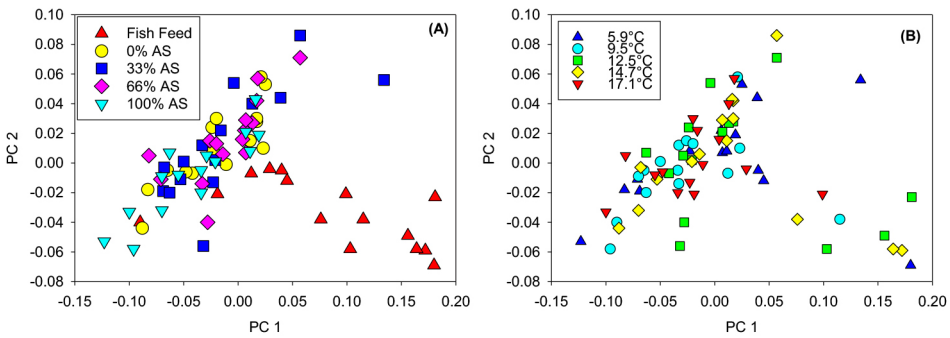

Figure 1: Principal Component Analysis (PCA) on total fatty acids composition of *H. diversicolor* fed different diets at different temperature visualized by (A) feed type (% AS in the diet or fish feed and (B) by the five different rearing temperatures (°C). PC1= 56%, PC2= 14%

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gradient table modified after Thomas et al. (1963) using a 18h:6h light:dark cycle. Worms (n=7-8) were stocked in glass beakers (800 mL) containing an 8 cm thick layer of sand and filled with sand- and bag-filtered (1 µm) seawater from the Trondheim fjord collected at 60 m depth. The worms were allowed to evacuate their guts in clean seawater for minimum 4 hours before each sampling and weighing. Water exchange and feeding was conducted every second day. Data analyses were performed using the inbuild statistical package of SigmaPlot v.14.5. Principal component analysis (PCA) on fatty acid composition of polychaetes was performed using PRIMER 5.2. Fatty acid levels were expressed as % of total fatty acids and were arcsine transformed before entering the PCA.

Results and Discussion

The main differences between the FF and side stream diets were the high concentrations of EPA and DHA in fish feed. EPA accounted for 15% of total fatty acids in FF, and about 1% in both, AS and SBD. DHA accounted for 8.5% of total fatty acids in FF, while it accounted for 2% in AS and 3.5% in SBD. Further, AS was richer in 16:0 (28% *vs.* 18% in SBD and 20% in FF), and it was also richer in 18:1n-9 (25% vs. 21% in SBD and 13% in FF). In sum, FF contained a lower percentage of saturated fatty acids (SFA) compared to AS and SBD (32% *vs.* 41%, respectively), and in turn a higher percentage of unsaturated fatty acids. No temperature- nor salinity-driven segregation patterns could be identified in the FA profiles of the polychaetes, however a clear diet-driven segregation was found between worms fed lipid-rich fish feed (control) and lipid-poor SS and SBD diets. The fatty acid composition of polychaetes fed side stream diets showed high concentrations of EPA (14–19%) comparable to the polychaetes fed fish feed (13%). Further, the percentage of PUFA was high in both (38–40%) also comparable to those fed fish feed (46%). However, DHA was higher in polychaetes fed fish feed (5%) than in polychaetes fed aquaculture sludge and SBD diets (1–2%). The pronounced differences in fatty acid composition found in the different feeds were not as pronounced in the polychaetes reared on these diets (Fig. 1). We here demonstrated that short-term (≤4 weeks) alterations of environmental parameters have neglectable effects on the fatty acid profile in wild caught *H. diversicolor* juveniles. Hence, our results indicate that the major influence on fatty acid composition in *H. diversicolor* is diet.

Acknowledgements

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OPTIMIZATION OF AEROBIC DIGESTION CONDITIONS OF SOLID WASTE FROM RECIRCULATING AQUACULTURE SYSTEMS FOR PRODUCTION OF LIQUID FERTILIZERS

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Introduction

The aim of the project was to test aerobic digestion as a potential environmentally friendly and sustainable bioprocess for the treatment of solid organic waste from recirculating aquaculture systems (RAS) used for rearing of African catfish (*Clarias gariepinus*). A further aim was to optimize the conditions of this process to maximize the mineralization of organic matter for plant nutrition purposes. Thus, the outcome of the test would be to find the optimal conditions for aerobic digestion (pH, temperature, test durationte, addition of external bioculture) and compare their effect on the progress and final degree of waste organic matter mineralization in fish farming. The concentrations of each element (N, P, Fe, K, Ca, Mg, Zn, Mn, Cu) in nutrient solution produced in this way were compared with the values at the inlet of the experiment

Materials and Methods

The source of organic waste from the rearing of African catfish was an experimental RAS at the Department of Fisheries and Hydrobiology, Mendel University in Brno (Czech Republic). A self-made separator based on the principle of centrifugation and sedimentation of suspended solids in water (vortex) was installed to the already functional system. The raw sludge was dewatered using a hand press, thoroughly homogenized and preserved by lyophilization. Accurately weighed samples of freeze-dried faeces (10 g) were transferred into sterile glass containers and topped up with distilled water to a volume of 1 litre. These laboratory bioreactors were placed in a thermal incubator to maintain a constant temperature. An adequate oxygen supply was introduced into each bioreactor by aeration. Additionally, air bubbling ensured the mixing of the sample in water. Several factors were tested and evaluated. Temperature: three temperature constants of 20 °C, 25 °C and 30 °C were maintained, pH: pH values of 6 and 7 were maintained using a buffer. External bacteria: an external culture of micro-organisms for biological treatment of sewage sludge was added to one variant and their effect on the mineralisation efficiency was monitored. Time: the total duration of aerobic digestion was 21 days. For each temperature there was always a variant without buffer (control), without buffer + external bacteria, buffer pH 6 and buffer pH 7. Each variant was tested in triplicate. Water samples were collected regularly on days 1, 5, 9, 13, 17, and 21 of the experiment. The $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ contents were evaluated spectrophotometrically using a PhotoLab 6600 UV-VIS and other elements by ICP-OES method.

Results and discussion

$N\text{-}NH_4^+$ production showed an increasing tendency over time for the pH 6 variants at all temperatures tested. The highest $N\text{-}NH_4^+$ level (166 mg/l) was reached on day 21 of testing in the pH 6 variant at 25 °C. In all bioreactors without pH adjustment, $N\text{-}NH_4^+$ production was several times lower than in the case of pH adjustment at 6 and 7. $N\text{-}NO_3^-$ production was lowest in the variants with pH 6 at all temperatures tested, in contrast to $N\text{-}NH_4^+$. The lowest nitrification was observed in the bioreactor at pH 6 and 20 °C. The highest $N\text{-}NO_3^-$ value (101.27 mg/L) was obtained on day 17 of the experiment in the control bioreactor without pH adjustment and without external bacteria at 30 °C. However, at higher temperatures (25 and 30 °C), the $N\text{-}NO_3^-$ content in the solution decreased gradually. In contrast, the increase in $N\text{-}NO_3^-$ content was most evident for the pH 7 variants at all temperatures. The highest $N\text{-}NO_3^-$ content at the end of the experiment was reached in the bioreactor with pH 7 at 30°C. Mineralization of phosphorus (P) was more efficient at higher temperatures. The highest P content (99.93 mg/L) was found at the end of the experiment in the control bioreactor without pH adjustment at 30 °C. Also in the case of the other macro-elements analysed, mineralization proceeded best under these conditions. When comparing the bioreactors with pH adjustment, metal mineralisation was more efficient at pH 6 than pH 7, however, the most efficient mineralisation of metals was achieved in the control bioreactor already mentioned. In all bioreactors, the elements Cd, Pb, Co, Cr and Ni were below the detection limit. Mineralization of organic solid waste without pH adjustment at 30 °C seems to be the most promising. This experiment will be extended in the future by comparing the resulting organic fertilizer with a selected commercial mineral fertilizer based on a hydroponic test. The growth of leaf lettuce (*Lactuca sativa*) seedlings would be tested in the Nutrient Film Technique (NFT) hydroponic system for 35 days.

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Figure 1. Macronutrients content in the control bioreactor without pH adjustment at 30 °C at the end of the experiment

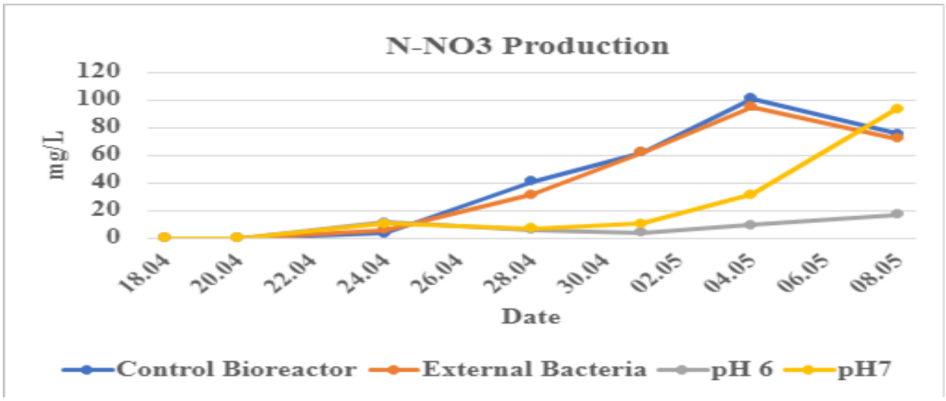
Macronutrients	**P**	**K**	**Ca**	**Mg**	**S**	**Na**
(mg/L	99.3	26.64	207.00	15.60	25.43	59.92

Figure 2. Micronutrients content in the control bioreactor without pH adjustment at 30 °C at the end of the experiment.

Figure 1. Time course of $N\text{-}NO_3^-$ content change during the experiment in all bioreactor

Micronutrients	**Fe**	**Cu**	**Zn**	**Mn**	**B**	**Mo**	**Si**	**Na**
(mg/L)	0.051	0.027	0.050	0.047	0.096	0.0062	9.86	59.92

variants at 30 °C



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THE GENOME REGULATORY LANDSCAPE OF ATLANTIC SALMON LIVER THROUGH SMOLTIFICATION

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Introduction

Atlantic salmon begin their lives in freshwater habitats before migrating to sea to grow and mature. To prepare for life at sea, salmon undergo behavioral, morphological, and physiological transformation, collectively known as smoltification. Key aspects of this life stage transition, such as remodeling of gill function, are well studied and known to be in part controlled by environmental queues like photoperiod (1). One key aspect of adaptation to life at sea is large-scale changes in lipid metabolism and energy homeostasis referred to as metabolic remodeling (2). Relatively little is known about this important seawater adaptation, specifically the timing of metabolic remodeling (pre-adaptation or reaction to life at sea), the impact of photoperiod on gene regulatory programs, and the role of epigenetic changes in smoltification of the liver.

To address these questions, we conducted a time course experiment where Atlantic salmon were exposed to either short- or long-day length, allowed to smoltify in freshwater, and transferred to seawater. We sampled the livers of fish at each key life-stage and measured changes in gene expression, DNA methylation, chromatin accessibility, and transcription factor binding. We test if photoperiodic history affects the smolt liver phenotype at the level of gene expression and use chromatin accessibility data to identify putative regulatory pathways and transcription factors (TFs) involved in life-stage associated changes in liver function from the juvenile stage in the freshwater environment to an adult fish in seawater.

Materials and methods

Atlantic salmon were reared on commercial diets throughout the experiment. Samples of liver were first taken 21 weeks after first feeding (week 1). Fish were then exposed to either “winter-like” (8 hours per day) or “summer-like” (24 hours per day) photoperiod for 10 weeks and sampled (week 10). Another sample was taken after smoltification in freshwater just before seawater transfer (week 19), and again after 6 weeks at sea (week 25). For each time point we performed RNA sequencing on four replicate fish. We also performed RRBS (DNA methylation) and ATAC-seq (chromatin accessibility) on two of the same individuals used for RNA-seq.

To identify gene expression patterns across smoltification we performed an ANOVA-like differential expression test between all four time points of fish exposed to artificial winter. We then generated expression clusters to identify sets of genes that are co-regulated across the smoltification gradient. To identify genes that respond to altered photoperiod, we performed an exact test across different photoperiod groups at weeks 10 and 19. To link DNA methylation status to gene expression during smoltification, we identified differentially methylated regions across the time course using an ANOVA-like analysis and correlated this to associated gene expression changes. To study genome wide transcription factor binding dynamics we identified dips in ATAC peak read depth at predicted TF binding sites (TF footprinting) across all time points.

Results

We find that metabolic remodeling is a preparatory adaptation to life at sea, as large-scale expression changes in metabolic genes occur primarily in freshwater smolts at week 19 before transfer to seawater. We observe a systematic reduction in expression level of genes with a metabolic function, such as lipid metabolism, and an increase in expression of energy related genes in pathways such as oxidative phosphorylation. We also find no impact of photoperiod history on smolt development in the liver in contrast to previous studies in gill. TF footprinting reveals key transcription factor binding dynamics and highlights ZNF682, KLFs, and NFY TFs as important for driving the metabolic shift in liver from parr-like to smolt-like. While genome-wide TF dynamics were highly correlated to observed smoltification related gene expression changes, we did not find a link between DNA methylation status and gene expression patterns.

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Discussion

We find that metabolic gene expression decreases in the liver of freshwater fish before transition to seawater. Given that the availability of polyunsaturated fatty acids is higher in seawater environments than freshwater, it is likely that this is a genetically programmed preadaptation to life at sea. This is the first report of smoltification associated DNA methylation and TF binding in liver of salmon during smoltification. Our observation that DNA methylation status is not linked to smoltification gene expression, but TF dynamics are highly correlated highlights the relative importance of chromatin accessibility and TF regulation in the smoltification process.

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MICROBIOTA AT THE WATER-FISH INTERFACE: THE CASE STUDY OF RAINBOW TROUT FED WITH A NOVEL FEED FORMULATION CONTAINING *Tenebrio molitor* MEAL

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Introduction

Sustainable aquaculture is dependent on a variety of factors, including water quality and effective feed formulations. To increase sustainability in aquaculture maintaining fish health, alternative protein sources to replace fishmeal (FM) should be used. Insects represent a new world of sustainable and protein-rich sources for farmed fish diets. The yellow mealworm (*Tenebrio molitor*) is one of the most popular insect species used in aquafeeds. The larvae from this insect are an excellent substitute for FM (Chemello et al., 2020; Terova et al., 2021). While great research efforts have been made to evaluate feed formulations, focusing especially on the effects on fish gut microbiota, few studies have explored host-environment interactions. Here, we evaluated the effects of a *T. molitor* -based novel feed formulation on the microbiota at the water-fish interface, in an engineered ecosystem farming rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Rainbow trout of about 80 g mean initial weight were randomly distributed into 400 L tanks (3 tanks/diet, 21 fish/tank) and fed with either a control diet without insect meal (IM) (diet A), or diets B, C, and D formulated with *T. molitor* larvae meal at 5%, 10%, and 20% inclusion, respectively. Fish were fed twice a day (at 8 am and 3 pm), 6 days per week. Water samples and water tank biofilm samples were collected to quantify bacterial DNA load by using qPCR. For each water sample, total nitrogen, nitrites, nitrates, ammoniacal nitrogen, and phosphate were measured with a spectrophotometer. At the end of the trial, six fish/diet were sampled from the groups fed with diet A (without IM), and diet D (with 100% FM/IM replacement). The skin mucus microbiota was obtained by gentle scraping of the fish body with a cotton swab, whereas the gut autochthonous microbiota was obtained by scraping the mucosa of the entire intestine. Using 16S rRNA metabarcoding, we comprehensively analyzed the microbiota of water inlet, water, tank biofilm, fish mucus, fish cutis, and feed samples. Raw sequencing data was processed by QIIME2. The Kruskal-Wallis H test for all and pairwise tests were used to compare the groups. Statistical significance between groups was determined by the ADONIS (permutation-based ANOVA, PerMANOVA) test with 10000 permutation-based Bray-Curtis.

Results and Discussion

The microbiological and chemical analysis of water showed no significant differences due to different feeds consumed by fish, proving that FM/IM substitution does not affect water quality. Microbiota analysis revealed the presence of a highly reduced core microbiota, constituted by *Aeromonas* spp., for both the control group and the novel feed test group. Looking at tank biofilm, *Acinetobacter*, *Pseudomonas*, *Rhodococcus*, and *Candidatus* Amoebophilus were significantly more abundant in tanks in which diet A was administered. The skin microbial community composition of two dietary groups A and D displayed distinctive features. A decrease of *Proteobacteria* and specifically of the *Deefgea* genus in the skin mucus-associated microbiota was found in trout fed with IM. Similarly, at the gut mucosa level, the dietary *T. molitor* meal inclusion led to a significant reduction of gut *Proteobacteria* phylum, predominantly belonging to the *Gammaproteobacteria* class. Furthermore, with respect to the control fish group, feeding IM led to a lower abundance of *Acinetobacter* genus, another potential pathogen in aquaculture, commonly known as a microorganism transmitting antibiotic resistance genes. Therefore, altogether our findings on gut microbiota indicate that feeding trout with IM has a positive effect through inhibiting the growth of potential Gram$^-$ pathogen bacteria. By network analysis, we showed that the major driver of microbial community structure was the sample source, with the main differences found between environmental vs host-associated samples. Network analysis indicated that samples clustered based on sample source,

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with no significant differences related to the feed formulation. Thus, different feed formulations seemed to not affect the environment (water and tank biofilm) and the fish (skin and mucus) microbiota. Trying to disentangle the contribution of feed at a finer scale, we performed a differential abundance analysis, and we observed differential enrichment/impoverishment in specific taxa, comparing the samples belonging to the control diet group and the insect-based diet group (Figure. 1).

Conclusions

Our results highlight a link between the environment and the fish, and subtle but significant differences due to feed formulation.

Acknowledgements

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IN-SITU MEASUREMENTS OF PARTICLE DYNAMICS IN A RECIRCULATING AQUACULTURE SYSTEMS (RAS) FACILITY – A CASE STUDY

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Introduction

The accumulation of suspended solids in recirculating aquaculture systems (RAS) is a major challenge, as they may affect the health of fish and rearing water quality negatively. In RAS, suspended solids are primarily introduced with the feed and consequently converted by biological processes, such as fish faeces excretion in the fish tank or by the microbiological processes i.e., the biofilters within the water treatment module. Commonly, suspended particles are reported as total suspended solids (TSS), given as dry mass concentration per volume (mg L^{-1}). In RAS, the TSS load strongly depends on the re-use rate, stocking density, fish species, feed quality and the treatment efficiency for solids removal [1]. At exchange rates below 10% per day, the majority of particles can be attributed to excreted faeces, uneaten feed and microbial growth, listed in decreasing order to the contribution to the total load [2]. However, TSS does not give any information on important physical parameters, such as the actual particle size distribution. To accurately determine the particle size distribution, an in-situ approach is necessary, as any sample handling will disrupt the fragile biogenic particles or floc together and thus bias the results.

Objectives

Despite the importance of limiting the concentration of suspended solids in RAS water, little is known about the actual particle size distribution (PSD), concentrations and/or dynamics found in large scale operated RAS facilities. This is important, as each RAS exhibits site-specific particle dynamics because the processes of settling, fragmentation and aggregation are influenced by the local flow conditions [1]. Thus, determining the site-specific PSD is important for optimizing the design of RAS, especially for effective solids removal.

Material and methods

This case study reports in-situ measured dynamics of particles in the range of 0.1 – 500μm in a RAS facility, measured by Laser In-situ Scattering and Transmissometry (LISST). PSD and associated volume concentrations were determined in a single RAS facility, operating two structurally identical RAS units, stocked with 81.000 and 85.000 kg Atlantic Salmon, and fed continuously for 24h with 360 kg and 352kg, respectively. The RAS water treatment module consisted of a drum filter with a mesh size of 60μm, up flow fixed bed biofilters, a CO_2-degasser and ozone treatment. Furthermore, the drum filter discharge was flushed to a plate separator for solids removal, while the effluent was led through an up-flow fixed bed denitrifying biofilter. LISST measurements were taken directly from the fish tank, before and after the drum filters, after the nitrifying and denitrifying biofilters, after the degasser, and at the effluent. However, the high- concentrated sludge discharge from the drum filters had to be diluted prior to LISST measurements. To estimate the mass concentrations of the volume concentration, water samples were taken in selected sites of both RAS and analysed for total suspended solids (TSS).

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Conclusions

Results of this case study provide an important first insight into the particle dynamics in RAS with identical construction and similar stocking/feeding loads, as well as daily operations. Results provide vital information for improving water and solids treatment design and understanding particle dynamics in RAS facilities.

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EFFICIENCY OF A WASHOUT STRATEGY WITH MICROALGAE MIXTURE OR FISH OIL ON RECOVERING THE OMEGA-3 FATTY ACID PROFILE OF EUROPEAN SEA BASS, *Dicentrachus labrax* FED WITH RAPESEED OIL

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Introduction

In the search for sustainable replacement of marine ingredients in aquafeed, relatively high levels of plant oils (PO) have been successfully included in the diet of freshwater and/or herbivorous fish species without affecting fish growth or health as long as requirements of n-3 long chain polyunsaturated fatty acids (LC-PUFA) were covered by the basal levels of marine ingredients (Sankian et al., 2019). The level of feasible inclusion is however lower in marine and/or carnivorous fish due to their inability to desaturate and elongate C_{18} PUFA, rendering them more prone to n-3 LC-PUFA deficiencies (Tocher, 2010). Below a certain dietary level of fish oil (FO), the fatty acid (FA) profile of the fish fillets produced are affected by the richness of PO in C_{18} FA and their deficiency in n-3 LC-PUFA. However, concomitantly to the 'healthy' FA, FO may also bring persistent organic pollutants. Because of the more sustainable and lower cost of PO and their low contaminant concentrations, it makes sense to increasingly use PO in aquafeeds (Belanger-Lamonde et al., 2018) but a strategy must be applied to recover the n-3 LC-PUFA rich FA profile of fish fillet, so important for human health. Such strategy involves the replacement of PO by marine ingredients in finishing diets some months before the fish commercialization. These 'wash-out' strategies have been tested in several fish species using oils rich in n-3 PUFA such as FO or microalgae (AO). The aim of the present study using such strategy in a marine carnivorous fish species of great economic importance in the Mediterranean region, the European sea bass (*Dicentrarchus labrax*), was to investigate if the potential drawbacks of long-term feeding with plant oil can be reverted by switching the fish to FO or AO diets for 11 weeks before reaching their commercial size.

Materials and Methods

Two microalgal species, *Nannochloropsis sp. and Schizochitrium sp.,* were chosen for their richness in eicosapentaenoic acid (EPA, 4.5%) and in docosahexanoic acid (DHA, 27.2%) respectively. The three experimental isonitrogenous (44.5%), isolipidic (17.5%) and isoenergetic (21.8MJ/kg) diets consisted in a control diet containing 9% FO and 6% rapeseed oil (RO) (FO diet), one diet containing 3% FO and 12% RO (PO diet) and a microalgae diet containing 7.2% *Nannochloropsis*, 3% *Schizochitrium*, 3% FO and 9% RO (AO diet). The microalgae mixture composition includes not only lipids but also high amount of proteins so the amount of soybean concentrate and wheat meal of this diet were reduced compared to that of the FO and PO diets. Diets were formulated and produced by extrusion at HCMR.

Table 1: Fish growth performance, feed utilization and morphometric indexes

	FO-FO	AO-AO	PO-PO	PO-FO	PO-AO	*P* values
Wi	200.22±3.85	195.86±6.92	196.97±3.51			ns
WG	93.17±9.42	90.16±6.59	81.48±1.90	91.01±6.56	76.47±13.13	ns
FCR	1.52±0.11	1.56±0.06	1.68±0.03	1.53±0.07	1.76±0.21	ns
FA profile						
Sat	23.89±0.5^{c}	21.94±0.2^{b}	20.75±0.4^{a}	22.49±0.6^{b}	21.84±0.1^{b}	***
Monosat	40.08±0.7^{a}	40.66±0.6^{ab}	45.18±0.9^{c}	40.96±0.9^{ab}	42.22±1.0^{b}	***
ω-9	32.33±0.7^{a}	33.03±0.33^{ab}	38.16±0.8^{c}	33.71±0.9^{ab}	34.37±0.94^{b}	***
ω-6	14.96±0.92^{a}	17.28±0.5^{bc}	18.39±0.34^{c}	16.21±0.14^{b}	17.33±0.5^{bc}	***
ω-3	21.06±1.04^{c}	20.12±0.9^{bc}	15.69±0.9^{a}	20.34±0.6^{bc}	18.61±0.9^{b}	***
ARA	0.7±0.07^{ab}	1.0±0.22^{b}	0.5±0.16^{a}	0.7±0.13^{ab}	0.8±0.13^{b}	**
EPA	4.2±0.18^{d}	3.7±0.11^{bc}	2.9±0.12^{a}	3.9±0.05^{c}	3.5±0.13^{b}	***
DHA	12.1±1.1^{c}	11.6±0.7^{bc}	7.5±0.86^{a}	11.5±0.62^{bc}	10.1±0.8^{b}	***
ω-3/ω-6	1.4±0.14^{c}	1.2±0.09^{b}	0.9±0.03^{a}	1.3±0.04^{bc}	1.1±0.07^{b}	***

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European sea bass individuals of 198 g initial body weight were distributed in 12 nets of 0.84 m^3 in 4 cement 5 m^3 tanks in an open-flow system. Diets FO and AO were fed to triplicate nets while PO diet was supplied to 6 nets, all diets supplied twice daily to satiation over a period of 13 weeks. Thereafter, fish were allocated to new diets in duplicate nets for the washout phase of the trial which lasted for another 11 weeks (24 weeks in total). Fish fed FO or AO continued being fed the same diet while fish fed the PO diet were switched to FO or AO or maintained on PO. Feed consumed (g) was recorded daily. Fish were weighed individually at the beginning, intermediately and at the end of the experimental trial. The evaluation of the wash out strategy was done in terms of feed efficiency, fish growth and nutritional composition, FA profile recovery, digestive histology and immunology.

Results and discussion

The growth and feed utilization of the fish (Table 1) showed no significant difference between the different experimental groups at any sampling point, as all diets were well balanced and covered the basic needs of the fish. However, as expected, the FA profile of the fillets of all experimental groups mirrored the dietary profile. Indeed, after 24 weeks feeding with the PO-based diet, the FA profile was significantly affected with decreased levels of EPA and DHA to 69 and 62% compared to FO-fed fish respectively. The diet switch from PO to FO or AO diets 11 weeks before the end of the trial enabled the recovery of 93% and 83% omega-3 polyunsaturated fatty acids respectively.

The microalgae-based diet which may enable aquaculture professional to lower their dependence on fish oil and thus to lower the pressure on wild fish stocks, enabled partial recovery of the FA profile. Moreover, this AO diet increased the antibacterial activity of the fish sera with increased complement bactericidal activity in fish serum. Liver morphology was similar in all groups with slightly higher fat deposition in the PO group. Partial tissue recovery was observed in fish switched to fish oil or microalgae containing diets.

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ENHANCING MATURATION RATE OF ATLANTIC SALMON (*Salmo salar*) FEMALES THROUGH DIETARY MANIPULATION DURING GONADAL RECRUDESCENCE

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Introduction

The feeding practices in farmed salmon have traditionally relied on fishmeal and fish oil as primary protein and fatty acid sources due to their high bioavailability 1,2. However, the need for sustainability and the expansion of aquaculture necessitate the exploration of alternative resources to reduce dependence on marine ingredients and enhance feed efficiency and economic viability 3. To address this challenge, plant-based alternatives to marine ingredients have gained attention 4,5. In the salmon breeding industry, achieving high production of high-quality oocytes, embryos, and larvae is crucial, and the nutritional requirements of the broodstock play a fundamental role in this process 6,7. Nevertheless, the substitution of fishmeal and fish oil with vegetable ingredients, poses potential challenges to achieving optimal broodstock nutrition. Consequently, it is essential to assess the impact of dietary changes during gonadal recrudescence on reproductive efficiency and embryo quality in Atlantic salmon (Salmo salar) females, with the aim of optimizing the management of this species in freshwater environments.

Materials and methods

Four diets were employed in this study to assess their impact on the reproductive performance of Atlantic salmon (*Salmo salar*) during gonadal recrudescence. The diets included: marine ingredients diet (Diet 1); partial substitution diet where marine origin meals and oils were replaced by terrestrial animal and plant sources (Diet 2) and two commercially available Atlantic salmon broodstock diets used for comparison purposes (Diet 3 and 4). Each diet was administered to duplicate groups (circular tanks of 5,000 m^3 each) of 15 female and 5 male *S. salar* for six months during gonadal recrudescence. Upon reaching the spawning stage, several zootechnical parameters and reproductive characteristics were evaluated. These included the measurement of weight, length, condition factor (K-factor), hepatosomatic index (HSI), and gonadosomatic index (GSI). Additionally, spawn mass, absolute and relative fecundity, oocyte quality (diameter and hydration percentage), fertilization rate, and blastomere symmetry were assessed. Furthermore, the survival and quality of embryonic development were evaluated by examining specific parameters such as microphthalmia, spinal malformations, siamese twins,

Among the various zootechnical parameters assessed, including weight, length, K-factor, and GSI, no significant statistical differences were observed between the diets (Table 2). However, a trend was observed where commercial diets tended to exhibit higher weight and length compared to the experimental diets. Furthermore, Diet 1, which was based on ingredients of marine origin, displayed a significantly higher HSI compared to the other diets.

Discussion and conclusion

In conclusion, the lower maturation rate and subsequent decrease in the number of spawned females observed in the groups fed commercial diets may be attributed to a potential nutrient imbalance. It is noteworthy that these diets had higher gross energy content compared to the other experimental diets. This finding aligns with existing knowledge that low protein diets in carnivorous fish can extend maturation time, reduce reproductive performance, and decrease ovulation frequency [8].

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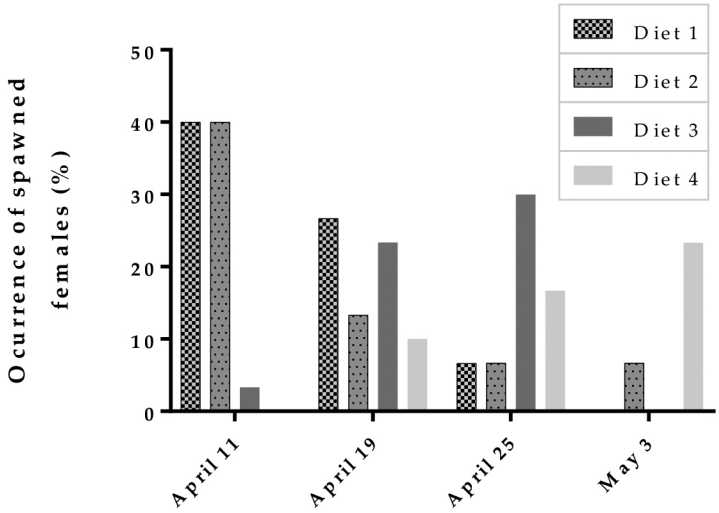

Figure 1. Frequency of mature Atlantic salmon females in each spawning period. The values presented are derived from the number of females that successfully spawned on the respective dates, divided by the total number of females considered in each diet throughout the trial (n=30 females per diet). Diet 1: Marine ingredients, Diet 2: partial substitution of marine origin ingredients with vegetable and animal sources, Diet 3 and 4: commercial diets.

Table 1. Linear regression equations for total fecundity (dependent) and spawn mass (independent). Confidence interval (95%) for the equation slope.

Diet	Spawn mass (g)	Total fecundity	R^2	Equation	95% CI	p-value
Diet 1[1] (n= 22)[2]	1210 ± 275	12271 ± 2748	0.800	y= 1429.17 + 8.92 (x)	[6.59, 10.35]	<0.001
Diet 2[1] (n= 20)[2]	1251 ± 337	13067 ± 3544	0.892	y= 609 + 9.96 (x)	[8.77, 11.88]	<0.001
Diet 3[1] (n= 16)[2]	1309 ± 442	13198 ± 4354	0.927	y= 803.53 + 9.46 (x)	[8.35, 10.42]	<0.001
Diet 4[1] (n= 15)[2]	1188 ± 268	12600 ± 2974	0.819	y=555.80 + 10.09 (x)	[7.69, 12.22]	<0.001
p-value*	0.64	0.82				

[1] The values shown are the mean ± SD
[2] Number of spawned females by diet

The significance of nutrition in gonadal development is crucial and extends beyond its effects on maturation. It also plays a pivotal role in other biological responses, such as fecundity. Hence, the results underscore the importance of reevaluating diet formulations for Atlantic salmon broodstock, as it holds the potential to enhance reproductive efficiency in the industry by increasing the number of mature females and improving embryo production.

Consequently, further research is warranted to investigate the optimal inclusion levels of nutrients, particularly energy, or the incorporation of other additives that can enhance the utilization of these nutrients within diet formulations. Exploring these aspects will provide valuable insights for optimizing the nutrition of Atlantic salmon broodstock and improving their reproductive performance in aquaculture.

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ALOE VERA NATURAL EXTRACT HAS A POSITIVE IMPACT ON ZEBRAFISH TISSUE REGENERATION AND IMMUNE RESPONSE AFTER CAUDAL FIN AMPUTATION

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Introduction

Aquacuture is a main economic activity worldwide and specially in Chile, where the farm fish production is very intensive, which expose fishes to conditions that facilitates the attack of ectoparasites which produce skin lessions that constitute potential entry points for secondary pathogens, increasing the risk of diseases and stress, therefore affecting animal welfare and also production [1]. In this context, tissue regeneration processes are fundamental and the use of natural compounds and extracts that could improve the animal regenerative capacity and immune system, is of great interest [2]. In this work we evaluated the benefits of Aloe vera (*Aloe barbadensis miller*, AV) extracts diluted directly in the medium and included as a food additive in different formulations with a protein base of animal and vegetable origin [3]. While is known that feeds with a vegetable protein base cause intestinal inflammation in several fish species [4] and that this effect can be reduced by AV [5,6], the impact of AV on tissue regeneration on wounds or other types of external insults, has not been investigated to date. To assess if AV administration improves the regenerative capacity on fish larvae, we applied a protocol to induce a wound and controlled tissue damage. To that end we took advantage of the zebrafish (*Danio rerio*) teleost model, and evaluated tissue regeneration, the cellular innate immune response and the transcriptional activity of genes implicated in these processes.

Material and methods

To quantify the impact of AV in tissue regeneration, a caudal fin and tail tip amputation protocol was used in zebrafish larvae exposed through immersion at 3 days post fertilization (dpf) and orally at 9 dpf. In the first instance, AV was administrated by immersion from 24 h before (pre AV) and until 3 days after (pre + post AV) amputation. Also 5 hours post amputation (hpa) the recruitment of phagocytic cells was quantified in the amputated area, and to further characterize the tissue response at the molecular level, we also evaluated transcriptional activity of genes implicated on immunity, regeneration, and oxidative stress by RT-qPCR. Finally, 5 days post amputation (dpa) the regenerated area was quantified. On the other hand, for feeding experiments, two basal diets were used: one with protein of animal origin (FM) and other with vegetable origin (SBM). In these experiments the amputation protocol was established at 9 dpf with 4 days of previous feeding, and the regenerated area was measured at 16 dpf (12 days of total feeding). Additionally, immune cells recruitment was quantified at 5-6 hpa.

To achieve this a zebrafish transgenic reporter line expressing the red fluorescent protein (DsRed) under the control of the Lysozyme C gene promoter, Tg(lysC:DsRed) was used. This reporter line allowed us to see and track *in vivo* leukocyte cells (macrophages and neutrophils) in the amputated area, and therefore to evaluate *in real time* the cellular response of the innate immune system after injury.

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Results and conclusions

The results of AV in immersion experiments showed a greater leukocyte arrival to the amputated area in both groups previously incubated with AV, the same groups also showed a significant increase in the regenerated area at 5 dpa in relation to the control group. The molecular analysis showed that by 6 hpa the transcription of inflammatory genes was increased in the pre incubated groups, as were the genes that code for antioxidant enzymes, compared to the control group. At 24 hpa, genes involved in regeneration processes were significantly increased in the pre AV and pre + post AV groups, while transcription of the inflammatory and antioxidant genes remained elevated. To stablish the protocol for diet trials, regeneration by 5 and 7 dpa was evaluated, obtaining significant differences between the basal diets FM and SBM at 7 dpa, where FM showed a greater regenerated area in the tail tip and caudal fin in relation to the SBM inflammatory diet. Later, the effects of a FM diet supplemented with AV (FM+AV) versus FM was evaluated, and results showed that regeneration is significantly higher in the FM+AV group. Similarly, a SBM diet supplemented with AV (SBM+AV) was evaluated, and we were able to detect that a greater recruitment of immune cells to the wound at 5 hpa, and a greater regenerated area in this group in comparison to the group feeded with SBM diet. Our results suggest that AV, both dissolved in the medium and added to feed formulations, enhances the regenerative process in zebrafish larvae, and triggers a significant immunostimulatory effect that could explain this enhancement in cell proliferation and tissue repair. Trials with diets and immersion are still ongoing to reproduce and extend our results and solve questions about how AV is producing this effects and how this extracts can be optimally included in fish farm practices to improve animal health and specially wound recovery after skin damage.

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EFFECT OF DIFFERENT PHOTOPERIOD ON GROWTH, SURVIVAL AND CANNIBALISM DURING WEANING OF EURASIAN PERCH *Perca fluviatilis* L. LARVAE REARED IN RAS SYSTEMS

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Introduction

Intensive perch culture in recirculating aquaculture system (RAS) provides optimal culture conditions for rapid fish growth, high survival rate, shorter production cycle, reduction of fish stress and cannibalism. In order to ensure a high productivity and reduced production costs, several rearing conditions have to be optimized. Larvae of percid species are strongly phototactic therefore, the varied photoperiod is a factor that can significantly affect the efficiency of rearing. Current knowledge in this matter for Eurasian perch larvae is still very limited.

Material and methods

Larvae from wild Eurasian perch spawners were obtained followed reproductive protocol and reared up to 13 DPH according to standardized procedure described by Palińska-Żarska et al. (2020). Afterwards, 28 days experiment was set, where four variants of different photoperiod (L:D – light:dark) i.e. 24L:0D; 20L:4D; 16L:8D and 12L:12D were tested. Throughout the entire rearing period, the intensity of light, measured at the water surface was 900 lux. The sudden weaning procedure at 21 DPH was used, 7 days after photoperiod variants were established. At the end of the experiment 30 perch post-larvae from each group were euthanized, individually weighed, measured and relevant growth parameters were assessed: coefficients of body length variation [CVL(%) = 100 SDL L^{-1}, where L is the mean body length and SDL is the standard deviation of body length]; coefficients of body weight variation CVM(%) = 100 SDW W^{-1}, where W is the mean body weight and SDW is the standard deviation of body weight]; Fulton's condition factor $K = 100\ W \times L^{-3}$, where W is the mean body weight and L is the mean total length]; specific growth rates SGR (% day^{-1}) = $100 \times (Ln{\cdot}WF - Ln{\cdot}WI)\ t^{-1}$, where WF is the mean final body weight and WI is the mean initial body weight (g); t is 28 days]. Moreover, cumulative mortality were calculated including the percentage of fish prey of cannibals. The data expressed in percentages were *arcsin* transformed before the statistical analysis. Data were compared using one-way ANOVA. A non-parametric Kruskal-Wallis' test was used to evaluate the differences in analysed parameters ($p<0.05$). Analyses were performed using Statistica software (StatSoft).

Results

The analysis revealed no statistically significant differences ($p<0.05$) between groups for final length and weight of larvae, Fulton's condition factor, SGR index, as well as in mortality (Table 1). On the other hand, such differences in the final values of coefficients of body weight and length variation were revealed with the smallest value recorded for the 20:4 photoperiodic regime.

Discussion

Lack of significant differences of perch larvae growth parameters reared under different photoperiod conditions suggest the limited influence of this abiotic factor on rearing efficiency. However, increased mortality of perch post-larvae after the change in the type of food and the violent transition from *Artemia* nauplii to artificial feed was confirmed. According to data Kestemont et al. (2015) perch larvae cultured under 24L:0D light conditions had significantly higher survival (56.2%) compared to those that had 12L:12D and 16L:8D photoperiod (45 and 49%), respectively, as well as on the final fish mean mass. It is likely that increased day length impacts the behaviour of both potential cannibals and potential prey. The day length did not significantly influence the survival of Eurasian perch but the proportion of mortality due to cannibalism was significantly reduced when day length was decreased from 24:0 LD to 8:16 (Kestemont et al. 2003; 2015) which was also reported in the present study.

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Table 1. The values (mean ± SD) of the studied indexes of perch larvae at the end of the experimental rearing. Data in rows marked with different letter subscripts indicate statistically significant differences between groups ($p < 0.05$).

parameter	group / photoperiod			
	24:0	**20:4**	**16:8**	**12:12**
initial body weight (g)	0.0047 ± 0.0012			
initial total length (cm)	0.71 ± 0.058			
final body weight (g)	0.151 ± 0.066	0.153 ± 0.045	0.138 ± 0.059	0.134 ± 0.063
final total length (cm)	2.39^a ± 0.32	2.46^a ± 0.22	2.23^b ± 0.32	2.30^b ± 0.31
Fulton's condition factor	0.61 ± 0.19	0.62 ± 0.13	0.60 ± 0.21	0.56 ± 0.18
CVL (%)	13.9^a ± 1.9	9.2^b ± 0.8	15.0^a ± 2.2	13.5^a ± 1.7
CVM (%)	53.0^a ± 23.1	32.3^b ± 10.0	52.5^a ± 24.8	56.0^a ± 22.8
SGR (% d^{-1})	12.06 ± 1.6	12.30 ± 1.06	11.73 ± 1.62	11.65 ± 1.53
survival (%)	40.4 ± 6.6	38.3 ± 10.2	38.7 ± 7.7	39.3 ± 4.9
cannibalism (%)	15.3 ± 4.6	11.8 ± 5.8	12.5 ± 6.0	10.7 ± 3.6

Acknowledgment

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ASSESSING THE PERFORMANCE OF WATERBORNE FEEDING SYSTEM IN A LAND-BASED POST-SMOLT FACILITY

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Introduction

Waterborne feeding systems have gained significant attention in land-based aquaculture due to their potential to improve feed distribution flexibility, reducing microplastic emissions and energy consumption. This feeding system involves delivering feed pellets through water instead of traditional feeding methods such as airborne feeding. By replacing the transporting medium with water, the system is gentler on the feeding pipes and reduce the breakage of feed pellets. Less microplastics is released, thus reducing the environmental impact from the facility.

These systems rely on pumps, pipes, and spreaders to distribute feed throughout tanks, allowing for precise control over feeding rates and reducing feed waste. Waterborne feeding systems can be automated, allowing for more consistent feeding and freeing up labor resources. However, waterborne feeding systems require technical expertise and specialized knowledge to operate and maintain, and can be subject to malfunction, potentially affecting fish health and growth.

With capacities of up to 50 kg feed per minute from each line, the opportunity to schedule feed based on peaks and lows in appetite during the day is far greater. This allows the growth potential to increase and feed conversion rate (FCR) to reduce.

Method

At Hofseth Aquas facilities in Tafjord, Norway, we have a permission of raising 2,000 tons in biomass of rainbow trout ranging from 125g to 1000g. The post-smolt facility contains six tanks with a size of 18m x 4,85m (1234m^3). Seawater from depths of 55m and 35m is treated with UV-filters which is used both in process and through the feeding-system. Water within tanks is renewed each hour using 80% new treated water, and 20% recirculated water using degassers.

Tests of waterborne feeding systems and adjusting numbers of feeding outlets to the tanks initiated in January and February. Two tanks had four feeding points installed, and two tanks had eight points installed. We observed visually and using submerged cameras how the fish behaved and how feed pellets were distributed from the feeding points.

To measure the effect of the feeding system, growth rate, feed conversion rate and energy consumption is compared to existing data from our facility which uses traditional feeding systems.

Results

One of the challenges with a waterborne feeding system has been its sensitivity to changes in water pressure. Malfunctions such as low water pressure can temporarily stop the system from operating and can result in lost opportunities for feeding and growth.

Distribution of feed through water has also been difficult compared to airborne distribution. The amount of feeding points in tanks had to be high enough to ensure a good distribution of the areal, while maintaining the water pressure for equal amounths of water and feed to reach each point. With fewer points of feeding, fish gathered in high density, creating empty areas in the tank. With more points of feeding, fish was distributed better in the tanks area, but feed pellet distribution was not satisfactory due to unequal water pressure.

A combination of a high amount of feeding points at different debts and reducing the pipe dimension to maintain optimal water pressure will be ready for testing in May, and results and further development will be presented at the conference alongside results of growth rate, FCR and energy consumption.

Paecilomyces variotii IN NOVEL FEEDS FOR ATLANTIC SALMON: EFFECTS ON PELLET QUALITY, GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND UTILIZATION, AND IMMUNE-RELATED BIOMARKERS IN THE DISTAL INTESTINE

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Introduction

Filamentous fungi are promising microbial ingredients (MI) for use in aquaculture feeds due to their high protein content and bioactive components. *Paecilomyces variotii* (also known as PEKILO® mycoprotein) is a β-glucan- and nucleotide-rich MI with a crude protein content of about 60-70% (Bajpai, 2017). The objective of the current study was to evaluate the effects of *P. variotii* produced from sulfite stillage derived from forest by-products on pellet quality and growth performance, nutrient digestibility and utilization, and expression of immune-related biomarkers in the distal intestine of Atlantic salmon (*Salmo salar*) reared in freshwater.

Materials and methods

Four isonitrogenous, isolipidic, and isoenergetic diets were formulated. Diet 1 was a control diet formulated with fish meal, soy protein concentrate, and wheat gluten meal as protein ingredients. Diets 2, 3, and 4 were formulated so that *P. variotii* replaced 5, 10, and 20% of the crude protein content of the diets, respectively. Groups of 40 fish (initial average body weight of 24 g fish^{-1}) were fed the experimental diets *ad libitum* in triplicate tanks for a period of 9 weeks. Daily feed intake in each tank was quantified by collection of uneaten feed using wedge wire screens. Fish were batch weighed at the start and end of the experimental period. Following the 9-week period, six fish per tank were randomly sampled and a small section of the distal intestine closest to the anus was collected and secured in RNAlater for gene expression analysis. Moreover, a pooled sampled of 20 whole fish at the start of the experiment and five fish per tank at the end of the experiment were randomly sampled for chemical analysis. The remaining fish in each tank were carefully stripped for fecal collection from the posterior intestine for determination of nutrient digestibility.

Results and discussion

Increasing levels of *P. variotii* in the feeds was associated with changes in physical pellet quality, including significant linear and/or quadratic decreases in pellet length, pellet width, expansion, and durability. Conversely, significant linear and quadratic increases in water activity, bulk density, sinking velocity, and water stability index were associated with increasing dietary levels of *P. variotii*. The changes in physical pellet quality may be attributable to the high total fiber and β-glucan content of *P. variotii*. There were no significant differences in weight gain, growth rate or feed intake among fish fed the experimental diets, but a significant linear improvement in feed conversion ratio with increasing dietary inclusion level of *P. variotii* was observed (Table 1). The apparent digestibility coefficients for crude protein and gross energy of the experimental diets decreased linearly with increasing dietary levels of *P. variotii*. Similar results were observed for the essential and non-essential amino acids. Nonetheless, linear increases in nitrogen, energy, and mineral retention efficiencies were observed with an increasing inclusion level of *P. variotii*. This has also been reported in salmon fed other MI including bacterial meal and yeast (Øverland et al., 2010; Øverland and Skrede, 2017). Interestingly, dietary inclusion of *P. variotii* resulted in significant upregulation in the expression of several cytokines (*tnfa*, *ifng*, *il10*, and *tgfb*), effector molecules (*inos*, *arg1*, *sod*), and transcription factors (*irf4*) in the distal intestine (Figure 1). These results suggest that *P. variotii* can induce the activation and control of the immune response through promoting pro- and anti-inflammatory responses, supporting immune homeostasis in the distal intestine, a mucosa-associated lymphoid tissue (MALT) (Morales-Lange et al., 2022).

Conclusion

Overall, replacement of up to 20% of the crude protein content of the feed with *P. variotii* improved feed conversion ratio and nutrient utilization efficiency of Atlantic salmon juveniles reared in freshwater. In addition, *P. variotii* also showed immunomodulatory effects in the distal intestine. *P. variotii* is a highly promising alternative ingredient for use in salmon feeds.

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Table 1. Growth performance parameters of Atlantic salmon fed the experimental diets with increasing inclusion level of *P. variotii*.

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	SEM[1]	P_{linear}[2]
Weight gain (g fish^{-1})	85.3	87.2	84.9	91.6	3.53	0.2599
Feed intake (g fish^{-1})	64.0	64.9	63.1	66.7	2.38	0.4750
SGR (% day^{-1})	2.37	2.40	2.36	2.46	0.05	0.2495
TGC [g$^{1/3}$(°C × day)$^{-1}$]	0.202	0.205	0.201	0.211	0.01	0.2724
Feed conversion ratio	0.75	0.74	0.74	0.73	0.00	0.0173

[1]Standard error mean.
[2]Significance of the linear orthogonal polynomial contrasts across experimental diets containing increasing inclusion of *P. variotii*.

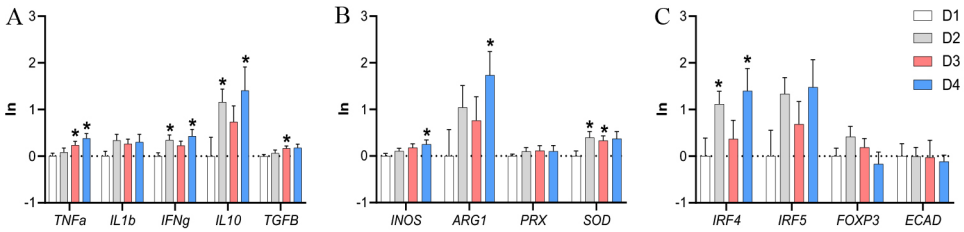

Figure 1. Gene expression of (A) cytokines, (B) effector molecules, and (C) transcription factors and surface molecules in the distal intestine of Atlantic salmon fed the experimental diets.

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A SYNTHESIS – STARCH, AN ANTINUTRITIONAL FACTOR FOR YELLOWTAIL KINGFISH *Seriola lalandi*

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Introduction

Farming yellowtail kingfish (*Seriola lalandi*) in recirculating systems is challenging due to its instable and fine faecal particles, which hampers the faecal solid removal. Accumulation of total suspended solids potentially affect fish health, system performance and environmental eutrophication negatively (Reid et al., 2009). Feeding yellowtail kingfish with raw natural feed items resulted in distinct faecal pellets and short strings (Horstmann et al., 2023). When these natural feed items were included (freeze dried and ground) in pelleted diets containing 15% starch, faecal pelleting was absent. A study with juvenile white sturgeon (*Acipenser transmotanus*) by Hung et al. (1990) suggested that starch could negatively affect the reabsorption of water in the distal tract due to the high water binding capacity or lead to fermentation processes (indicated by production of volatile fatty acids (VFA)), thus negatively affecting faecal characteristics. Three studies were conducted with the focus of investigating the effect of dietary starch content on fish performance, faecal removal efficiency (both by settling and drum filtration) and digestion kinetics along the intestine.

Materials and Methods

Two diets varying in starch content were produced by either including 0% or 20% gelatinized wheat flour. Juvenile yellowtail kingfish were fed restrictively (study 1 and 2 on DM basis, study 3 on crude protein basis). Study 1 and 2 aimed to investigate the effect of starch level on faecal removal efficiency by settling and drum filtration, respectively. Study 3 aimed to investigate underlying mechanisms for observed differences throughout previous studies.

Results

Study 1: Low starch diet had a higher faeces removal efficiency by settling by 62% compared to the high starch diet ($p < 0.001$; Fig. 1a). Fish fed low starch diet excreted faecal pellets, while high starch diet resulted in poor faecal integrity (Fig. 1b).

Study 2: Faecal removal efficiency by drum filtration was not affected by dietary starch level (Fig. 2a). However, to achieve similar removal efficiencies at high starch systems, the drum filter frequency had to be on average 210% compared to low starch systems

Study 3: Feed intake on crude protein and fat was equal among treatments, but fish fed high starch diets had a higher energy feed intake. High starch diets did not result in an improved growth, even high starch diet resulted in a numerically lower growth. High starch diets resulted in significantly higher chyme moisture content in the proximal and mid gut, but not in the distal gut. Butyric acid (VFA) was significantly higher at fish fed high starch diet.

Conclusion

Supplying additional energy in form of starch does not positively affect fish growth. High starch level induce a poor faecal integrity in yellowtail kingfish, hampering its removal by settling and drum filtration. Results study 3 suggests that this might be related to fermentation processes.

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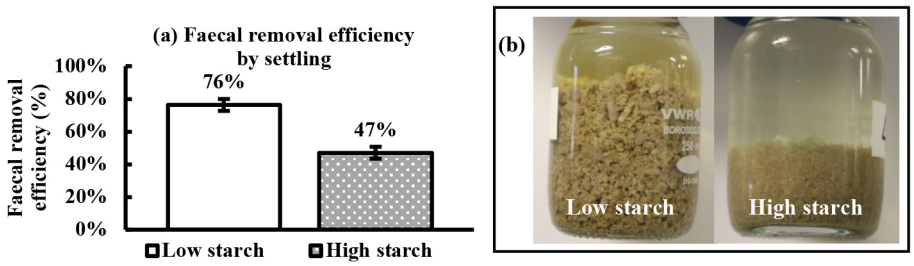
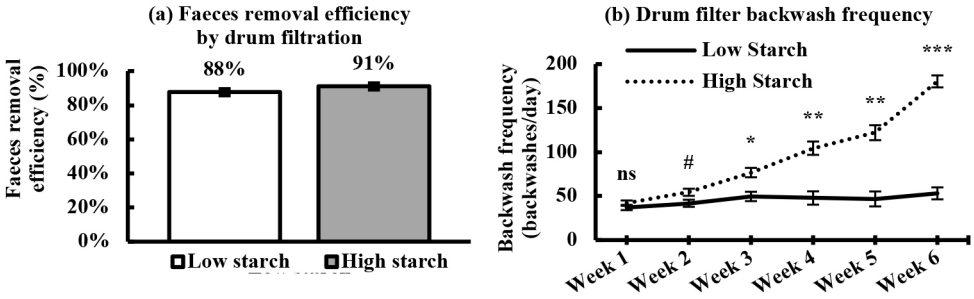


Figure 1. Study 1 – (a) Faeces removal efficiency by settling (%; $p < 0.05$) and (b) collected faeces of yellowtail kingfish fed low (left picture) and high starch (right picture) diet; error bars indicate standard error of means.



(Fig. 2b).

Figure 2. Study 2 – (a) Faeces removal efficiency by drum filtration (%; $p > 0.05$) and (b) Drum filter backwash frequency of yellowtail kingfish fed low and high starch diets; error bars indicate standard error of means.

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EVALUATION OF MICROBIAL GROWTH DURING STORAGE OF GILT-HEAD SEA BREAM, SHAOOR AND SALMON AND EFFECT OF COOKING ON FLESH COMPOSITION AND NUTRITIONAL VALUE

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Introduction

Worldwide, aquaculture represents about 44% of the total fish production (FAO, 2022) and it is estimated that about 70% of aquaculture production is leaning on commercial feed sources. Fish meal contains high levels of dietary essential amino acids and essential fatty acids (ω6 and ω3 highly unsaturated fatty acids) that promote rapid growth. Nutritionally well-balanced diets/feeds play an important role to ensure high/optimal fish performance, maintaining good health and minimizing waste production. The objective of the study was to evaluate the shelf life of three different fish (i.e. gilthead sea bream, shaoor and Atlantic salmon) during refrigerated storage and to investigate the impact of conventional cooking in an oven on the composition and nutritional value of fish flesh. For farmed gilthead seabream, two alternative diets were also investigated, based on 45/12 or 43/16 protein/fat ratio, in order to evaluate the effect of fat content in diet on the nutritional value of farmed fish.

Materials and methods

Farmed gilthead seabream (*Sparus aurata*) was obtained from Tabuk Fisheries Co., wild shaoor (*Lethrinus mahsena*) and imported Atlantic salmon (*Salmo salar*) were purchashed from the local marked (fish weight: 1 kg) and within 24 h samples were transported in polystyrene boxes with appropriate quantity of flake ice. For farmed gilthead seabream, two alternative diets were investigated, based on different protein/fat ratio, i.e. F1: 43/16 protein/fat ratio, F2: 45/12 protein/fat ratio, in order to evaluate the effect of FA compo-sition of fish feed on the nutritional value of fish flesh. Conventional cooking in an oven for 45 min at 180°C was considered as a representative meal preparation for the evaluation of the nutritional value of the final product. The total nitrogen content was determined on 200 mg of sample using a micro system of Kjeldahl method. The profile of amino acids was determined by LC-MS/MS. The profile of amino acids was determined by GC/MS. For shelf life evaluation, fish was isothermally stored at 4°C. At predetermined times, a representative sample of 90 g of fish flesh were homogenized with 225 mL sterilized Ringer's solution (Ringer tablets, Merck, Darmstadt, Germany) for 60 s with a Stomacher (BagMixer ® interscience, France). Samples (0.1 mL) of 10-fold serial dilutions of fish homogenates were transferred into the appropriate media on Petri dishes for the enumeration of Total Viable Count (TVC) and *Pseudomonas* spp. TVC was enumerated on plate count agar (PCA, Merck, Darmstadt, Germany) after incubation at 25°C for 72 h. *Pseudomonas* spp. were enumerated on Cetrimide agar (CFC, Merck, Darmstadt, Germany) after incubation at 25°C for 48 h. The microbial growth was modelled using the Baranyi Growth Model (Baranyi and Roberts, 1995). For curve fitting the program DMFit (IFR, Institute of Food Research, Reading, UK) was used (available at http://www.combase.cc/index.php/en/). Kinetic parameters such as the rate (k) and lag phase (λ) of microbial growth were estimated.

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Results

The content and the composition of specific fatty acids in fish flesh are of high importance for assessing fish quality and nutritional value. Regarding ω3 fatty acids, EPA, DPA, ALA and DHA are essential fatty acids for human health that cannot be synthesized by the human body, so they have to be sourced from food. In the present study, sea bream contained important contents of ω3 fatty acids and especially fish fed the F2 diet (2400-2900 mg/100g sample). Trans fatty acids were not detected in any of the tested samples. The observed values of protein content are within the reported levels for fresh Mediterranean fish. For gilthead seabream fed the alternative diets, protein content was similar to the values reported in the literature for *Sparus* spp. fish (19,0-22,8%). Regarding the essential amino acids fraction, glutamic acid, aspartic acid were the most abundant, followed by methionine. In fact, fish contains high amounts of protein and balanced proportions of all amino acids relative to human requirements. No significant differences were observed in gilthead seabream fed the two alternative diets (i.e. F1 and F2). *Pseudomonas* spp. dominated spoilage in all aerobically stored samples. Based on the microbial growth data and considering a limit of acceptability as 10^7 cfu/g for total viable count, the shelf life of from gilthead seabream, shaoor and salmon at 4°C was estimated as 8, 8, 8 and 7 days, respectively (Tsironi et al., 2020). No statistically significant differences were observed in gilthead seabream fish fed the tested alternative diets (data not shown).

Discussion

The results of the study indicated that thermal processing did not affect the beneficial amino acids and unsaturated fats in fish tissues, thus conventional cooking in an oven for 45 min at 180°C retained the nutritional value of all the tested fish products.

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NON-GMO MICRONUTRIENT FORTIFICATION FOR ON LAND BIVALVE SYSTEMS

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Current estimates suggest over 2 billion people world-wide suffer from a micronutrient deficiency (MND). This figure is expected to increase with a growing global population unless existing food practices change. With limited agricultural land and increasing evidence that common farming practices negatively impact the environment and human health, there is an urgent need for novel farming techniques. In particular, the development of novel onshore aquaculture approaches, such as marine bivalve micronutrient fortification, offers considerable promise for easing these global challenges. This work explores the strong potential value bivalves present for food security. We first compare three bivalves, oyster, mussel, and clam nutrient profiles against top alternative protein nutrient profiles before laying the groundwork for developing optimised feeds for tailored micronutrient uplift in marine bivalves. This all in the setting of onshore bivalve aquaculture facilities holds a significant promise for addressing MNDs whilst mitigating the current human health risks associated with consumable bivalves.

Bivalves as a Solution to Malnutrition

It is important that highly nutritious foods are farmed and produced at scale. Comparing the nutritional profile of ocean-based livestock (OBL): bivalves, salmon, and lobster, reveals bivalves are rich in protein, but also in micronutrients (MNs) that are consistently lacking ? within the global population. Furthermore, growing awareness of the environmental impact of meat and dairy farming has led to increasing interest in novel alternative protein sources (APs) to support food security. Nutritional profiling of bivalves compared to the five top alternative proteins: cricket meal, tofu, tempeh, Quorn, and meatless beef; show bivalves contain comparable levels of protein (Table 1) and generally much higher amounts of key micronutrients than other APs (Table 1).

As aquaculture feed is the main contributor of carbon emissions in aquaculture, we have also developed algal feeds as a nature-based solution to bivalve micronutrient fortification. Algae are the predominant source of food for bivalves in the ocean, and naturally contain various micronutrients. Optimised algal feeds show strong promise to reduce the carbon footprint of urban bivalve aquaculture and offer a more sustainable means of micronutrient support for deficient individuals within the human population.

Table 1: **Comparison of nutritional profiles of main alternative protein substitutes and aquaculture products reveals bivalves as a highly competitive nutrition source.** Nutritional profile comparison between APs show that protein levels remain competitive amongst bivalves, but other APs are not complete in the main micronutrients found to underlie the main global micronutrient deficiencies, unlike bivalves.

Nutritional profile of alternative proteins and aquaculture products								
	Cricket	Tofu	Tempeh	Quorn	Meatless Beef	Oyster	Clam	Mussel
Nutrient								
Protein (mg $kcal^{-1}$)	150.0	114.0	105.7	97.3	122.0	117.0	171.0	146.0
Omega 3 (mg $kcal^{-1}$)	0.00	2.41	1.04	2.80	1.28	9.90	1.20	4.80
Iron (μg $kcal^{-1}$)	7.20	18.7	14.1	26.8	21.7	63.0	18.6	81.4
Zinc (μg $kcal^{-1}$)	0.00	10.3	5.73	4.33	19.8	205.0	5.80	15.2
Vitamin B_{12} (ng $kcal^{-1}$)	51.1	0.00	0.52	0.00	16.6	198.0	131.0	114.0
Vitamin A (ng $kcal^{-1}$)	0.00	0.00	0.00	0.00	0.00	1000	1047	565.0
% Daily Value* per 100g								
Protein	48.8	7.38	15.9	9.65	14.9	7.4	11.5	12.0
Omega 3	0.00	12.5	12.5	21.9	12.5	50.0	6.25	31.3
Iron	37.5	19.4	33.8	42.5	42.5	63.8	20.0	106.9
Zinc	0.00	7.73	10.0	5.00	28.2	150.9	4.54	14.54
Vitamin B_{12}	8.88	0.00	4.17	0.00	108	666.7	470.0	500.0
Vitamin A	0.00	0.00	0.00	0.00	0.00	9.00	10.0	6.59

*Percent daily values based on a 2,000kcal a day diet

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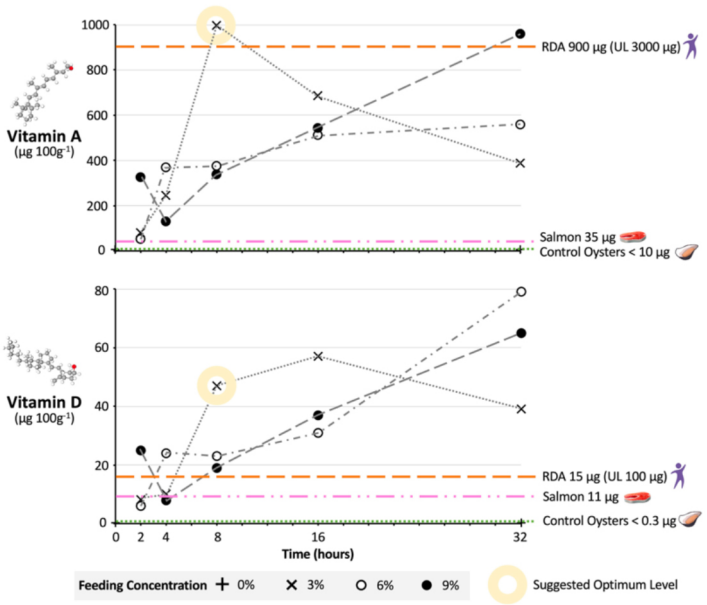

Figure 1. **Vitamin A & D Fortification through non-GMO methods in *Crassostrea gigas*.**
Figure from Willer & Aldridge, 2020, demonstrating *Crassostrea gigas* fortification.

Conclusion & Future Directions

The present work highlights the promising role of fresh, marine bivalves in addressing current population MNDs, as well as being a highly nutritious food source. The development of a novel urban marine bivalve aquaculture systems paired with non-GMO fortification not only sets the groundwork for its translational use towards addressing global food security, but also aids in addressing fundamental questions that are still unknown in bivalve mariculture, and MND reduction within the human population.

Future work will include optimizing bivalve growth, decreasing bivalve related health hazards, and expanding micronutrient fortification capabilities within an artificial setting. We believe elucidating these methods is a necessary step towards developing scalable technologies for onshore urban marine bivalve aquaculture, gaining consumer trust around bivalve consumption, and ultimately delivering the nutritional promises marine bivalves have to offer.

CONSCIOUSNESS INDICATORS AND CARDIAC RESPONSES OF EUROPEAN SEABASS TO DIFFERENT SLAUGHTERING PROCESSES

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Introduction

Harvesting and slaughter are the final steps in the production cycle for all farm animals used for human consumption. During the process, a large number of actions can affect fish welfare. The key point of humane slaughter is to ensure that the animals become unconscious rapidly and that this state lasts until death. Slaughter is generally a two-step process, stunning and killing. In industrial seabass and seabream aquaculture, the most commonly used slaughter method is suffocation and immersion in ice water (ice-slurry), where the animal remains conscious for a long period (several minutes) during which there are indicators of distress (inferred through physiological and behavioural responses) before death (EFSA 2009). Regarding seabass, there are diverse studies comparing different stunning and killing methods, with a strong component on flesh quality assessment (e.g. Papaharisis et al. 2019; Simitzis et al. 2014; Zampacavallo et al. 2015; Acerete et al. 2009; Tulli et al. 2015). Most of these studies pointed towards the necessity to improve the knowledge of slaughtering methods to reduce stress and suffering of fish. Thus, the goal of this study was to assess levels of unconsciousness and cardiac response of seabass exposed to different slaughtering procedures.

Material and Methods

This study was carried out in the experimental facilities of IRTA located in La Rápita (Spain). European seabass of about 1 kg in weight were randomly selected, and surgically implanted with bio-loggers capable to measure heart rates and internal temperature (DST milli HRT, 13 mm × 39.5 mm, 11.8 g, Star-Oddi®, Iceland, www.star-oddi.com). For the surgery, we followed the steps developed by Mignucci et al. (2021). Right after the surgery the fish was placed in a quarantine tank and monitored until full recovery. All tagged fish were kept in rearing tanks for several weeks before the slaughtering experiment. The experimental approach consisted on a combination of pre-slaughtering procedures and slaughtering techniques in order to assess the cardiac response through heart rate variations before and after the slaughtering moment.

Level of unconsciousness through observational indicators (turnover, prick, fin reaction, opercular movement) were assessed on three slaughtering techniques: overdoses by anaesthesia (with 2-phenoxyethanol), ice-slurry (common method applied at farms, water temperature: 0-1º C) and *Ikejime* (japanese immediate slaughter technique). In addition, fasting (seven days) and crowding (three hours) were separately applied on some tagged seabass as pre-slaughtering procedures and combined with slaughtering techniques. These combinations resulted on seven groups: 1) Anaesthesia overdoses, 2) Ice-slurry, 3) *Ikejime*, 4) Fasting + Ice-slurry, 5) Fasting + *Ikejime*, 6) Crowding + Ice-slurry, 7) Crowding + *Ikejime*. Those groups with pre-slaughtering processes were done in duplicate at different temperatures/seasons (winter and summer time). Bio-loggers intensively recorded heart-rates and internal temperature 30 minutes before and 60 minutes after the slaughtering.

Results and Discussion

Observed indicators of consciousness differed among assessed slaughtering techniques. Individuals euthanized by ice-slurry took significantly longer to stop moving their fins and stop breathing than individuals euthanized by overdose of anaesthesia or *ikejime*. Regarding the bio-loggers, mean heart-rate values and patterns differed during the various combinations. The time until the heart rate signal becomes undetectable varies depending on the slaughter technique. Heart rate of seabass individuals slaughtered using *ikejime* showed a high peak instantly after slaughter, but heart rate dropped immediately, stopping after 30 minutes. For seabass slaughtered with anaesthesia, heart rate values decreased gradually to basal values, being undetectable after 35-40 minutes after slaughtering. Regarding seabass individuals slaughtered in ice-slurry, the heart rate dropped under basal value, but was detectable up to 45-50 minutes after the start of slaughtering. It is worth to mention that in this latter case, the internal temperature of tagged seabass individuals decreased gradually (not immediately) when exposed to such cold conditions. If we add the effects of the pre-slaughter methods (fasting and crowding) on the cardiac response after slaughtering, it can be seen that the time when heart rate can be detected is reduced in both cases, being furthermore distinct at different seasons (winter and summer). Therefore, in this study we demonstrate the effects of the different slaughtering methods on the behavioural, mental and cardiac response of seabass, providing relevant information for the future development of more humane techniques.

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THE TEMPERATURE FOR HOLDING OF FEMALE BROODSTOCK CAN AFFECT THE INCIDENCE OF AUTOTRIPLOIDY IN STERLET (*Acipenser ruthenus*)

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Introduction

Artificial propagation is an essential source of sturgeon progeny for both commercial and conservation purposes. However, in culture settings sturgeons often spontaneously produce offspring with altered ploidy – mainly autotriploids having in somatic cells 1.5-fold more DNA than 'normal' fish. Autotriploidy can impact reproductive performance, the presence of autotriploid rather than normal individuals may thus decrease farm productivity or wild population recovery (Schreier et al., 2021). In this three-year study, we tested whether the temperature for holding female broodstock (female-holding temperature; FHT) and/or the temperature for fertilization of eggs and subsequent incubation of embryos (fertilization and incubation temperature; FIT) contribute to the incidence of spontaneous autotriploidy in sterlet (*Acipenser ruthenus*).

Material and methods

Due to the capacity for holding females at individual temperatures and the availability of ripe females, the study was performed in five experiments. Two experiments were designed to assess A) the effect of various FHTs (FHT-experiments), and three experiments to study B) the effect of various FHTs, various FITs, and potential FHTs to FITs relationships (FHT+FIT-experiments). The respective FHTs were reached several days before hormonal stimulation and kept until egg collection. In FHT-experiment 1 and FHT+FIT-experiment 1, we used several temperatures from the optimal range for spawning of females (11–17 °C, Detlaff et al., 1993), i.e. 12, 14, and 16 °C. In FHT-experiment 2 and FHT+FIT-experiment 2, FHT from the optimal range, 15 °C (preferred temperature at the authors´ workplace), and two above-optimal FHTs, 18 and 20 °C, were used. An additional experiment was conducted with FHT below the optimal range, 10 °C, and FHT 15 °C: FHT+FIT-experiment 3. In each experiment, the samples of eggs from three females at a given FHT were utilized, except for FHT 20 °C in FHT+FIT-experiment 2 with the eggs obtained from a single female only. Eggs were always collected within 2h post-ovulation and fertilized within 30 min using a common methodology (Gela et al., 2008). In FHT-experiments, the eggs of each female were fertilized individually (single female at a time) using water at 15 °C, the same temperature was used for subsequent incubation. In FHT+FIT-experiments, the eggs of the females at the same FHT were pooled, mixed properly and the resulting egg mixture was split into aliquots, each of them being subsequently fertilized and incubated at 12, 14, and 16 °C (FHT+FIT-experiment 1); 15, 18 and 20 °C (FHT+FIT-experiment 2); or 10 and 15 °C (FHT+FIT-experiment 3). Ploidy was analyzed using flow cytometry in 2-3d post-hatch prelarvae according to the methodology of Hubálek and Flajšhans (2021).

Results and discussion

Only low proportions of autotriploids (0–1.1%) were detected in the progeny of females held at 10, 12, 14 and 16 °C, which was the case also for females held at 15 °C. However, a considerable part of females from above-optimal temperatures – two out of three females at 20 °C and at least two out of six females at 18 °C – produced significantly higher proportions of autotriploids (FHT+FIT-experiment 2, FHT-experiment 2), with the highest proportion observed in a female held at 20 °C: 53.3% (FHT-experiment 2). We hypothesized *in vivo* egg ageing to be the most probable cause behind this observation. High temperatures can generally accelerate ageing in fish eggs and consequently reduce their developmental capacities (reviewed in Alix et al., 2020). Post-ovulatory ageing-associated changes in the cytoskeletal organization of the egg, which subsequently alter the fertilization mechanism and cause the failure of the second polar body extrusion and formation of autotriploid individual (Piferrer et al., 2009), may also appear sooner at higher temperatures. This theory is consistent with Aegerter and Jalabert´s (2004) study on rainbow trout (*Oncorhynchus mykiss*), as these authors suggested that autotriploid fry could frequently and spontaneously occur when broodfish are checked for ovulation at low frequency and/or are held at high temperatures.

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When each of the female-holding temperatures 12, 14 and 16 °C was combined with 12, 14 and 16 °C for fertilization and incubation (FHT+FIT-experiment 1), the holding temperatures 15, 18 and 20 °C were combined with 15, 18 and 20 °C (FHT+FIT-experiment 2), and holding temperatures 10 and 15 °C were combined with 10 and 15 °C (FHT+FIT-experiment 3) – the proportions of spontaneous autotriploids remained statistically unchanged. The exposition of eggs to certain species-specific temperatures after gamete activation can block the second polar body extrusion by disrupting the microtubules of the meiotic spindle, and effectively induce autotriploidy in sterlet (Fopp-Bayat et al., 2007; Flajšhans et al., 2020), but the temperatures used for fertilization and incubation in our experiments were probably too close to the physiological optimum (or directly within optimal range) to cause this phenomenon.

Conclusion

We conclude that the holding of sterlet female broodstock at temperatures of 18 °C or higher can often result in a remarkable occurrence of spontaneously autotriploid progeny, while the holding of females at 10–16 °C and/or using of water at 10–20 °C for fertilization and incubation do not seem to affect the incidence of autotriploidy.

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SALMOSIM-DIGEST: AN IN-VITRO ASSAY DEVELOPED TO PREDICT THE DIGESTIBILITY OF COMPLETE DIETS FOR SALMONIDS

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Introduction

The ability of aquaculture to provide safe and nutritious fish products to consumers is highly dependent on the diets provided to cultivated fish, as aquafeeds are the principal operating cost of production. In times of climatic turbulence, rapid testing and deployment of novel aqua feeds that improve resilience and fish welfare are key. Moreover, the depletion of marine resources and numerous biotechnological innovations spur the inclusion of sustainable terrestrial sources in aquafeeds to replace traditional marine fish protein and fish oil. As wild salmonids are obligate carnivores, the concentration and quality of proteins present in salmon and trout pellets are crucial to their health and growth. Unfortunately, feed raw materials vary substantially in terms of protein digestibility and absorption, impacting the feed-conversion ratio. Previously applied to analyse the digestibility of ingredients, here we present a redeveloped version of SalmoSim-Digest for *in-vitro* testing of complete diets where we reveal the absorption of amino acids *in-vitro* and compare the *in-vitro* protein digestibility with *in-vivo* digestibility trial data.

Results and Discussion

The apparent digestibility coefficient values (Figure A) generated for each pelleted diet by The SalmoSim-Digest™ showed a highly significant correlation ($p = 0.04$) with the *in-vivo* digestibility coefficient values observed during a traditional feeding trial with rainbow trout (*Oncorhynchus mykiss*). Regression analysis ($R^2 = 0.83$) indicated SalmoSim-Digest has predictive power to estimate the digestibility of complete feed pellets containing protein from various sources. SalmoSim is being offered to producers of aquafeeds as an

Material and Methods

A total of seven diets based on commercially relevant protein raw materials with assumed contrasting effects on protein digestibility were formulated. The *in-vivo* study comprised a 5-week feeding trial using rainbow trout (start weight 55g). Fecal collection was conducted by stripping. SalmoSim-Digest is comprised of bioreactors where pH and temperature are maintained and cocktails of salmon bile and enzymes are added to mimic the digestive tracts (first stomach, then pyloric caecum, midgut and finally hindgut). Aquafeed pellets were characterized by nutritional composition, ground, sieved, and then applied to SalmoSim-Digest for simultaneous digestion and absorption of small molecular weight molecules. Each diet formulation was analysed by technical triplicate bioreactors (n=3). The amount of absorbed amino acids was quantified after each phase of *in-vitro* digestion using a phthalaldehyde assay (absorbance read at 340nm). The apparent digestibility coefficients were calculated by comparing crude protein content (as determined by the Kjeldahl approach) for both *in-vivo* and *in-vitro* assays.

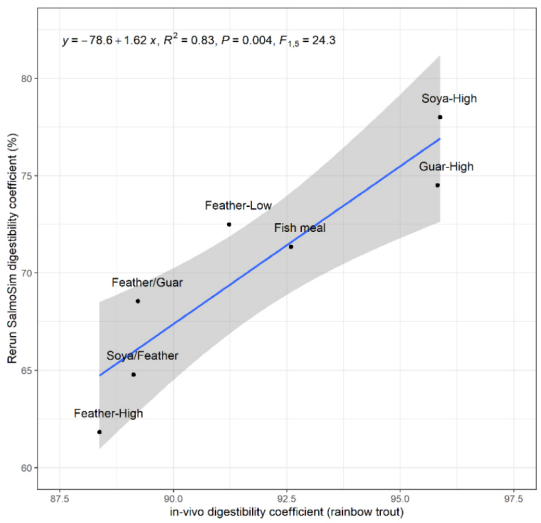

Fig A: Comparison of *in-vivo* and SalmoSim-Digest crude protein ADC values

in-vitro pre-screening test and promises to aid nutritional science in achieving the 3Rs (reduce, reuse and refine) the number of animals used in science. Moreover, SalmoSim-Digest presents an economically advantageous approach to evaluate the crude protein digestibility of novel feed pellet formulations.

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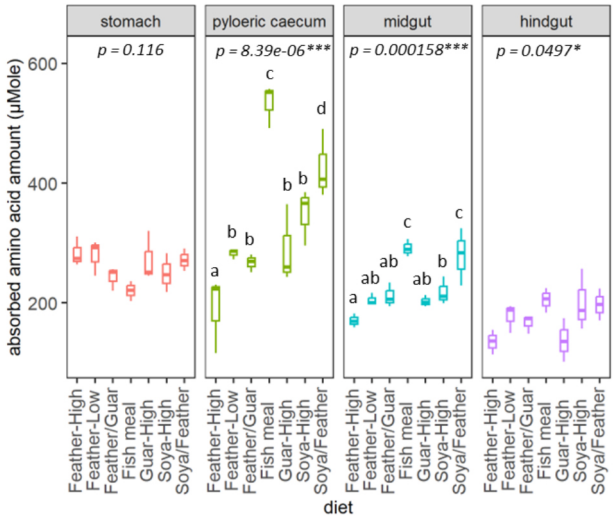

Fig B: Quantity of amino acids absorbed during *in-vitro* phases of digestion.
Comparing each feed formulation regarding the amount of absorbed amino acids revealed significant differences at the pyloric caecum phase and midgut phase with the protein sources of fish meal and soya/feather being significantly higher than the others (Figure B).. SalmoSim-Digest *in-vitro* absorption allows repeated testing of individuals in a manner which would be unethical or impractical in *in-vivo* trials. Furthermore, the precise amino acid composition of the absorbed fraction can be readily ascertained to establish the bioavailability of the amino acids and optimise feed formulations where necessary.

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EFFECT OF FEEDING TIME ON THE INTRADAY VARIATION OF AMMONIA EXCRETION RATE IN NILE TILAPIA *Oreochromis niloticis*

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Introduction

Evolution has selected organisms that were able to anticipate fluctuations of their environment and adapt themselves accordingly (Dunlap et *al.*, 2004). As a result, organisms have developed specific biological clocks that act as natural timing devices, regulating their metabolism according to the cyclic changes in conditions (Dunlap and Loros, 2017). These biological clocks respond to a broad range of parameters known as zeitgebers. Photoperiod and feeding time are some of the foremost zeitgebers controlling farmed-fish metabolism. The present study aims to investigate the variation of intraday rhythms of ammonia excretion in the commonly farmed Nile tilapia (*Oreochromis niloticus*) exposed to 3 different feeding protocols (ML group: fed during the middle of the light phase; MD group: fed during the middle of the dark phase; aleatory group: no fixed feeding time).

Materials and methods

A total of 189 fish were used in this study (8.87 ± 1.23 cm total length, and 9.39 ± 2.23 g total weight) equally distributed among the 3 different experimental groups. The 3 groups were exposed to the same 12:12 LD (light:dark) photoperiod. ML group was fed at ZT6, 6 hours after the beginning of the light phase, MD group fed at ZT18, 6 hours after the beginning of the dark phase, and the aleatory group were fed at a different time everyday).

After 3 weeks of treatment, the sampling took place. In order to measure ammonia excretion rate at a given time, 9 fish were selected per treatment and isolated in metabolic tanks. The ammonia concentration was measured in the metabolic tanks right before adding the fish, as well as 4 hours after the introduction of the fish. The differences in ammonia concentration allowed us to calculate ammonia excretion rates throughout these 4 hours. After 4 hours, the fish were sacrificed. The same methodology was consecutively repeated 7 times in total, allowing us to measure ammonia excretion rate throughout a period of 28 hours.

Additionally, in order to track ammonia excretion down to the molecular level, gills and liver samples were collected from the sacrificed fish. qPCR analyses were performed to measure the intraday variation of the expression of key genes involved in ammonia metabolism in fish (*glsn* and *gludmit* in the liver; *ca*, *rhag*, *rhbg* and *rhcg* in the gills).

Results and discussion

NB: qPCR results are not yet available, they should be released by the end of the month, so the current section will only cover ammonia excretion rate.

The data collected proved the rhythmic nature of ammonia metabolism in Nile tilapias.
Statistical analysis (one-way ANOVA and its post-hoc Tukey test) have showed significant
differences in the daily variation of ammonia excretion rate. Fish in the MD group had their highest ammonia excretion rate at ZT 20 (2 hours after their feeding time), ML group displayed its highest ammonia excretion rate at ZT 16 (10 hours after feeding time) and the aleatory group had its peak ammonia excretion rate at ZT 16 as well (17 hours after the last feeding time). Interestingly, this group is the only one that displayed a second minor peak of ammonia excretion at ZT 8. The experimental groups displayed different rhythmicity but the magnitude of their excretion rates were also significantly different (Student test, $p<0.05$), with the Aleatory group having the highest mean excretion rate (43.73g/kg/h) followed by MD (21.51g/kg/h), and ML (12.40g/kg/h).

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These preliminary results showed the strong endogenous nature of ammonia excretion in this species. Even though ML and MD had their feeding times shifted by 12 hours (ML was fed at ZT 6, MD was fed at ZT 18), their resulting peaks of ammonia excretion were only shifted by 4 hours. The aleatory group received its last meal at ZT 23 the day before the sampling began and displayed a peak of ammonia excretion at ZT16 the day of sampling. This group distinguishes itself from the others by having a second minor peak at ZT 8. When considering mean ammonia excretion rates, the aleatory group has the highest value, more than twice the value observed for MD and almost 4 times the mean ammonia excretion rate in ML. This difference may be the result of higher stress level in fish exposed to this aleatory feeding protocol, which would trigger a higher protein catabolism rate and consequently increase the amount of ammonia excreted.

When available, qPCR results will allow to follow daily variations of selected gene expression, and provide us an insight on the molecular mechanisms involved and their relation with photoperiod and feeding time. It is possible that some of these genes are sensible to light while others depend on feeding time, this would explain the strong endogenous nature of the results.

The main objective of this project is to gain knowledge on ammonia metabolism in order to control nitrogen cycles in aquaponic systems.

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COMMUNICATION CAMPAIGNS TO PROMOTE OCEAN LITERACY AND INTEGRATED MULTITROPHIC AQUACULTURE IN TIERRA DEL FUEGO, ARGENTINA

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Many of us are unaware of how our daily actions affect the health of the ocean, its sustainability and many of the resources we depend on. In addition, most of us do not know about the global reach and importance of the sea and oceans in medical, economic, social, political and environmental terms. These aspects could be counterbalanced by providing or improving access to accurate and reliable education about the marine environment. Ocean Literacy's main goal is understanding the ocean's influence on humans and the influence of humans on the ocean. Lately, many international communities have assigned a large portion of their budgets and efforts to research and innovation programs, in order to reinforce science activity on different topics.

In this context, the All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture (ASTRAL) arises. ASTRAL is focused on Integrated Multi-Trophic Aquaculture (IMTA) farming, aiming to define, support, and promote sustainable aquaculture production across the Atlantic area. IMTA is the farming of species from different trophic levels in a way that allows one species' uneaten feed and wastes to be used as inputs (fertilizers and feed) for another species. Sharing knowledge and capacity development are among ASTRAL priorities, as well as to build a collaborative ecosystem along the Atlantic Ocean with industrial partners, scientists, policymakers, social representatives, and other relevant stakeholders.

ASTRAL is committed to increasing the public acceptance and awareness of aquaculture by fostering public understanding of the value of aquaculture and especially IMTA, as a sustainable way to produce aquatic products. In Tierra del Fuego (Argentina), southernmost South America, we had to face the distrust of the local community on aquaculture as a production alternative due to a previous "no to salmon farming" campaign which was very popular. Therefore, several interviews in local radio and TV programs (at a local and national scale) were held in order to communicate and introduce society to other aquaculture systems. The project works with a multilayer stakeholder approach; therefore, we design different Ocean Literacy activities to be held in Tierra del Fuego, mainly in Ushuaia city and a nearby, small fishing village, Puerto Almanza to ensure that the message reaches all the sectors of interest. Some of the activities performed were oriented mainly to primary and secondary schools receiving kids and teachers at the research institution (CADIC-CONICET), organizing and participating in science fairs, giving lectures where we told stories, and we presented native species (that could be used in IMTA systems locally) through pictures and video images (Figure 1). Other activities include an apprenticeship program for early university students at CADIC-CONICET, workshops with local authorities, and training courses with technicians of the fisheries secretary, among others. It is worth mentioning an international course on the actual state of the art of aquaculture in Argentina that was held in Ushuaia on May 2023.

Up to the day, ASTRAL continues to interact with the local community by contributing to an educational project called "A window to the sea" (*Una Ventana al mar*), orientated to teachers for first educational levels, whose main goal is to teach Ocean Literacy, introduce native species and has a special chapter on sustainable and regenerative practices such as IMTA (Figure 2).

ASTRAL is a HORIZON 2020 project (GA 863034) financed under the Blue Growth program. Further information: www.astral-project.eu/

ELECTROLYZED WATER TREATMENT FOR THE CONTROL OF THE ZOONOTIC PATHOGEN *Vibrio vulnificus* AND ITS APPLICATIONS IN AQUACULTURE

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Introduction

Vibrio vulnificus (*Vv*) is a septicemic zoonotic pathogen native to warm, brackish water ecosystems that is considered a biomarker of climate change. Recently, it has been shown that fish farms act as evolutionary drivers of this species by favoring the emergence of new pathogenic variants (Amaro *et al*., 2020). Establishing *Vv* control measures on farms would serve to reduce the risk of animal and human infection as well as the emergence of new variants that are dangerous to animal and public health. Saltwater electrolysis is an environmentally friendly control method that exhibits transient disinfectant properties due to the formation of hypochlorous acid (HOCl). HOCl attacks and destroys bacterial membranes and therefore has a bactericidal effect (Wang *et al.*, 2020). In this work we explore this strategy to control the presence of *Vv* in water as a preventive measure or even treatment combined with antibiotic therapy.

Material and methods

Bacterial strains and culture. Representative strains of the five phylogenetic lineages of *Vv* were used in the study. The strains were grown in LB-1 (1% NaCl Luria Bertani broth) or on TSA-1 (1% NaCl Tryptic soy agar) plates at 28°C for 24 h. Bactericidal assay. An electric current was applied to a saltwater solution to obtain EW (electrolyzed water). Overnight cultures in LB-1 were inoculated (1:10 ratio) in freshly obtained EW (final volume of 100 ml) and bacterial viability at different times was tested by drop plate counting on TSA-1 plates. Different water salinities (0.5, 1.5 and 3%), chlorine concentration (5 to 125 ppm) and pH (3.5, 5, 6.5 and 7.5) were tested. All experiments were performed in triplicate. Toxicity. European eels were exposed to different concentrations of chlorine (from 5 to 20 ppm) for 15 min to assess toxicity. EW conditions mimicked farm conditions (pH 6.5 and salinity 0.5%). For each test condition, 8-10 individuals with an average weight of 8 g were used.

Results and discussion

At concentrations of 125 ppm chlorine, EW was highly bactericidal for all strains tested, producing a 100% reduction of the bacterial population regardless of salinity and pH. In contrast, at concentrations of 20 and 25 ppm, the bactericidal effect depended on water salinity and/or pH. Thus, at intermediate salinity (1.5%) there was a 99.99% reduction in the population when water was acidic (pH 5, Fig. 1 and 2) and a 90-60% reduction when pH of water was around 7 (Fig. 2). At low salinity (0.5%) again, the bactericidal effect was high and independent of pH with a population reduction of 99.99% in all cases.

EW with chlorine 20 ppm resulted highly toxic for eels when treatment lasted 15 min but it was safe when chlorine concentration was up to10 ppm. Animal trials are planned for short-term treatments.

Conclusions

Since EW was highly bactericidal against *V. vulnificus*, this technology could constitute a complementary and sustainable strategy to antibiotic treatment during vibriosis outbreaks in aquaculture facilities. Furthermore, EW could be used as a preventive measure during stressing periods to reduce the load of pathogenic bacteria in farm water.

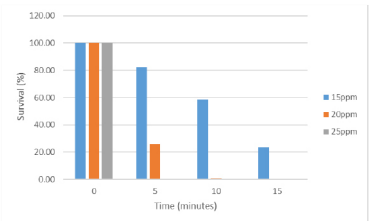

Figure 1. Survival of *V. vulnificus* (strain CECT 4999) in electrolyzed water (pH 5; salinity 1.5%) with different chlorine concentrations.

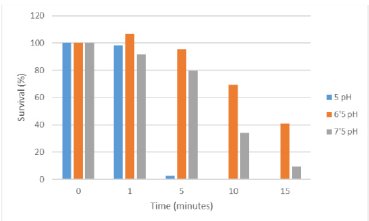

Figure 2. Survival of *V. vulnificus* (strain CECT 4999) in electrolyzed water (1.5 % salinity and 25 ppm of chlorine) at different pH values.

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EFFECT OF AN ALTERNATIVE DIET BASED ON THE DIATOM *Phaedactylum tricornutum* ON THE GROWTH AND HEALTH OF RAINBOW TROUT *Oncorhynchus mykiss* – PRELIMINARY RESULTS

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Introduction

Proper nutrition is essential for immune system function, disease resistance, growth, and overall well-being of farmed fish. Alternative feeds in aquaculture can play a significant role in maintaining fish health and minimizing the environmental impact of fish farming. Although some potential benefits are already known, much work must be done to characterize their composition and the effect on the fish's physiology and immune system. This study is part of the FISHEALTH project (financed by the Spanish Centre for the Development of Industrial Technology – CDTI), where 4 research institutions (ANFACO, AZTI, CETGA, and CTAQUA) are working together to develop tools and methods to diagnose, prevent and treat the primary infectious diseases that affect marine and continental Spanish aquaculture. In particular, this study aimed to include active ingredients from alternative sources that may enhance the fish's immunity and improve the overall fish health status.

Materials and Methods

At the ANFACO-CECOPESCA aquaculture facility (recirculating aquaculture system, 6.5 m^3, 14°C), 20 fish per tank (8.34 cm and 6.71 g) were stocked at a 0.40 kg.m-3 density. For 45 days, rainbow trout (*O. mykiss*) were fed 4 different diets (by triplicate) based on: (A) microalgae (the diatom *Phaeodactylum tricornutum*; 5.0% inclusion), (B) seaweed (the Pepper dulse, *Osmundea pinnatifida*; 5.0% inclusion), (C) fungi's fermented fruits biomass (0.5% inclusion) versus a control diet (D). At the end of the experiment, 54 fish per diet were anesthetized and sacrificed by phenoxyethanol overdose and measured. Then, different target organs (liver, kidney, spleen, distal gut, and skin mucosa) were taken for ulterior analysis: antioxidant and immunomodulatory activities, differential gene expression (transcriptomic analysis), and gut microbiome composition.

Results

Different growth and performance indicators were measured at the end of the study, not showing any significant difference between the four treatments (growing at a specific rate of 4.36% and reaching a size of 15.95 cm and weight of 48.09 g, average) (Figure 1). A feed conversion ratio of 1.00.

Only Fulton's condition factor (k) was different, being significantly higher for the fish under diet A (*Phaeodactylum*) ($p = 0.049$, ANOVA test; $p < 0.05$, Tukey, LSD, and Bonferroni tests) compared to the control diet (Figure 2). Also, when the immunomodulatory activity expressed by the levels of inflammatory cytokines (Interleukin 6 (IL-6) and Tumour necrosis factor (TNF-α)) was measured, we found that the fish being fed with the *Phaeodactylum*-rich diet (A) also had significantly low levels of IL-6 in liver and spleen compared to the rest of the groups ($p = 0.031$, Kruskal-Walis test) (Figure 3).

ANFACO's previous work evaluated the antioxidant activity of *P. tricornutum* bioactive compounds. Focusing on the levels of phenolic compounds, our results showed higher levels in diets containing algae biomass: A, *Phaeodactylum* (3.11 CFT, µg/mg sample) and B, *Osmundea* (3.46), compared to C (2.50) and D (2.65). Moreover, the analyzed microbiome in the fish's gut fed with microalgae and seaweed was more diverse.

Discussion and conclusions

Although our results are still preliminary, we can already conclude that the tested alternative diets show, in general terms, good results in terms of growth and performance. Moreover, one of the diets, based on the diatom *Phaeodactylum tricornutum* at 5% inclusion, may contain natural active compounds related to the expression of some immunomodulatory molecules, such as IL-6.

Our next step is to identify the nature of these compounds, connect those results with the previous (phenolic compounds content and gut microbiome diversity), and add more information from the samples already collected (mucosal antioxidant activity and distal gut transcriptomic analysis). With all this information, we will be able to assess the suitability of this alternative source/s for aquaculture.

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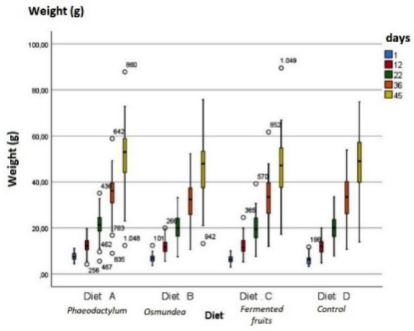

Fig. 1 – Fish weight for the different diets in the study

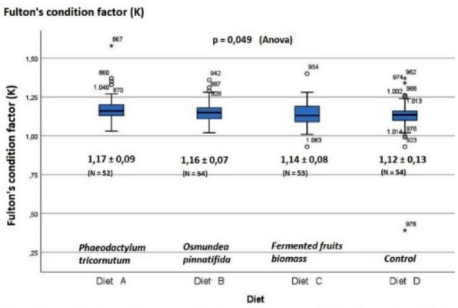

Fig. 2 – Fulton's condition factor (k) for the fish under the three experimental diets and the control

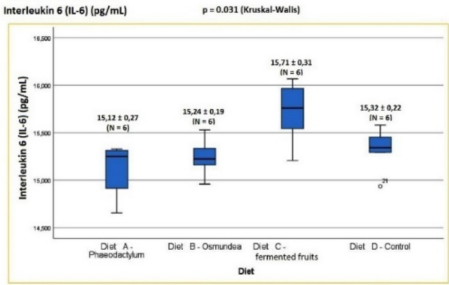

Fig. 3 – Interleukin 6 (IL-6) levels in the liver and spleen of the fish under the three experimental diets and the control

TOWARDS BETTER LUMPFISH: CHANGES IN SIZE VARIATION, CATARACT DEVELOPMENT, BEHAVIOUR AND SEA LICE GRAZING THROUGH SELECTIVE BREEDING

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Introduction

The biological control of sea lice using cleaner fish including lumpfish (*Cyclopterus lumpus*) has become a feasible option due to the increased occurrence of resistance towards medical treatments in salmon lice, *Lepeophtheirus salmonis*. For lumpfish a significant individual differences in feed intake and preference for sea lice has been seen (Imsland et al., 2014a), and genetic influence has been suggested to be a possible factor (Imsland et al., 2021). A series of studies (called Phase I, II, III and IV) were conducted over a four-year period where distinctive lumpfish families have been established initially from wild caught mature fish and latterly from established breeding lines. The aim of this study was to evaluate whether sea lice grazing efficiency, behaviour, size variation and cataract development can be improved through selective breeding of lumpfish.

Material and methods

Four subsequent trials (called: Phase I-IV) with ten families of lumpfish (N = 480) with a mean (± SD) weight of 46.4 ± 9.4 g (Phase I), 54.8 ± 9.2 g (Phase II), 42.0 ± 7.4 g (Phase III) and 31.3 ± 2.4 g (Phase IV) were distributed among ten sea cages (5 × 5 × 5 m) during autumn 2018 to spring 2022, each stocked with 400-404 Atlantic salmon with an average initial mean (± SD) of 387 ± 9 g (Phase I), 621 ± 15 g (Phase II), 280 ± 16 g (Phase III) and 480 ± 66 g (Phase IV). All the ten cages were stocked with 48 lumpfish (12% stocking density). In all four phases sea lice grazing efficiency, behaviour, size variation and cataract development was monitored and compared.

Results

The increase in incidence of cataracts from start to end of each trial phase was significantly reduced from Phase I (16%) to Phase IV (2%) (Fig. 1A). In all phases there was a large inter-family variation of lice grazing of lumpfish of *both L. salmonis* and *C. elongatus*. When sea lice grazing was scaled in relation to sea lice infestation levels on the salmon the highest sea lice grazing activity was found in Phase IV (Fig. 1B) and in particular in families sired from farmed parents. There was a general trend for mean start weights and standard deviations to decrease as the phases continued. A significant increase was found in frequency of behaviour associated with feeding on natural food sources and grazing sea lice from salmon during each subsequent phase.

Discussion and conclusion

There were clear differences in sea lice grazing efficacy, size variation, behaviour and cataract prevalence between the families tested in the different phases of the study. Further, when sea lice grazing was scaled in relation to sea lice infestation levels in each experimental phase a trend of increased sea lice grazing in the 1G breed fish used in Phase IV. Juvenile lumpfish exhibit a limited palette of behaviour types (12-14 types) with the majority based on food location and/or feeding (Imsland et al. 2014b, 2021). These behaviour types were classified and grouped into "positive/cleaning"; "negative" and "normal" types for the purpose of this study. Positive behaviours are associated with feeding on natural food sources withing the cage environment, feeding on feed blocks and grazing sea lice from salmon. This behaviour type increased significantly during the subsequent Phases. One of the breeding targets of this study was to lower the incidence and development of cataract. This target was achieved as the increase in incidence of cataracts from start to end of each trial phase was reduced from Phase I (16% increase) to Phase IV (2% increase). Overall, present findings showed that sea lice grazing of both *L. salmonis* and *C. elongatus*, size variation, cataract prevalence and behaviour types can be enhanced through selection and targeted breeding programs.

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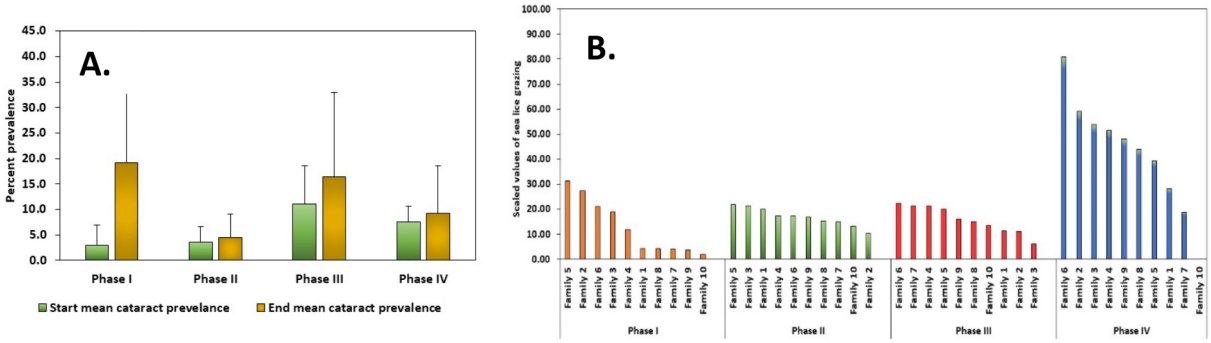

Fig. 1. (A). Percentage cataract prevalence (mean ± S.D) at the start and end of each phase. (B). Comparison of how much lice found in lumpfish stomachs related to the amount available.

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QUANTIFYING BEHAVIOUR DURING CROWDING ACTIVITIES TO ASSIST AQUACULTURE WELFARE ASSESSMENTS OF ATLANTIC SALMON (*Salmo salar*) REARED IN EXPERIMENTAL TANKS

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Introduction

Farmed aquatic animals are subjected to numerous handling operations that can be challenging for their health and welfare. One such operation is crowding, where fish are subjected to reduced rearing volumes to facilitate handling, which is key in the rearing cycle. If fish are repeatedly crowded, there may be potential cumulative effects upon fish welfare and these effects can be detrimental (Espmark et al., 2015; Grøntvedt et al., 2015; Roth, 2016; Gismervik et al., 2017). Methodologies based on operational welfare indicators (OWIs), Laboratory-based welfare indicators (LABWIs), health indicators and new emerging technologies have been developed to monitor and audit the welfare of farmed animals, including fish (Noble et al., 2018). Its application can help audit, refine, and optimize crowding procedures. The main goal of this study was to quantify and audit fish behaviour during crowding operations in order to create automatic frameworks and protocols that can assist in documenting/optimising fish welfare during crowding operations.

Materials and Methods

The effect of four different crowding intensities on Atlantic salmon welfare was explored by the project Crowd Monitor (Norwegian Seafood Research Fund, pr. num. 901595) by means of 12 tanks of 3300 l, holding ca. 120 fish (>500g) within a clockwise water flow. Each crowding level was triplicated (4x3 design) and a GoPro hero4 black was installed over the tank to capture the entire water surface in the field of view. Each tank was recorded for ca. three hours: around half an hour before crowding, two hours during crowding and half an hour after crowding. One tank with intermediate crowding intensity was used for the present study. After observing the original video, both swimming structure impacted by crowding onset and its gradual increase during crowding were hypothesized. Hereafter, fish swimming behaviour was quantified through computer vision (Python 3.0, OpenCV library). Firstly, median frames were calculated for the 30-minute period before and after crowding. The 2h crowding period was split in four 30-minute periods and the median frames were also generated (Fig. 1A). Theoretically, a still and more structured fish swimming would be mirrored in median frames showing fish silhouettes oriented towards the water flow, whereas chaotic swimming and low swimming structure would render blurred median frames. Further metrics were quantified for median frames' validation. Five frames of every 30-minute period were subsampled and the coordinates from heads, anterior dorsal fins and tail peduncles were extracted (ca. 50 fish per frame) to calculate a) individual swimming orientations and ii) swimming angles as proxies of swimming structure. Heart rate as a proxy of activity/stress to cross-validate mean frames, swimming orientation and swimming angle was obtained from intraperitoneal tags (n = 4 fish). Eventually, fish swimming behaviour was continuously quantified over the whole sample video by mean histogram values at three distinct levels, *i.e.,* within the crowding area, rear- and front-zone of the crowding area. The fish abundance in the front and rear zone of the crowder were also calculated (Fig. 1B).

Results and discussion

The largest proportion of individuals swimming against the water flow was detected before crowding, followed by the post-crowding period. The crowding period showed the largest variability in swimming orientation and swimming angle. Overall, and consistent with median frames observation, the mean histogram colour unveiled a gradual increase in fish abundance in the front of the crowder, in contrast to the rear zone (Fig. 1B). This is consistent with the visual analysis of median frames, revealing clearer fish silhouettes swimming against the water flow in the crowd front, and avoidance of the rear zone. Both the largest shift in the number of fish swimming against the water flow and a decreasing heart rate coincided one hour after the crowding onset. This could be linked to a decrease in activity/stress during crowding. Overall, the current behavioural approach detected fish gradually coping with crowding conditions by structuring swimming and avoiding the rear zone of the crowding area. Uncontrolled factors such as chaotic swimming and/or interactions with the crowder might jeopardize fish welfare status. Therefore, further research could focus on the optimal thresholds of duration, repetition and/or the speed of crowding activities regarding welfare status.

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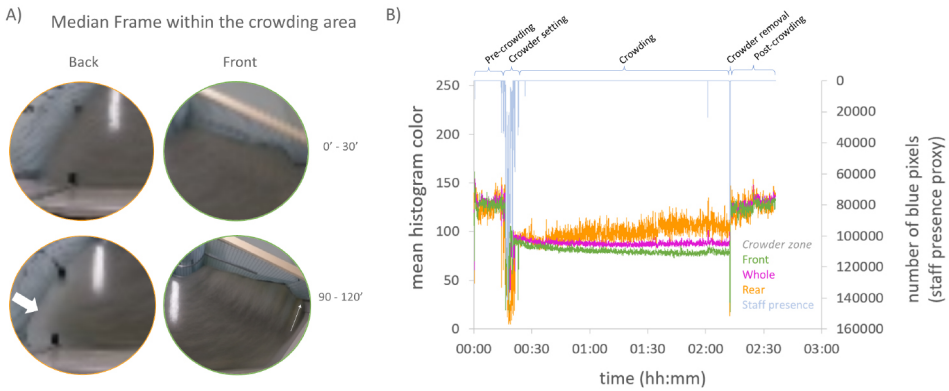

Figure 1. A) Fish activity based on median frames. Activity in the rear versus the front zone of the crowding area is represented in the left and right column respectively, whereas the first 30 minutes of the two-hour crowding period versus the last 30 minutes correspond, respectively, to the top and bottom row. B) fish activity depicted by mean histogram values over time (0 = black, 255 = white). White arrows in A) indicate an empty area in the back of the crowd (bottom-left) and an example of a fish silhouette swimming against the water flow (bottom-right).

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MOPSEQ-DB: THE REFERENCE DATABASE AND VISUALISATION PLATFORM FOR MARINE MOLLUSC PATHOGENS GENOMES

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Introduction
Pathogen surveillance and diagnostic methods are constantly evolving. Sequencing can provide critical information to diagnose diseases and inform control and mitigation strategies by identifying genetically distinct pathogen variants that may have different host reservoir species or geographic distributions.

During the last decade, with the development of high throughput sequencing methods, reference laboratories and research groups studying marine mollusc diseases have advanced considerably in sequencing-based analyses. However, data management is still unconventional as the community lacks dedicated databases and tools, despite a considerable increase in data volume.

Material and methods
We therefore developed a user-friendly web-platform, called MoPSeq-DB, which references curated genomic data related to mollusc pathogens. It gives users opportunities to navigate through data, interactively visualise genomic structure and variation, provide integrated analysis tools, and allow to download data in various file formats. Since marine bivalve molluscs can be affected by viral, bacterial and eukaryotic pathogens, MoPSeq-DB is designed to be used with a large panel of genome particularities (e.g. size, architecture).

Results
MoPSeq-DB, is an open-source tool based on the Python web-framework Django enabling convenient and fast sequencing data exploration and visualisation in an intuitive and user-friendly way, particularly for non-bioinformaticians. It has minimal hardware requirements and is easy to install, host, and update.

While MoPSeq-DB folder structure enforces systematic yet flexible storage of genomic data of bivalve mollusc pathogens, including associated metadata, the platform could easily be declined to pathogens of any other organisms. The application can be deployed using a Docker container, and runs on all modern browser engines (Firefox, Chrome, Safari).

Availability
Source code and documentation are available at https://gitlab.ifremer.fr/bioinfo/mopseq-db.

A public web server will be online at: https://mopseq-db.ifremer.fr by end 2023.

MISLABELLING OF STURGEON CAVIAR – A DRAWBACK FOR LEGAL TRADE AND SPECIES CONSERVATION

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Introduction

In response to dramatic declines in many sturgeon populations globally – in large parts due to overharvesting – and with the aim of ensuring that the trade in sturgeon products is sustainable, all 27 species of Acipenseriformes have been listed in the Appendices of CITES[1] and international trade is strictly regulated. In 2000, a universal labelling system for caviar was introduced to allow the identification of the source. The system has been revised and updated on a number of occasions since then. The labelling system[2] should be implemented for all types of caviar – both wild-sourced and derived from aquaculture – for both international and domestic trade. Labels must be non-reusable, i.e. they cannot be removed undamaged or transferred to another container, and they must seal the container, or if not, the packaging should permit other visual evidence of any opening. They must provide a minimum amount of information, in a defined code of letters and numbers: the species or hybrid of origin, the source code of the caviar ("W" for sturgeon harvested from the wild, "C" for captive-bred sturgeon etc.), the country of origin, etc.

Despite these trade regulations and legal protection of recent years and despite the large increase of caviar from aquaculture, the decline of critically endangered sturgeons in the wild continues, and poaching and illegal trade have been reported in many sturgeon range countries globally.

Materials and methods

Information on illegal trade in sturgeon and the reasons for it (e.g. incorrect caviar labelling) was collected from own research, published literature, seizure reports, investigations of experts and journalists, etc. to understand and address deficiencies in labelling implementation and enforcement and close entry points for illegal activities.

Results

Market survey

A large-scale market survey – including wildlife forensic analysis to determine species and source of sturgeon meat and caviar – demonstrates the widespread occurrence of caviar mislabelling and sturgeon tracking in the Lower Danube Region (Bulgaria, Romania, Serbia and Ukraine)[3].

Apart from 27 samples (19% of all samples) that originated from wild-caught sturgeons, 17 samples (12% of all samples and 29% of all caviar samples) were caviar sold in violation of CITES regulations. Of these, 2 caviar samples were illegally imported into the country, 4 caviar samples were sold without mandatory CITES labels in EU Member States (where CITES caviar labelling is legally required both on the international and on the domestic market), and 11 mislabelled caviar samples were sold in EU Member States:

- 7 of these were determined (with genetic analysis) to have a wrong species code,
- 3 were determined (with isotope analysis) to have a wrong code for the country of origin,
- 1 had a wrong code for species and country of origin.

On 10 of the caviar samples which were found to provide wrong information in their CITES codes, all labels also failed to meet mandatory CITES requirements (not sealing or providing visual evidence of opening).

In many instances, consumers were misled by e.g. products from lower-priced sturgeon species being misrepresented as coming from higher-priced ones or caviar from hybrids as deriving from pure species, but also vice versa.

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Other findings of caviar mislabelling

Official investigations, seizures, court cases, trade suspension, market surveys, journalist investigations, etc. revealed several cases of incorrectly labelled or mislabelled caviar, e.g.:

- According to a presentation by a German CITES representative in 2006, falsified marks and labels for over 3000 kg of caviar were seized, and smuggled caviar had been sold to different traders – including reliable companies – all over Europe[4].
- In an organised crime investigation in Germany, a witness stated that Russian caviar was smuggled into the EU and commercialized as Bulgarian farmed caviar. Isotope analysis of samples taken in 2009 supported this claim and found caviar labelled as coming from a registered Bulgarian aquaculture operation actually to originate from the Caspian Sea. The extensive evidence showed that more than 100 kg of illegally obtained Beluga caviar were sold to several top restaurants in the course of a year[5].
- In 2009, the UK Border Agency seized twenty tins – in total two kilograms – of Beluga caviar after suspicions based on poor labelling[6].
- Between December 2017 and January 2018, French enforcement authorities led 32 inspections aiming to control trade in sturgeon caviar. 17 controls detected non-compliance to the caviar labelling provisions, e.g. CITES labels being removable undamaged. 329 primary containers corresponding to 9,750kg of caviar, for a value of approximately 15000€, were seized. The caviar had come from processing plants in France and China, repackaging plants were located in France, Germany and Spain[7].
- Published in 2015, journalist investigations found that a company in Lithuania sold caviar with counterfeit CITES labels from a registered Bulgarian aquaculture operation to EU Member States such as Germany. Isotope analysis of three caviar samples with CITES labels and country code BG for Bulgaria showed that one sample ordered directly from Lithuania and one bought from a German repackager did not derive from Bulgaria but likely from the Caspian Sea region[8].

Discussion

Nearly a third of all caviar samples tested in targeted market research did not meet CITES requirements and were therefore illegal. All these samples were from farmed sturgeons. However, misdeclarations are a breach of CITES regulations and undermine the aim to ensure that the international trade in sturgeons does not threaten their survival in the wild. Furthermore, any misuse or incorrect implementation of the labelling is a drawback for the confidence in the labelling and for all legally operating facilities.

Current CITES regulations are not sufficient or not adequately enforced to detect and stop illegal trade. Some national CITES Management Authorities accept labels that do not fulfil all CITES labelling requirements or do not control sufficiently the implementation by caviar processing and repackaging companies and, in general, law enforcement authorities do not further investigate labelled caviar containers unless with initial suspicion (most seizures are of un- or mislabelled tins or due to infringements of the personal or household effects exemption).

CITES caviar labelling requirements must be correctly applied, including on domestic markets, and improved (considering new technologies such as QR codes). Furthermore, effective and secure traceability systems throughout the trade chain are needed (including database systems such as blockchain). To this respect, e.g. a feasibility study is currently underway to determine if individual analytical signatures can be identified to reliably trace sturgeon products in trade back to the aquaculture facility of origin.

Strengthened enforcement measures are indispensable and need to include inter-agency and cross-border cooperation, and controls have to cover the whole trade chain and include forensic analysis of samples. Effective combating of illegal activities also fundamentally requires the cooperation of the sturgeon industry (aquaculture producers and traders).

A Brief History of Sturgeons and CITES

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APPLICATION OF MICROALGAE FOR RAS WASTEWATER TREATMENT: NUTRIENT REMOVAL AND BIOMASS PRODUCTION

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Introduction

Recirculating aquaculture system (RAS) has advantages compared to flow, and semi-flow-through systems, such as reusing treated wastewater and requiring lower water volume. Despite these advantages, nitrate accumulation is a significant problem of RAS wastewater (RASWW). The nitrate accumulation can be toxic to fish in the RAS system and cause eutrophication (1). Therefore, microalgae can be introduced as it can utilize nutrients from RASWW, and harvested biomass can be used as fish feed.

Materials and methods

Nannochloropsis oculata and *Chlorella vulgaris*, were ordered from Norwegian Institute for Water Research (NIVA) culture collection, inoculated in a 2 L photobioreactor containing 0.5 L medium at 23 ± 2 ºC. Light irradiance source was white neon tube LED with light intensity of 115 µmol/m^{2}s^1 and photoperiod of 12:12 (otherwise stated). Microalgal growth was measured by filtering a specific volume of microalgal culture on to filter paper followed by drying at 60 ºC in a hot air oven. The microalgal dry cell weight (g/L) was measured calculating the difference between the empty filter paper before and after filtration. The concentration of nitrate (NO_3^-) and phosphate (PO_4^{-3}) was measured by ion chromatography (Shimazu). The concentration of total nitrogen (TN) and non-purgeable organic carbon was measured by TOC/TN Analyzer (multi-N/C 2100S). The nutrient removal efficiency was calculated by equation 1, where C_i is the initial and C_f is the final concentration of respective nutrients.

$$R\% = \frac{(C_i - C_f)}{C_i} * 100 \quad (1)$$

Experimental design

Experiment 1: Screening of different strains (*N. oculata* and *C. vulgaris*) and RAS wastewater (RASWW 2 ppt and 14 ppt) for optimum biomass.
Experiment 2: Effect of different photoperiods (light:dark period, L:D) (8L:16D, 24L:0D, 0L:24D) and light wavelengths (red, blue, white) on microalgal growth.
Experiment 3: Effect of autoclaved and non-autoclaved RASWW on microalgal biomass accumulation.
Experiment 4: Effect of nitrogen:phosphate molar ratio (NPR) (N:P, 5N:P, 10N:P, 20N:P) achieved by phosphate (K_2HPO_4) addition to RASWW on microalgae growth.
Experiment 5: Effect of different salinity (14, 20, 25, and 34 ppt) for microalgal growth.
Experiment 6: Effect of optimized condition (*N. oculata* in RASWW 14 ppt, with 24-hour white light, 25ppt salinity and with NPR of 20:1) based on the experiment 1-5.

Results and discussion

Experiment 1: *N. oculata* is a marine microalga so it preferred 14ppt over 2ppt RASWW and 14ppt RASWW over commercial media f/2 possibly due to its nutrient composition.
Experiment 2: White light resulted in optimum growth as it is composed of all light spectra essential for microalgal growth. Previous studies have also shown the optimum growth of some microalgae species obtained in white light and 24 h photoperiod (2).
Experiment 3: The dry cell weight accumulation showed a similar pattern in RASWW with and without sterilization. Therefore, nonsterile RASWW was chosen as it is economically favorable.
Experiment 4: The optimal nutrient ratio required for biomass accumulation and nutrient uptake can be depicted by microalgal cell's elementary composition ($C_{106}H_{263}O_{110}N_{16}P$). In this regard, previous studies have also shown the important role of NPR in microalgal growth (3). In the current study the optimal biomass was accumulated with NPR of 20N:P.
Experiment 5: Salt concentration is required at an optimum level otherwise it might disrupt the osmoregulatory mechanism of microalgal cells. In the current study salinity of 25 ppt provided optimum biomass accumulation.
In experiments 1-5, The dry cell weight ranged from 0.55 g/L to 0.62 g/L.
The highest dry cell weight obtained was 0.62g/L, in salinity experiment at 25 ppt salinity.

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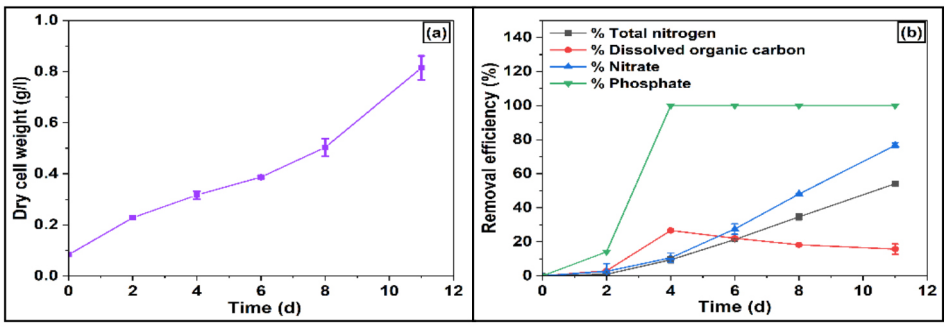

Figure 1. The growth profile (a) and nutrient removal profile (b) of *N. oculata* cultivated in RASWW.

Experiment 6: This study was based on the optimum dry cell weight obtained in experiments 1-5. The maximum dry cell weight of 0.81g/L was obtained, which had significant increase of 30.64% than that in the highest biomass obtained in the experimental variations. The nutrient removal profile on day 11 consisted of total nitrogen removal of 54.1 %, dissolved organic carbon removal of 15.76%, nitrate removal of 76.65%, and total phosphate removal of 100%.

Conclusion

N. oculata can remove nutrients and organic carbon from RASWW and produced algal biomass can be used as fish feed. Thus, microalgae can be introduced as a carbon-neutral option for achieving a circular bioeconomy to solve the issue of nutrient accumulation in RASWW.

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Acknowledgment

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COMPUTER VISION METHODS FOR FISH DETECTION AND TRACKING

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Introduction

Aquaculture is crucial for global seafood production as it addresses the growing demand resulting from population growth. Developing robust and efficient autonomous solutions such as unmanned underwater vehicles (UUVs) is vital for a safe and cost-effective aquaculture industry [1]. However, unsuitable technologies or underdeveloped automation can lead to unexpected negative consequences. Among important aspects, vehicle design plays an essential role to ensure fish welfare. In other words, understanding fish-UUV interactions is essential for designing UUVs that do not scare or hurt fish. This study explores the application of computer vision and stereo vision tools to study such interactions, to examine the behavior of Atlantic salmon (Salmo salar) in industry-scale fish farms. Specifically, the study aims to analyze how fish respond to static structures, seeking to determine the distances that fish maintain from these structures.

This work was carried out in collaboration with SINTEF Ocean, specifically SINTEF ACE-Robotic Lab and the CHANGE project [2].

Materials and methods

To collect the data, a structure of varying shape and color was fitted with two Lucid TRI032S-CC GigE vision cameras and two BlueRobotics Ping360 echo sounders before being lowered into the water. The structure was maneuvered around before being secured, and data was captured for 12 minutes. This procedure was executed six times for each structure variation in two distinct cages at depths of 8 and 12 meters, respectively. Additionally, stereo calibration videos featuring a moving chessboard in front of the structure were captured in order to enable 3D position estimation from the resulting trial videos. The data were derived from computer vision techniques to estimate the distance from the fish and monitor its behavior.

The employed method extracts frames from a video and performs object detection in the left image frames utilizing YOLOv8, a state-of-the-art object detection model [3]. SuperGlue [4] is subsequently employed for feature extraction and matching, with the resulting point matches being used to associate detections in the left frame with objects in the right frame. To maintain tracking of individual fish across multiple image frames, object tracking capabilities are incorporated through StrongSORT [5]. Lastly, the 3D positions and distances relative to the camera are determined through triangulation, using the positions and disparities of the corresponding left and right objects and the calibration data.

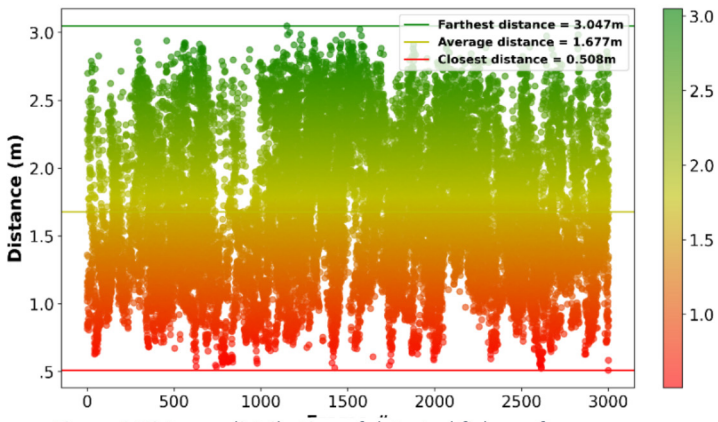

Figure 1 Distance distribution of detected fish per frame. Most fish keep one to three meters distance.

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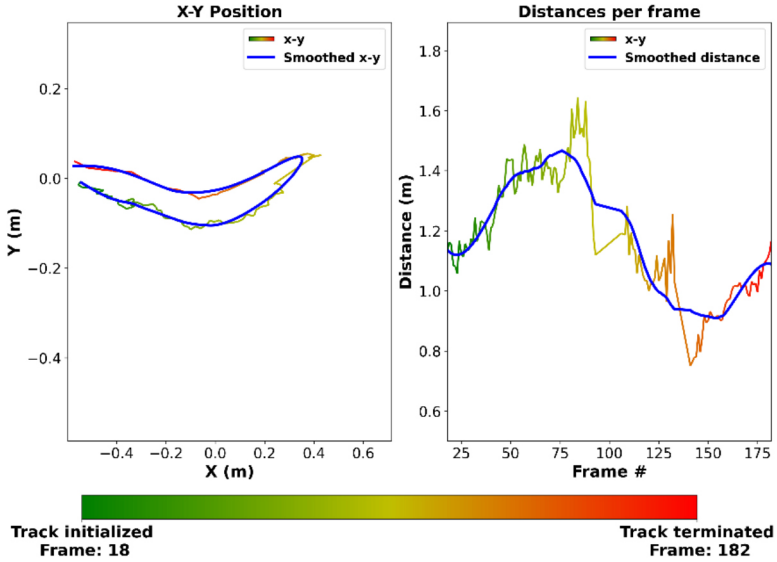

Figure 2: Estimated position and distance track of a single fish. The trajectory is colored to show where it starts (green) and ends (red).

Results

The distribution of distances for detected fish in a video with a white cube structure is shown in Figure 1. In ideal conditions like this scenario, most fish maintain a distance of one to three meters from the structure. However, when current and water pollution increase, the estimation quality declines, leading to many unrealistic distance estimations. This degradation can be attributed to inconsistent epipolar geometry when the structure moves due to current or challenges in correcting distortion with increased suspended particles in the water.

Figure 2 instead illustrates the estimated 3D X-Y movement and corresponding distance to the camera for a single fish. The position and distance estimations depend on disparity, which can vary between frames based on detection and feature matching performance, resulting in fluctuations in raw estimations. In challenging conditions, these fluctuations become more pronounced. Consequently, raw estimations are smoothed to reveal movement trends, represented by the blue lines in Figure 2. The fish's movement in the video aligns with the trajectory shown in the left graph. Additionally, the fish initially swims away from the camera, then turns toward it, and finally swims away again, as depicted in the right graph, demonstrating that the method accurately captures the movement trend.

Conclusion and future work

The results show that under ideal conditions most fish maintain a distance of one to three meters from the structure. However, in challenging situations with increased current and water pollution, the quality of the estimations deteriorates due to inconsistent epipolar geometry and challenges in correcting distortion. Despite this, by smoothing the raw estimations, the method proves capable of capturing movement trends. Future efforts should focus on mitigating the impact of adverse conditions and ultimately using the method to analyze fish behavior in response to various structures. Gaining such insights will contribute to the development of innovative autonomous tools that can further advance the aquaculture industry.

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REAL-TIME OPTIMIZATION IN RECIRCULATING AQUACULTURE SYSTEMS

Jamal, A.

Introduction:
The aquaculture industry is rapidly growing and has become a crucial part of global food production. Land-based recirculating aquaculture systems (RAS) are increasingly important due to their efficient water use, stable conditions, solids removal, and effluent treatment (Spiliotopoulou et al., 2018). Efficient management and optimization of RAS protocols are essential to achieve optimal growth outcomes (Seginer 2016). Optimizing various factors that impact the process, including feeding, heating, and oxidation, is necessary to achieve optimal growth. However, most existing RAS management methods are based on pre-determined solutions that may not be optimal for specific cases. Furthermore, existing solutions are yet to be applicable, as they fail to incorporate a proper description of the processes in the system such as water quality and fish growth processes (Chahid et al., 2021).

Materials and Methods:
In this study, I propose a novel decision support system (DSS) that provides optimal decisions regarding the feeding rate, temperature, and oxygen for maximizing profits in RAS. The proposed method utilizes a simulation-optimization framework that ensures reliable application in real-time (Jamal et al., 2018). A holistic simulation is developed by integrating fish growth simulations as well as water quality simulations. The fish growth simulations are based on bioenergetics models in which the fish growth is estimated by the metabolism rate with time as a function of current weight and the water quality of un-ionized ammonia, temperature, and dissolved oxygen (Ursen 1967). The water quality is simulated to describe the dynamics of the water quality with time while accounting for the impacts of the feeding, temperature, pH and bio-filtration. The relation between these factors and the water quality factors are well-known in the literature (Ebeling and Timmons, 2010). The coupled simulations of fish growth and water quality are used as a simulation for the processes that occur in RAS. The accuracy of the simulation is enhanced through a closed loop based on observations for oxygen, temperature, ammonia and weight. The simulation is coupled with the optimization algorithm (e.g. genetic algorithm) to estimate the value of several decisions (several levels of feeding rate, temperature and oxygen) within a simulated forecast period to the future. The decision that comes with the optimal expected profit in the forecast period is the optimal decision which is applied for the current time step. The process is repeated on a daily basis. Overall, this approach provides a reliable and customized solution for RAS management, enabling efficient decision-making to maximize profits. Figure 1 describes the overall framework.

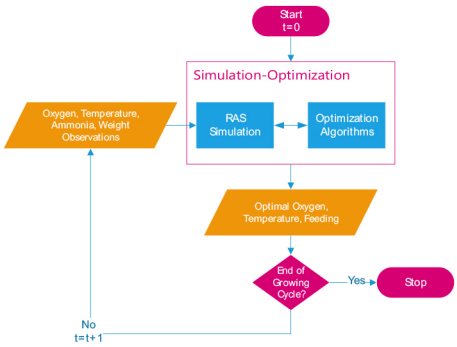

Figure 1: the overall flowchart.

Variable	Conventional	Optimized
Profit [$]	**13.7**	**59.4**
Fish Revenue [$]	225.2	183.8
Feeding Cost [$]	204.4	120.2
Oxygen Cost [$]	7.0	4.2

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Case Study:
A case study of Tilapia for feeding optimization for maximizing profits was conducted. The oxygen and temperatures were not optimized and remained constant throughout the growing cycle. The optimized results were compared to a conventional feeding schedule based on proportions (between 1% to 5%) of the current weight of the fish. The bioenergetics model parameters were used from Yi (1998).

Details:
Tank volume: 6 cubic meter; Number of fishes: 3000; Initial weight: 7 gr; Temperature: 28 C; Fish price: 1.5 $/kg; Oxygen price: 0.016 $/kg; Oxygen level: 0.6 mg/l; Bio-filter surface area: 500 ; pH level: 7.

Results:
Although lower revenue was obtained using the optimization method, the profits (the objective) was higher due to the saving in the feeding.

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EMPOWERING ATLANTIC AQUACULTURE. INSIGHTS FROM H2020 AQUAVITAE PROJECTS KNOWLEDGE MANAGEMENT AND CAPACITY BUILDING INITIATIVES

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AquaVitae is a four and half year H2020 EU funded project which started in 2019. It has 35 partners from all around the Atlantic, focusing on Europe, Brazil and South Africa. The project has two main aims. The first is to increase the production from low trophic value chains across the Atlantic. The second is to contribute to the All Atlantic Ocean Community, particularly in regards to the sustainable production of food from the Atlantic.

The project focusses on 5 value chains selected for their potential for sustainable production and their significant impacts, specifically:

- Macroalgae production; New species, offshore production, and post-harvest processes
- Integrated Multi-Trophic Aquaculture (IMTA); land-based and sea-based, biofloc systems
- New echinoderm species: Sea urchins and sea cucumbers
- Existing shellfish species: Oysters and mussels
- Optimised production of selected existing finfish species; freshwater and marine

These value chains are implemented through 13 case studies (including two cross cutting case studies) where practical, hands-on innovation and exploitation work has been carried out by industry and research participants. In addition, a significant part of the research activities in AquaVitae have focussed on cross-cutting issues applicable to many of the CSs. This includes research on: biosensors, Internet of Things and data management, product characteristics, consumer attitudes and market potential, sustainability, circular economy, environmental monitoring and risk assessment, and value chain analysis, profitability and socio-economic aspects. Issues related to policy and governance are essential and AquaVitae has contributed to various policy dialogues and produced EU policy briefs.

Knowledge, network-building, training, and communication is crucial when it comes to ensuring project impact both inside and outside the consortium. In AquaVitae this includes the development of relevant stakeholder platforms, exchange programs as well as a Massive Open Online Courses (MOOC) in low trophic aquaculture. The knowledge and Capacity Building activities will be the focus of this presentation together with their impact on Europe and the wide Atlantic Ocean Community.

DEVELOPING USES FOR SEA URCHIN BIMASS REMOVED FROM AREAS TO REINSTATE MACROALAGE FORESTS

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There are many places around the world where there are issues with overpopulation of both endemic and invasive sea urchin species. Generally, this leads to large mono populations of sea urchins that are known as 'sea urchin barrens'. These areas have reduced diversity, overall ecosystem production and reduced ecosystem services. There are many initiatives to remove the sea urchins and re-establish the macroalgae forest in the place of the sea urchin barrens. Along with developments in harvest technology and the possibility of 'enhancement' of some of the extracted sea urchins there are initiatives to find a productive and economically viable use for the sea urchin biomass extracted from sea urchin barrens. In addition, there are approximately 700,000t of sea urchins harvested and processed every year around the world. The byproduct of this processing could also be used in various forms.

This presentation will summarise the results from three trials which have investigated various uses for sea urchin biomass. The first of these is to make a hydrolysis of the biomass to extract marine based protein powder. This trial identified some basic processes and chemical characteristics of the hydrolysis from sea urchin crush. The results are published and will be presented.

The second was a Master student project investigating the use of sea urchin crush as a nutrient source for macroalgae seedlings. This proved challenging, primarily in respect to bioavailability of nutrients to the seedlings. The results of this trial will be presented.

Finally, the third was to develop and use sea urchin crush as an agricultural bio stimulant for agricultural production. This involved two harvesting events from 4 sites close to Tromsø in northern Norway to characterize the biochemical content of the sea urchin crush and any changes that occur because of harvesting from different sites and at different times of the year. Additionally, a plant trial has measured the efficacy of the sea urchin crush as a natural bio stimulant. The results from these trials will be presented.

The work in this presentation was funded by the InEVal Project, funded through the Blue Bio Era Net call.

FISH & SHRIMP HYDROLYSATE: ALTERNATIVE INGREDIENTS FOR SUSTAINABLE AQUAFEED

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Introduction

Today, there are various questions to address – from how to tackle global challenges such as climate change, biodiversity loss, resource depletion, water scarcity, waste, and pollution to how to build a resilient model, good for business, people, and the planet. The circular economy model helps to address these stakes, by applying three principles: reduce waste, circulate products, and regenerate nature.

Circular economy

Symrise Aqua Feed employs a circular model to bring marine co-products to their highest value. This helps the industry to tackle challenges for more sustainable aquafeed. Our solutions can create value to the aquaculture industry. (Figure 1)

100% co-product raw materials

Symrise Aqua Feed collects raw materials such as shrimp heads, tuna viscera, tilapia heads and bones or other marine by-products, which are not used for human consumption. These are usually waste by-products from fish and shrimp processing plants, that contain many precious proteins.

These sites are located next to the plants where fish and shrimp are processed, guaranteeing the freshness of the raw material. The locations also help to optimise the logistic and minimise the carbon footprint of the raw material transport.

The process, optimisation of nature.

Consequently, there is the processing and transformation to increase the functional value of the by-products. We master enzymatic hydrolysis,aiming at highly standardised hydrolysates in liquid and powder form, with multiple benefits such as nutrition, palatability, health.

We also pay attention to our own organic waste. We try to valorise left-over materials in the feed chain as much as possible, with the further processing into fish or shrimp meals. When the process into meal is not the best option, other alternative solutions come into play, such as composting with anaerobic digestion Overall, it is always seeking to find the highest value of fish and shrimp by-products.

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Combined benefits on palatability, nutrition, and health in the aquafeed.

The ingredients use in aquafeeds help to substitute fish meal, enhance feed palatability, standardise fish and feed performance by reducing deviation, enhance fish health statusand fish resistance to environmental and pathogen challenges. With better digestibility and better absorption, the ingredients help to reduce “fish in” while maximise “fish out” ratios (FIFO

Sustainable aquafeed for sustainable farming.

The zootechnical performance of Symrise Aqua Feed’s ingredients are proven in Aqualis: performance measurement centres, by scientific partners and in aquaculture farms. While guaranteeing high performance, the ingredients also help to reduce the environmental impacts in aquaculture. With high palatability, the company ensures high feed consumption while reducing feed wastes. With good nutrition, they improve the whole feed digestibility to reduce the release of non-digested feedin the environment and improve FIFO. With health benefit thanks to bioactive compounds, they enhance resistance to stressful events, improve gut health, increase survival, and help to reduce synthetic inputs.

Conclusion

Using this circular model, Symrise Aqua Feed produces alternative ingredients with a low carbon footprint and high positive social impact.

ENHANCING AQUACULTURE WITH AN OPTICAL AND ARTIFICIAL INTELLIGENCE BASED SYSTEM FOR MICROPLASTIC DETECTION

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Introduction

The presence of microplastics (MPs) in marine water is one of the main contamination sources nowadays, becoming a significant threat to marine ecosystems [1]. MPs detection and removal methods are essential to control water quality and implement measures to ensure the preservation of the environment. This paper proposes a work developed within the frame of ASTRAL European project and based on the design and development of an optical MP sensor capable of identifying these particles, differentiating them from other natural particles present in seawater. The technology will address some of the issues and limitations of current commercial alternatives [2], such as low flow rate or high cost, by combining fast off-the-shelf industrial cameras, tunable optics, image processing and deep learning models.

ASTRAL is a European project that aims at developing and providing innovative tech-niques and species combination to improve Integrated Multi-Trophic Aquaculture (IMTA). Within ASTRAL, a circularity assessment is being carried out to provide evidence-based metrics to evaluate how the new aquaculture systems performs in the context of the circular economy. Particularly, for the specific case study in Scotland, focused on the cultivation of seaweed and bivalves, we have identified the major drivers to increase the circularity in infrastructure.

Microplastic Sensor

The designed device can inspect a continuous water flow to search for microscopically-sized polymer particles, or MPs, and differentiate them from other natural particles (e.g., sand, algae) that may be present in marine water. The sensor's operation consists of a pump circulating water through a pipe which, in a stretch, passes through an optical detection system based on a laser light and a photo-diode. If the water contains a particle, the photo-diode stops receiving the laser beam, allowing the image acquisition system to take multiple images with different focus configurations. The collected images are analyzed to identify the detected element and determine its nature: a MP, a grain of sand, a bubble in the water, etc. The design and development of the microplastic sensor intends to achieve several objectives:

• Reliable MP detection. The optical system must properly detect particles so that they can be subsequently analyzed, allowing the sensor to provide helpful information about water quality. The image acquisition system must capture high-sharpness focused images to detect the particles and distinguish MPs from other common elements in water (e.g. sand, algae) using image processing algorithms.

• Efficient operation. The design must ease the deployment in marine environments, achieving low-power operation, robustness, and effective protection against hazardous en-vironmental conditions (corrosion, water inflows, humidity, etc.)

• Automatic and manual control. The manual control allows to configure, test, and vali-date the operation in offline mode, and the automatic control allows the sensor to operate without continuous supervision and online after the deployment.
A dedicated house is designed to ensure portability and long-term operation without con-tinuous supervision or maintenance. The design is based on commercial components to create an affordable sensor but capable of analyzing a permanent water flow and uploading results to a cloud database with low latency. The conducted validation reports a promising efficiency for the identification of MPs, which must be confirmed after the final deploy-ment in a real location.

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Results

A partial validation of the microplastic sensor prototype has been achieved at LEITAT lab-oratory by using synthetic samples based on the most common polymers (microplastics) located in sea water (such as polystyrene, propylene, etc). Furthermore, samples gathered nearby Norway (NORCE) and Scotland (Scottish Association for Marine Science), has en-riched image datasets to assist LEITAT in the development. First results have shown that the microplastic sensor is capable of detecting samples from 350um and upwards, with the current lenses and AI configuration.

Conclusions

Preliminary validation of an optical microplastic sensor, combined with artificial intelli-gence (deep learning) models and algorithms has been conducted in order to identify mi-croplastic particles (from to 350um and upwards) and to differentiate them from other par-ticles located in seawater, at laboratory. Final validation in a real environment is planned to be performed in the upcoming months.

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THE EFFECTS OF WOODCHIP BIOMEDIA AND DIFFERENT CARBON SOURCES IN DENITRIFICATION SYSTEMS COUPLED WITH SHRIMP RAS

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Nitrate continually accumulates in most RAS and has been shown to reduce production in high concentrations, thereby limiting long-term water reuse. Denitrification, a process in which facultative anaerobic microbes reduce nitrate to harmless dinitrogen gas, may allow for greater water reuse. Many denitrification filters are media based and may be unsuitable for systems with high solids concentrations such as biofloc-style techniques. Reactors relying on heterotrophic microbes also require organic carbon additions to fuel microbial activity, which increases costs for producers. In addition, some media types can be expensive. Low- cost alternatives such as wood chips for media and utilizing endogenous solid waste for carbon may lower costs while maintaining high rates of denitrification. The purpose of this study was to evaluate the potential for using no media versus woodchips in in-line denitrification bioreactors as well as to evaluate carbon sources, including fermented sludge supernatant, ethanol, and no added carbon.

The study consisted of six treatments with four replicates each and lasted 90 days. Each system included a 1 m^3 shrimp tank stocked at 250 shrimp m^{-3}. Water from each shrimp tank was pumped through a 3 L foam fractionator, an 18 L settling chamber, an 18 L moving bed biofilter, and a 28.7 L denitrification column. Sludge was collected from the settling chambers and allowed to ferment for a week prior to additions. Ethanol was added to maintain a 3:1 C:N ratio based on the daily nitrogen additions through feed. The concentrations of TAN, nitrite, nitrate, phosphate, alkalinity, turbidity, and TSS/VSS were measured weekly. Temperature, DO, pH, salinity and ORP were each measured twice daily.

Results indicate that nitrate was significantly lower in the WC treatments than in the NM treatments and in the ethanol treatments than in the no-C and sludge treatment. Shrimp survival and average weight were greater in the no media treatment than in the woodchip treatment. The ethanol treatment had greater average weight but lower survival than both the no added carbon and the sludge treatments. This experiment indicates that there can be a range of implications based on media and carbon types in denitrification reactors. Some factors seem to benefit water quality, but may have negative effects on shrimp. Such considerations are important for producers who wish to balance nitrate remediation while optimizing shrimp production.

	Water		Shrimp	
Factor	Nitrate (mg/L)	TIN (mg/L)	Mean Wt. (g)	Survival (%)
NM	46.4[a]	47.6[a]	16.9[a]	82.6%[a]
WC	23.2[b]	24.1[b]	16.1[b]	75.4%[b]
Ethanol	18.8[a]	20.0[a]	17.1[a]	71.8%[a]
Sludge	43.0[b]	43.9[b]	16.3[b]	81.5%[b]
No C	42.7[b]	43.7[b]	16.1[b]	83.7%[b]

ARCHITECTURE OF LUMPFISH *Cyclopterus lumpus* L. SKIN AND DEVELOPMENT OF A DIGITAL HISTOMORPHOMETRY TOOL TO ANALYSE ITS QUALITY

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Introduction

Lumpfish (*Cyclopterus lumpus* L.) is used in the salmon farming industry to combat sea lice. The numbers produced and used have increased from 10 million in 2010 to 51 million in 2020. However, the ethical use of these animals in the salmon net pens is an ongoing discussion, and concerns on how they are being handled during production has been extensively reported. The health and welfare of lumpfish must be improved if continuation of using lumpfish for biological control of sea lice in salmon farming should proceed. This includes a better understanding of lumpfish biology, insights into the physiological responses of cleaner fish to stressors, and development of tools for a better accuracy of assessing lumpfish health. At least 40% of the cleaner fish used in Norwegian farms die during production. This mortality is mainly linked to delousing operations, injury during transport, or release of poor-quality fish. Stress and damage through these events increase disease susceptibility and high mortalities can follow. Despite their robust appearance, lumpfish are very susceptible to skin damage and incidence is increased by their habit of adhering to substrates from which they are at times forcefully removed. Fish skin provides protection against external stimuli, has a high capacity for healing and regeneration and its structural changes may offer insights into the quality of the production environment. In this study we have studied lumpfish skin to characterize it`s features comparatively in in pre-adult and adult fish, we have used the sample-sets to develop digital histomorphometry algorithm, and we have used this algorithm to study the effects of handling and delousing on lumpfish skin.

Material and methods

Lumpfish were collected from three different sources: Preadult lumpfish from a land-based hatchery, adult lumpfish from a salmon farm, and adult lumpfish before and after a delousing procedure using HydroLicer in a salmon farm. Skin samples were analysed histologically from five different regions in the two first sampling sets to characterize lumpfish skin: dorsal (1), anterior (2), posterior (3), tail (4), and suction disc (5). A digital histomorphometry algorithm was developed using Aiforia® and tested on all skin regions and on the sample set from the delousing event. The algorithm included basic layers typical for fish skin, like the epidermis, the dermis, and mucous cells. In addition, features specific for lumpfish skin were added, e.g. goblet cells and bone plates. 52 AB/PAS-stained histological skin sections were used to build the Lumpfish skin algorithm (AI Lumpfish skin). In these, there were 1403 training regions, with 12730 mucous cells and 6089 goblet cells identified. Samples sets were run in the AI and results manually inspected to verify the algorithm.

Results and discussion

Results showed that regions 1-4 had similar features in the adult and pre-adult lumpfish, with epidermis, dermis, and the bone plates. In the dermis, mucus cells, goblet cells and keratocytes were found. However, several features differed between adult and pre-adult lumpfish. One markable difference was the thickness of the dermis. Preadult fish have a thin layer of dermis, whilst adult fish had an extensive dermis layer in all regions except for the tail region. The dermis layer increased at both stages in regions with bone plates and fins. The dense connective tissue is also more pronounced in adult fish compared to the pre-adults. Furthermore, the mucous cells are more numerous in adult fish, reflected by a higher mucous cell: epidermis ratio, while the ratio goblet cell: epidermis is higher in the pre-adult fish. Lumpfish has no scales, but instead the skin is strengthened through numerous smaller and larger bone plates. The bone plates are located between the epidermis and the dermis. In adult fish, the epidermis makes a thin layer, consisting of one or two cell layers, surrounding the bone plates. In preadults, the epidermis covering these bone plates are thicker, with several cell layers. Lumpfish skin has few, but evenly scattered sensory cells in the skin, with increasing numbers in the head/ mouth region.
The analyses of lumpfish from untreated and treated (deloused) lumpfish showed an increase in fin and skin damage up to 14 days after treatment in the treated groups. Skin damage will leave the fish more vulnerable to infections. Aiforia based analyses showed significant increase in dark pigment and in mucus cells in treated fish 14 days after treatment, both indicative of a stress response, as earlier shown in Atlantic cod, Polar cod and Atlantic salmon. Opposite, the amount of

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goblet cells was significantly reduced. Goblet cells are known from other species to be responsive to stress and as first line of defence against pathogens. They have been shown to be responsive to environmental factors and production related stress like handling and transport, however the specific response to stressors might vary between species. Three weeks after delousing the mortality of treated lumpfish was increased and the fish were diagnosed with crater disease caused by *Tenacibaculum* sp. Indications are that effects from the handling and delousing treatment of the lumpfish made them more vulnerable to disease.

Conclusion

An algorithm based histological analyses for lumpfish skin has been developed and has been successfully used to describe the lumpfish skin architecture of pre-adult and adult fish. It has furthermore been tested on field samples from lumpfish subjected to delousing. Results show that delousing operations contribute to skin- and fin damage and increase responses in the skin known to be connected to production related stress and handling. Indications are that the effects of delousing on the lumpfish make them more susceptible to pathogens.

DIVING INTO ZEBRAFISH STRESS: SKIN MUCUS CORTISOL AS A STRESS BIOMARKER

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Introduction

Zebrafish are often used to study cortisol mediated responses, particularly acute or chronic stress responses. However, to extract cortisol researchers have been relying on the zebrafish trunk, which is terminal, inhibiting the possibility of new cortisol measurements (Midttun et al., 2022) or re-using the animals for other purposes. Skin mucus offers a non-terminal and minimally invasive option (Franco-Martinez et al., 2022). However, no study has demonstrated its usefulness for identifying changes in cortisol levels in stressed zebrafish. Here, we evaluated the ability of zebrafish skin mucus to quantify cortisol in acute and chronic stressful situations.

Materials and methods

Adult AB wild-type zebrafish (sex ratio 1:1; 24 animals/group) were exposed to no-stress (control), acute (5 min net chasing + 30 sec air exposure), or a two-week unpredictable chronic stress protocol (2 stressors/day; a total of 7 stressors). The animals were euthanized by rapid cooling (0-4ºC) either immediately (control), 20 min after acute stress or approximately 20h after the last chronic stressor. Alterations on physiological levels were evaluated through cortisol (trunk and skin mucus) and oxidative stress markers (brain and liver homogenates). The sample (n=8), consisting of a trunk or a pool of 3 fish (skin mucus, brains, or livers) was considered the experimental unit. Data was analyzed using the IBM SPSS Statistics 27.0 computer program and plotted in GraphPad Prism 7 for Windows. Data normality and variances homogeneity were evaluated using the Shapiro-Wilk and Levene tests, respectively. For the cortisol data, the Independent Samples t-test and Mann-Whitney U-test were used to assess differences between groups. The oxidative stress markers in the liver and the brain were compared across groups using the one-way ANOVA or the Kruskal-Wallis test. The significance level was set at $p<0.05$.

Results and Discussion

As previously seen in other tissues, acute stress significantly increased ($p<0.05$) ROS (e.g., Monteiro et al. (2021)) and AChE levels in the brain. However, no differences were observed in the liver. This could be due to the liver's robust antioxidant activity compared to the brain (Song et al., 2006). Results also showed that skin mucus cortisol strongly responds to acute stress, identically to the trunk cortisol ($p=0.002$ and $p<0.001$, respectively).

Oxidative stress parameters were not altered by chronic stress, probably due to the animals' adaptation to this stress, which is also supported by the lack of alterations in the trunk's cortisol. Nevertheless, the cortisol levels in zebrafish skin were heightened after prolonged stress exposure compared to non-stress animals ($p=0.043$), which is interesting since skin mucus is traditionally viewed as an acute stress biomarker (e.g., Fernández-Alacid et al., 2019; Guardiola et al., 2016). It is possible that changes in skin composition were induced during exposure to chronic stress (see Carbajal et al., 2019), and that alterations in cortisol levels could have been masked by the cortisol distribution in the zebrafish trunk. However, due to the data variability and p near 0.05, these results require further confirmation.

Conclusion

Overall, the data suggested that skin mucus cortisol is an acceptable biomarker for acute stress in adult zebrafish. However, its role as chronic stress biomarker requires further studies.

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DOCUMENTATION OF CHANGING FLUORESCENCE WITHIN THE RED KING CRAB *Paralithodes Camtschaticus*

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Introduction

Biofluorescence in decapods has been reported for several taxa. However, few studies have carried out a detailed examination under controlled conditions to describe fluorescence patterns and how it varies between individuals and under different conditions. Red king crabs *Paralithodes camtschaticus* (RKC) are an introduced species to Norway in the 1960s (Lorentzen et. al. 2018) that are routinely kept in long-term holding facilities prior to live shipping (Mota et. al., 2021). The aim of this study was to evaluate whether *P. camtchaticus* biofluoresces and to document the dynamics of fluorescence in the species under controlled conditions. The specific objectives for this study are to determine whether detectable changes in fluorescence levels occur in either the hemolymph or exoskeleton of *P. camtchaticus*.

Methods

The study of biofluorescence in male RKC was divided into two groups: hemolymph and exoskeleton. RKC (N=19) were sampled for hemolymph before and after they were transported and stored overnight in 20-liter styrofoam boxes. Each RKC had ~1.5 mL of hemolymph sampled and analyzed with a Duetta fluorescence and absorbance spectrometer. Fluorescence emissions were recorded in the EZ Spec Software. Each sample was recorded with excitation wavelengths from 250 to 350 nm with a 5 nm interval and emission wavelengths from 400 to 800 nm with a 0.5 nm spectral resolution.

The exoskeleton analysis scanned RKC (N=10 juvenile, 8 adults) on a conveyor belt illuminated with ultraviolet (~ 400 nm) and royal blue (~445 nm) LED lighting. After hyperspectral scanning, the crabs were placed overnight for post shipping recovery in an aerated 1000-liter holding tank at 6° C overnight. All hyperspectral images were taken with the HySpex VNIR-1800 hyperspectral camera. Raw data files collected with hyperspectral scanning were radiometrically calibrated with HySpex Rad v2.5 to produce radiance values in units of . The radiometrically calibrated data was uploaded for analysis in Breeze hyperspectral imaging software and inspected visually for fluorescence signals.

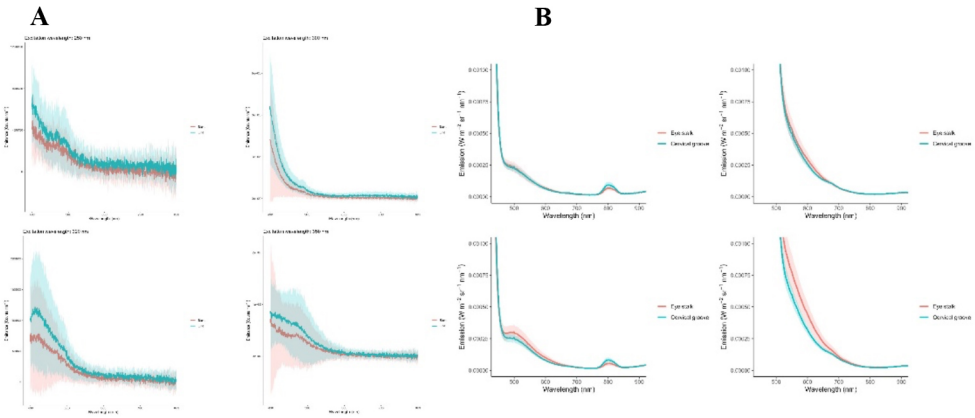

Fig 1. (A) Adult male king crab hemolymph fluorescing under 250, 300, 320, and 350 nm excitation light at 0 hours and 12 hours. The end point (blue line) of the experiment shows elevated fluorescence levels. (B) Fluorescence in the eye stalks of juveniles (left) and adults (right) during simulated shipping (start) and subsequent recovery (12 hours) in 6 °C seawater overnight.

Results

Biofluorescence was recorded in both the exoskeleton and the hemolymph in the species. We found that RKC show great variability in biofluorescence and that most displayed a green biofluorescence in their exoskeleton (~500 nm), with the greatest intensity occurring in the eye stalks and the cervical groove of the carapace. The arthrodial joints between the limbs fluoresced in red (~680 nm). Hyperspectral analysis detected both green and red fluorescence in juvenile (N=10) and adult (N=8) crabs. A decrease in fluorescence occurred in the eye stalks after a post transportation recovery period while the cervical groove remained constant (Figure 1B). After applying a live transport simulation to a second RKC group (n=19), we found a significant increase (Anova; $p = 0.008$) in the hemolymph fluorescence of animals tested before and after out of water transport (Figure 1A).

Conclusions

As both physiological parameters such as stress hormones and tools available for empirically documenting physiological changes in crabs remain less advanced compared to higher order aquaculture species, it is difficult to ascertain whether this change in fluorescence can be categorized as an indicator for specific levels of stress. A recent study exposing adult RKC to low oxygen levels documented anoxic stress in hemolymph through an increased concentration of lactic acid (Mota et. al., 2021). Imagery-based estimates of changing fluorescence, like the one demonstrated in this study, should be linked with such empirical approaches in future studies to further explore the utility of non-invasive techniques for assessing RKC welfare. These findings indicate that biofluorescence in RKC is dynamic and further investigation may reveal whether such fluorescence could be used to monitor the welfare of decapods in captivity.

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GENOTYPE ENVIRONMENT INTERACTIONS IN ATLANTIC SALMON, *Salmo salar,* REARED IN FRESHWATER RECIRCULATING AND LOCH SYSTEM

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Introduction

Atlantic salmon, *Salmo salar,* is a key aquacultural species, with Norway, Chile and the United Kingdom as the largest producers. There has been progressive uptake of land-based systems, ranging from flow-through to fully closed recirculating aquacultural systems (RAS). Scotland, that dominates salmon production within the UK, is the exception and there is still large-scale use of floating net-pens in freshwater lochs up to smoltification (Bergheim et al., 2009). In line with other countries, in Scotland there is now increased interest in the adoption of RAS as it allows greater husbandry control, mitigates environmental impacts, as well as providing flexibility in farm design and location, among others benefits (Clark and Bostock, 2017; Bostock et al., 2018). However, given the environmental differences in RAS compared to loch systems, it is necessary to assess the relative performance of selected stocks across the two environments. We therefore aimed to investigate the genetic architecture of growth traits as well as quantify genotype environment interactions (GxE) of salmon smolts when reared in each environment. Our findings will potentially help inform possible future breeding and husbandry choices.

Materials and Methods

~250,000 eggs from MOWI commercial stock were transferred to RAS in the northwest of Scotland. The population was co-reared for 9 months, undergoing vaccination and 10% cull. After this time half the population was transferred to a freshwater loch system. After 8 weeks of separate rearing 1,000 fish were sampled per environment, with weight length and condition factor recorded. Fish were genotyped to 50K SNP by Identigene Ltd, along with parental genotypes, were made available for analysis. Duplicated and unaligned SNPs were removed, with the remaining filtered by removing individuals missing >10% of SNPs, SNPs missing in >10% of individuals, SNPs that failed HWE at $p=10^{-6}$ and SNPs with minor allele frequency <0.005. Variance components (residual (V_r) and additive genetic (V_g) variance) and narrow sense heritability *(h^2,* calculated as V_g / V_p) were estimated using univariate animal model via implementation of a REML approach. GxE was quantified as the genetic correlation between a given trait measured in each environment, estimated by fitting a bi-variant model (Mulder et al., 2006; Sae-Lim et al., 2016). All models were fit with H matrix and performed in BLUPF90 software (Misztal et al., 2002).

Table 1: descriptive statistics for both the overall and environment specific populations.

	Weight (g)		**Length (cm)**		**K**	
	RAS	**Loch**	**RAS**	**Loch**	**RAS**	**Loch**
Mean	88.03	121.87	19.05	21.35	1.26	1.24
(S.D)	(17.87)	(27.11)	(1.25)	(1.67)	(0.08)	(0.11)
V_r	175.08	591.94	0.83	2.33	$0.38e^{-2}$	$0.15e^{-2}$
(S.E)	(13.26)	(35.11)	($0.62e^{-1}$)	(0.14)	($0.27e^{-3}$)	($0.50e^{-3}$)
V_g	127.1	129.58	0.55	0.43	$0.22e^{-2}$	$0.10e^{-1}$
(S.E)	(21.17)	(33.26)	($0.95e^{-1}$)	(0.12)	($0.39e^{-3}$)	($0.59e^{-3}$)
h^2	**0.42**	**0.18**	**0.40**	**0.15**	**0.37**	**0.13**
(S.E)	($0.55e^{-1}$)	($0.43e^{-1}$)	($0.55e^{-1}$)	($0.43e^{-1}$)	($0.52e^{-1}$)	($0.42e^{-1}$)

(Continued on next page)

Results and Discussion

55,357 and 65,774 SNPs were returned for parental and offspring genotypes, respectively. After filtering 45,751 SNPs, which were common to both, were used for analysis. Pedigree reconstruction identified 72 sires and139 dams, as well as 141 full-sib families, of which 134 were shared between environments. The greatest difference was seen in body weight and length, which were both greater in the loch compared to RAS population. However, though smaller the RAS population showed low estimates of variability as well as marginally greater condition factor (table 1). Differences between the populations reared in each freshwater environment were also seen in the genetic architecture of each trait, whereby the RAS population had comparatively greater heritability estimates.

Estimated GxE ranged from moderate to low. Specifically, the genetic correlation for body weight was 0.624 (s.e. 0.14), for length 0.778 (s.e. 0.15) and for condition factor 0.853 (s.e. 0.17). These values indicate that GxE may be of concern for weight as the genetic correlation fell below the break-even correlation, suggested in fish as < 0.7 (Mulder et al., 2006; Sae-Lim et al., 2016). This indicates that, if employment of RAS continues to increase in the UK, the performance of current commercial populations may be below what could be achieved if environment specific breeding programs were established. However, such conclusions are only based on statistical results and further investigation in use of RAS for current populations is necessary.

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EXPRESSION PROFILE OF HEMOPEXINS IN RESPONSE TO TEMPERATURE STRESS AND IMMUNE STIMULATION

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Changes in water temperature are one of the most important environmental factors that directly affect the physiology and behavior of ectothermic fish. In order for fish to adapt to a wide range of seasonal temperature changes, corresponding changes in physiology and gene expression are required. In previous studies, myosin, lactate dehydrogenase, Δ□-desaturase, and warm temperature acclimation related 65kDa protein (wap65) were reported as genes regulated to adapt to temperature changes. Wap65 is known as hemopexin (hpx) in vertebrates. Hpx is a glycoprotein that binds to heme with high affinity and transports it to hepatocytes. It has been reported to prevent free heme-mediated cell damage in plasma. In fish, Hpx (Wap65) consists of two isoforms. In this study, to infer the function of Hpxs in fish, we analyzed the gene expression pattern of the *hpx*s in zebrafish by temperature and immune stimulation.

Whole mount *in situ* hybridization analysis showed that *hpxa* and *hpxb* are primarily expressed in the liver of 2, 3, and 5 dpf embryos. Specifically, *hpxb* expression was observed in the liver at 2 dpf, while *hpxa* expression was not. In 5 dpf embryos, *hpxa* and *hpxb* expression was strongly maintained in the left lobe of the liver; however, compared with the overall expression of *hpxb*, *hpxa* was observed as a single spot on the limited hepatic region. Although *hpxa* and *hpxb* distribution differed in adult tissues, they were most strongly expressed in the liver, while the lowest levels were detected in the heart. When temperature stimulation was applied, it was confirmed that the expression of *hpxa* decreased and the expression of *hpxb* increased in low temperature. During artificial infection with *Edwardsiella piscicida* (*E. piscicida*), a major bacterial pathogen in fish, and viral hemorrhagic septicemia virus (VHSV), a viral pathogen, expression of *hpxa* decreased, while that of *hpxb* increased. Thus, zebrafish *hpxa* and *hpxb* may have important roles in *E. piscicida* and VHSV infection; however, are likely controlled by different regulatory mechanisms. The higher expression of zebrafish *hpxa* and *hpxb* in important immune-associated organs, as well as their significantly regulated expression in response to immune challenge and temperature stimulations, warrant a more detailed *in vivo* analysis of the immune-related role played by zebrafish Hpxa and Hpxb. The findings of the current study provide novel insights that may help elucidate the immunological role of Hpxa and Hpxb in fish.

AVOIDING FISH ESCAPES IN OFFSHORE AQUACULTURE THROUGH THE DIGITALIZATION OF MOORING SYSTEMS AND THE CONTROL OF THE SEA CAGE BEHAVIORS

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One of the main problems that offshore aquaculture is facing are losses and fish escapes caused by breaks in the sea cages. This problem arises mainly from failures in the mooring and the integrity of the system produced by storms, deficiencies in infrastructure supervision and maintenance, and lack of prediction of the sea cage behavior. In addition, sea cage ruptures have been intensified due to climate change.

This poster presents a pilot deployed in a meagre offshore fish farm in the Region of Murcia (Spain) within the project DigiSafeCage. The aim of the pilot is to take a first step towards a digital transformation in aquaculture production processes through monitorization and sensorization of infrastructure parameters such as deformations and tensions in the mooring lines, and oceanographic parameters such as waves and currents. Moreover, advanced statistical techniques and Artificial Intelligence analysis have been developed to predict sea cage movements under climate change scenarios.

Aim:
The aim of the project is to minimize the economic, environmental and social impact derived from sea cage escapes, caused by failures and breaks in the infrastructures. This poster presents a new digital system for the supervision of the offshore infrastructure that has been designed and, it is currently being tested to decrease the risk of ruptures based on the implementation of the sea cage digital twin.

Material and Methods:
To study the sea cage movements, sensors for monitoring the net deformations have been deployed (Figure 1 left) and load cells (Figure 1 right) have been installed in each mooring line. Wave parameters and currents data (direction and speed) have been correlated with the net movements and the tension of the mooring lines. The data analysis was done using the CRISP-DM (Cross-Industry Standard Process for Data Mining) methodology.

Results:
The load cell number shows an average value of 1507 kg and a minimum value of 593 kg (Figure 2).

The net sensors number 46 (Figure 3). “Pitch”; in green backwards movements and in red forwards movements (Figure 3 top). “Roll”, refers to side-to-side movements; in grey movements to the right and in blue movements to the left (Figure 3 bottom movements).

Conclusions:
Wave moments do not play a significant role in the sea cage net movement. Besides, there is a negative correlation between the net sensor’s movements and the speed of the current. These results mean that the speed of the current determines the movement of the sea cage net in one direction or another, in many cases regardless of the direction. Therefore, a continuous control of the currents in the location where the sea cage is placed would help to further understand the net behavior.

Regarding the load cell number 1, it shows values within the limits the mooring lines can hold, meaning that there is no risk of ruptures if the values are maintained as monitored. Nevertheless, these values were collected during the summer season without any storms. Loading cells should be deployed during winter season to understand the load the mooring lines are holding under adverse conditions to arrive to solid conclusions.

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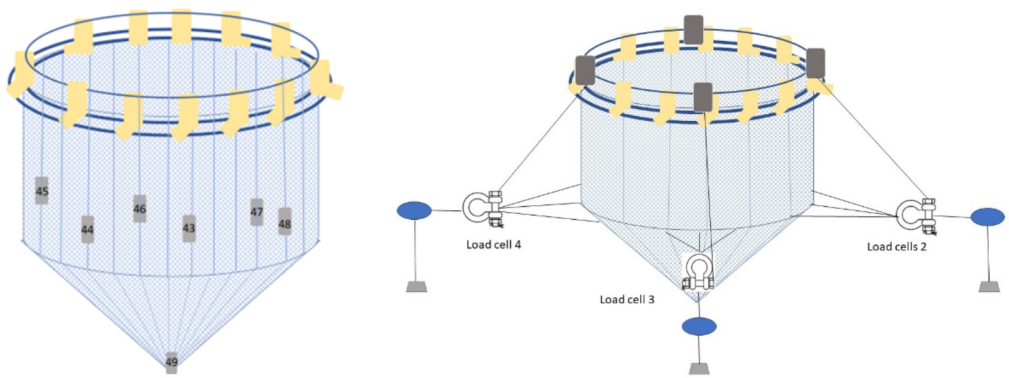

Figure 1. Deployment of the sensors in the sea cage. On the left; the net sensors, and on the right; the load cells in the mooring lines.

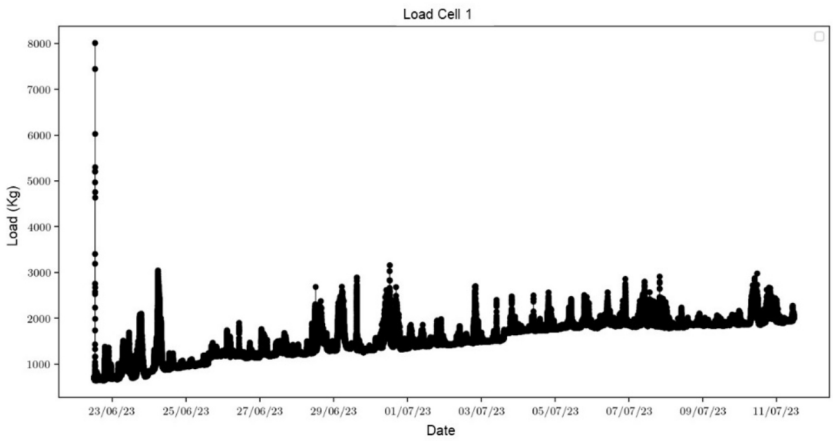

Figure 2. Kg supported by load cell number 1

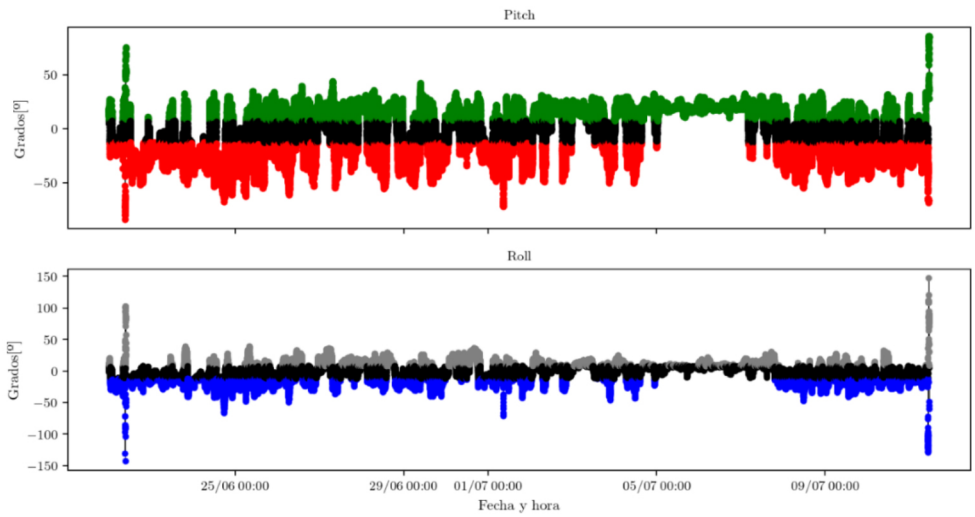

Figure 3. Net movements in degrees

POLYETHYLENE GLYCOL AND ALGINATE AS DELIVERY MATRICES FOR ORAL VACCINES: IMPACT ON RAINBOW TROUT (*Oncorhynchus mykiss*) DIGESTIVE TRACT AND SYSTEMIC RESPONSE

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Introduction

The limitations surrounding oral vaccines for aquaculture species pose challenges to both animal welfare and production efficiencies. Insufficient understanding of delivery matrices and histological effects further compounds these challenges. Immersion and injective immunization methods come with drawbacks, underscoring the potential of effective oral vaccines to confer lasting protection while reducing costs. Encouraging outcomes from experimental trials employing oral vaccines have been reported (Adelmann et al., 2008; Ballesteros et al., 2015). Overcoming existing obstacles necessitates the development of biocompatible matrices and defense mechanisms during administration. Notably, alginate and polyethylene glycol have demonstrated successful protection of vaccine components; however, comprehensive insights into the impact on fish intestinal histology are lacking (Mohapatra et al., 2019; Weber et al., 2006). The conducted study aims to assess the influence of polyethylene glycol and alginate on the digestive tract and systemic response of juvenile rainbow trout (*Oncorhynchus mykiss*). The findings provide valuable insights into the suitability of these compounds as secure and effective delivery matrices for bioactive substances, including oral vaccines.

Materials and Methods

Conducted at the Center for Aquaculture Research (ZAF) of the Alfred-Wegener-Institute Helmholtz-Center for Marine and Polar Research, Bremerhaven, the trial involved 240 juvenile rainbow trout (18.7 ± 0.1 g) acclimated to a temperature of 15°C for 14 days before the 22-day feeding trial commenced. The fish were randomly allocated to 16 rearing tanks within a recirculating aquaculture system (RAS). The four treatment groups were tested in quadruplicates:
Treatment 1 – Commercial pellet with 5% alginate inclusion
Treatment 2 – Commercial pellet with 18% polyethylene glycol inclusion
Treatment 3 – Commercial pellet with 17% polyethylene glycol and 5% alginate inclusion
Treatment 4 – Commercial pellet (control) The effects of the test substances were evaluated through histomorphological analysis of different intestinal sections and the quantification of inflammatory gene expressions including TNF-α, IL-1β, and IL-8.

Results and Discussion

Following the 22-day trial period, fish receiving the experimental diet with polyethylene glycol inclusion exhibited alterations in the intestine, notably a reduction in villus height and bioactive intestinal surface. Alginate inclusion resulted in a decrease in mucus-secreting goblet cells. Despite observable intestinal changes, analysis of inflammatory gene markers did not reveal significant systemic impairments. While negative intestinal effects were observed, their potential adjuvant impact on the immune response following oral vaccination, particularly for bioactive components such as oral vaccines, remains to be defined (Just et al., 2023).

Conclusion

Based on the outcomes of this study, all test substances showed immunocompetence and can be considered safe for the encapsulation and application of bioactive substances, including oral vaccines. These findings hold promise for advancing the development of effective and efficient oral vaccination strategies in aquaculture, contributing to improved animal health, welfare, and overall production outcomes.

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PERFORMANCE EVALUATION OF AQUACULTURAL WASTE-BASED BIOCHAR AS A CATALYST IN SEDIMENT MICROBIAL FUEL CELL FOR INTEGRATED MULTITROPHIC AQUACULTURE SYSTEMS

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Introduction:
The worldwide increase in the production of organic wastes has increased the need for treating and using them as manures, fertilizers, nutritive feeds or energy substrates. Integrated multitrophic aquaculture (IMTA) systems along with wastewater treatment techniques is a novel area of research which in future can be established as a cutting-edge technology in the field of agriculture and aquaculture. The sediment microbial fuel cell (SMFC) assisted wastewater treatment system has the potential of simultaneous agriculture/livestock/synthetic wastewater and aquacultural wastewater treatment along with promising power production for powering different low-energy up-taking sensors such as water quality or environmental sensors. Activated aquacultural biochar catalysts can be prepared from aquacultural wastes, which can potentially improve the performance of SMFC acting as cathode catalyst. Biochar-based catalysts are made of naturally available sources, mainly wastes which makes them a better sustainable alternative to metallic catalysts making them more cost-effective for large-scale applications. Waste minimization, wastewater treatment, water utilization and renewable energy production are the primary advantages of SMFC-assisted aquacultural systems.

Materials and methods:
A sediment microbial fuel cell (SMFC) reactor with carbon felt as the electrode was designed and fabricated for wastewater treatment and power generation studies (Fig. 1). After filling up the pond sediment and aquacultural wastewater, an anode electrode (carbon felt of dimension 18cm ×3cm ×0.5 cm) was placed at the bottom attached to the pond sediment and a cathode electrode (three carbon felt each of 6cm ×3cm × 0.5cm) which was stacked parallelly, was placed in the overlying water. The water quality parameters of the aquacultural pond water sample were analyzed and synthetic wastewater was prepared considering the maximum COD range that can occur in an integrated poultry-fish system for more accurate studies. The SMFC reactor was operated and monitored for more than 50 days (10 experimental cycles).

A novel activated aquacultural biochar catalyst was synthesized from aquacultural wastes and used as a cathode catalyst for improving the performance of SMFC. Material and electrochemical characterization studies were carried out in order to analyze the property of aquacultural biochar catalyst. Polarization studies were carried out by varying external resistance from 10 kΩ to 10 Ω to compare the performance of SMFC reactor with and without activated aquacultural biochar-based cathode catalyst in treating aquacultural and synthetic wastewater (poultry-fish based). A detailed design of an intensive poultry fish culture system assisted with SMFC-based wastewater treatment was proposed after validating the data from the experimental SMFC reactor's performance.

Results:
SMFC started producing an open circuit voltage (OCV) of 772 mV and an operating circuit current of 0.4 mA on the first day after its acclimatization phase. The stable operating voltage was generated until the COD value reached the optimal range and hydraulic retention time (HRT) was determined to be 4 days. An average COD removal efficiency of 86.31% was obtained over the 10 experimental cycles. SMFC generated a maximum operating voltage of 0.422 V when connected to an external resistance of 975 Ω on the 21st day of operation. The characterization studies of the synthesized aquacultural biochar catalyst such as cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS) exhibit the synthesized aquacultural biochar catalyst is an active electrocatalyst which can help in faster oxygen reduction reaction (ORR) rate which ultimately leads to higher power output and overall efficiency of the cell. Raman spectroscopy and X-ray diffraction (XRD) analysis suggest that the biochar shows good electrochemical activity and catalytic properties.

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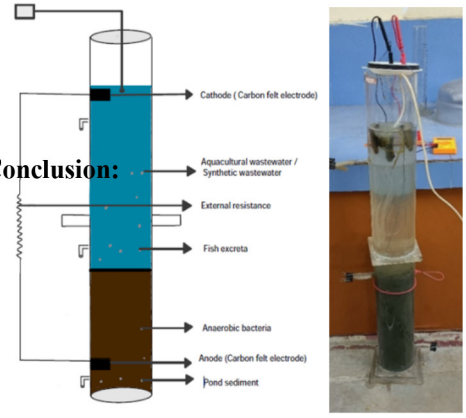

Fig. 1. Experimental SMFC reactor

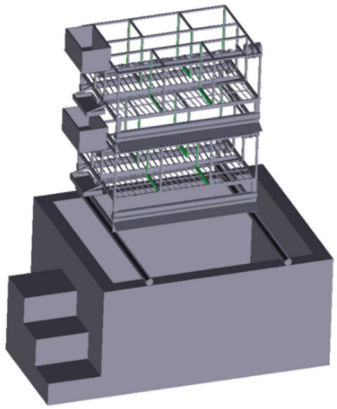

Fig. 2. SMFC assisted integrated poultry fish culture design setup

The SMFC with aquacultural waste-based biochar cathode catalyst treating the synthetic wastewater showed the highest maximum power density (MPD) with a range of 101.63 mW m^{-2} (1693.83 mW m^{-3}) and lowest internal resistance (49.34 Ω) which depicts the improved performance of the SMFC by the use of cathode catalyst. The results validate the reliability of using SMFC in actual aquacultural systems, thus proposing a system design of SMFC reactor-assisted small-scale integrated poultry fish culture system. SMFC used for the design study was proposed for the aquacultural fish tank of inner dimensions of 1.75 m ×2.1 m with a tank depth of 1.2 m. The design specifications for the integrated poultry fish culture are proposed in such a way that the poultry cage set-up should be installed on the top of the fish tank to easily pass the poultry droppings into the fish tank (Fig. 2). The poultry chickens will be kept inside the cages and the fish will be reared in the same fish tank where SMFC has been developed. SMFC equipped inside the fish tank will treat the wastewater and maintain the aquaculture water quality with simultaneous power production.

Conclusion:

This study represents the experimentation, analysis and validation of incorporating wastewater treatment techniques such as SMFC into IMTAs including integrated poultry-fish culture. The operating parameters such as pH, DO, temperature, SMFC reactor design, electrode material, and stocking density of fish and chicken are to be considered before incorporating SMFC into an integrated poultry-fish culture. SMFCs can be integrated into aquacultural systems, such to provide a complementary source of energy and improve water quality reducing the risk of disease outbreaks in aquacultural systems. SMFCs have the potential to act as power generators for different water quality and environmental sensors which in fact provides multiple advantages of assisting this reactor with the IMTA system. SMFC using aquacultural waste-based biochar catalysts can effectively be incorporated into IMTA systems resulting in a potentially valuable technology for sustainable IMTA systems. SMFC-based IMTA systems improve water quality, generate renewable energy, improve fish growth and survival and maintain sustainability.

STOICHIOMETRIC IMBALANCES OF AVAILABLE NUTRIENTS DRIVE CENTRAL EUROPEAN FISHPONDS – A REVISION OF CURRENT POND MANAGEMENT IS NEEDED

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Introduction

Fishponds, artificial ecosystems built exclusively for fish production, had been integral parts of the European landscape for centuries. The current fishponds have become less predictable regarding production processes, hence, also less cost-efficient, more stochastic, and greatly unstable systems. While human activities purposefully eutrophicated fishponds in the last century to increase their productivity, little is known about stoichiometric shifts of macronutrients available for planktonic food webs in these shallow ecosystems. Understanding the complex interactions between nutrient stoichiometry and ecosystem dynamics in fishponds is crucial for effective management and conservation efforts aimed at preserving these vital aquatic ecosystems while keeping them as protein-production units. We hypothesized that (a) the nutrient regime might vary both on regional and seasonal scales and (b) fishery management interventions might enforce stoichiometric imbalances. The long-term excessive nutrient loading may thus lead to stoichiometric imbalances and further worsen the bad nutrient state of fishponds yet affected by a region and a period of the vegetative season.

Material and methods

We surveyed 31 fishponds from lowland to highland regions of Czechia (altitudinal range from 170 to 730 m a.s.l.). All fishponds are polymictic, eutrophic to hypertrophic waterbodies (area 0.23–449 ha) with controlled fish stock. The fishponds under study were used for rearing ongrowing common carp exclusively. They were sampled monthly over a vegetative season from April to September (Apr–Sep). Data spanning the vegetative seasons were taken as observation units, herein referred to as pond-year, resulting in 150 pond-year cases. Dissolved inorganic N to total P ratio (DIN:TP) was used as an indicator for inferring in situ N versus P limitation of primary production from chemical data. DIN was calculated in-silico: DIN = NO_3-N + NH_4-N. We analyzed the data using generalized linear mixed models (GLMMs) with Gaussian distribution and log10 and log10+1 transformations to explore the drivers of chlorophyll-*a* and DIN:TP concentrations in water.

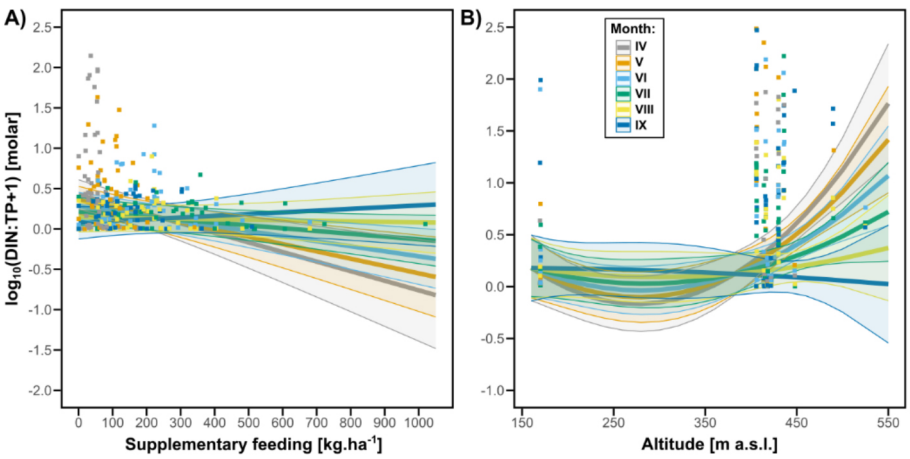

Fig. 1. Predicted effects of supplementary feeding (A) and altitude (B) during the season as identified by the most parsimonious model. Non-focal continuous values are fixed at the mean value in the dataset. Symbols: individual data, Colours: different months; lines and ribbons denote GLM model predictions with 95% confidence intervals.

(Continued on next page)

Results

We surveyed the seasonal trends of chlorophyll-*a* and the dynamic of available nutrients to the planktonic food web in 31 ponds used exclusively for fish production. Results of data compiled over various temporal and spatial scales suggested that fishponds might be stressed by seasonal and regional stochiometric imbalances of available macronutrients that could drive the concentration of chlorophyll-a (Fig. 1).

Discussion

Pond aquaculture is becoming progressively more marginal and challenged by many factors, such as prices, sustainability, environmental concerns, and resource utilization. Besides altitude, supplementary feeding (e.g., cereals and triticale; rich in phytate P) seems to be connected to the DIN:TP shift. The dynamics of available nutrients changed over the vegetative season with the cumulative input of supplemental feed. Most of the feed (65%) is fed in the second part of the growing season, at the time when the natural prey is over-grazed. We suggest that the current pond management practices deserve a revision, as warranted by changing nutrient status, stoichiometry, and climate.

INFLUENCE OF *Artemisia Arborescens* AS A FEED SUPPLEMENT ON PHYSIOLOGICAL AND IMMUNE RESPONSES OF GILTHEAD SEA BREAM (*Sparus Aurata*)

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Introduction

Fish infectious diseases are one of the main constraints of the aquaculture sector, representing a serious economic, social, and environmental challenge for the industry. Sea bream (*Sparus aurata*) is a teleost fish found in the Mediterranean Sea. It is economically important and natural stocks are subject to intensive exploitation raising future conservation and management issues. Due to its importance as high-quality food, many studies are focusing on enhancing its immune defense mechanisms, in order to improve its health in farming conditions. The use of medicinal plants as aquafeed additives provide a sustainable way of fish protection using safe, eco-friendly compounds in a cost effective way compared to antibiotics and chemical compounds currently used. Several studies have proved that medicinal plants have extensive antimicrobial, immunostimulant, antioxidant, anti-stress, and growth-promoting properties [Awad et al., 2017; Firmino et al., 2021]. Aim of the present study was the assessment of *Artemisia arborescens* feed supplementation effects on sea bream (*Sparus aurata*) physiological and immune responses in both lab-based experimental and field feeding trials.

Materials and methods

Two experimental diets containing different amounts of *Artemisia arborescens* (AA) essential oil extract (0.25 % AA and 0.50 % AA) and a positive control diet (commercial diet without AA) were used. Sea bream were assigned to experimental tanks and each diet was allocated in triplicate groups. The feeding trial was continued over a period of two months. At the end of the feeding trial, fish mucus was isolated, followed by measurement of fish weight and length. Blood was drawn by the caudal vein of fish and serum was isolated by centrifugation. Spleen tissues were removed aseptically and stored at – 80 °C. All animal handling and sampling procedures were conducted in accordance with Greek and EU laws and regulations.

Mucus and serum samples were used for biochemistry and immunological parameters assessment, according to well established protocols. Non-specific immune parameters (i.e. nitric oxide, lysozyme, myeloperoxidase, complement C3, proteases and anti-proteases), antibody responses (total antibodies, immunoglobulin M, anti-microcotyle and anti-*T. maritimum* antibodies), oxidative stress (CYP1A1, metallothionine (MTT)) and metabolism markers (glucose, alkaline phosphatase (ALP) were determined. Total RNA was extracted from fish spleen and real-time PCR assays were carried out to analyze the expression levels of genes related to antioxidants (SOD1, GPx1), cytokines (Il-10, TGFb1, Il-1b, TNFa), anti-bacterial peptide (Hepcidin) and heat shock protein (GRP75).

Results and Discussion

Fish weight, length and splenosomatic indexes showed no significant differences in tested diet groups. The total protein amounts in serum and mucus were significantly lower when the experimental diets were compared to control diet. Glucose and alkaline phosphatase levels weren't significantly altered by the experimental diets in serum, however the ALP mucus levels were decreased. Non-specific immune responses were assessed in fish serum and mucus by measuring the levels of nitrite ions, lysozyme, complement C3, myeloperoxidase, as well as protease and anti-protease activities. No significant differences were found in serum nitric oxide; however, mucus nitric oxide levels were significantly lower in experimental diets. Lysozyme and C3 levels were relatively stable in all groups. The myeloperoxidase levels were also not altered by the experimental diets. The remaining parameters (proteases and anti-proteases activities) were not affected by any of the experimental diets. The total antibody levels remained stably low, however the IgM levels was significantly increased with experimental diet 0.25% AA compared to the control diet. The cytochrome 1A1 was significantly lowered in 0.50% AA diet, while metallothionine was not affected by experimental diets. Those results indicate a mild modulation of oxidative stress by *A. arborescens*.

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Gene expression profile was analyzed to evaluate the modulation of immune-, oxidative stress- and metabolism-related genes in sea bream fed with the two experimental diets. The studied genes in sea bream spleen were related to cytokines (Il-1b, Il10, TGFb1, and TNFa), oxidative stress (SOD-1 and GPx1) and metabolism (hepcidin and GRP-75). All analyzed genes were differentially expressed to some extend but gene expression differences were not statistically significant for any of the tested groups. However, the 0.50% AA group appear to have increased expression of the immune and oxidative stress markers compared with control and 0.25% AA groups.

The 0.25% AA diet was also tested in a field feeding trial and it was found that the experimental and control groups had no statistically differences in any of the tested parameters, which were described above.

Concluding, serum and mucus tested parameters revealed that *A. arborenses* influences *S. aurata* immune and oxidative stress profile. The gene expression levels increased in diets with high extract concentration. The suitability of *A. arborescens* as efficient food supplement for immune status improvement was investigated and the results indicated that it could be used as dietary additive since it appears to have considerable potential as natural immunostimulant.

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DRY AND LIVE FEED FROM THE ONSET OF EXOGENOUS FEEDING: A VIABLE FEEDING STRATEGY FOR HATCHERY PRODUCED GILTHEAD SEABREAM, RED SEABREAM AND EUROPEAN SEABASS

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Introduction

Marine larval feed technology has greatly advanced and provides microdiets that cover the nutritional needs of newly hatched larvae, have high acceptability and palatability, water stability and low nutrient leaching (Hardy and Barrows, 2002). In the hatchery practice of gilthead seabream *Sparus aurata*, red seabream *Pagrus major* and European seabass *Dicentrarchus labrax*, dry feed is progressively introduced to fish larvae not earlier than 15-25 days post hatching (dph) with the concomitant use of live feeds (rotifers, *Artemia*) until weaning. However, compared to live feed, dry feeds appropriately manufactured (i.e. physical and nutritional properties) are expected to better provide for all nutritional needs of altricial fish species. Although the exclusive use of dry feeds is still inefficient, it is widely accepted that the longer the co-feeding period of live and dry feeds, the better the larvae performance at weaning (e.g. Cañavate and Fernández-Díaz, 1999; Khoa et al., 2020). In cooperation with commercial hatcheries, we previously investigated the introduction of dry feed, together with live feed, as soon as the onset of the exogenous feeding in gilthead seabream, red seabream, and European seabass larvae (Karakatsouli et al., 2019, 2021a, 2021b, 2022). The aim of the present study is to overview results obtained and present comparative data regarding three of the most important intensively reared fish species of Mediterranean aquaculture.

Materials and methods

Three experimental trials were conducted, one for each species. The experimental design included two stages: Stage 1, Hatchery rearing (Hr), was performed in a commercial marine fish hatchery. It is in this stage that the experimental treatments were imposed. Specifically, dry feed was introduced either on the first day of exogenous feeding (Experimental Feeding Protocol, EFP) or on the dph (days post hatching) that the specific hatchery protocol used (Typical Feeding Protocol, TFP). The trial was performed under actual production conditions and each treatment was duplicated. In all experimental tanks, the larvae were fed equal amounts of live feed according to the hatchery protocol. Samples were regularly taken, from hatching up to weaning, to monitor larvae growth and functional development of the digestive system (digestive enzymes analyses). Skeletal deformities were also evaluated. Stage 2, Laboratory rearing (Lr), was performed in a laboratory recirculating seawater system. It is in this stage that post-larvae growth performance (pre-growing) was evaluated by rearing larvae produced from Stage 1 under common and controlled conditions. The trial was decided to be performed under laboratory conditions, since, under production conditions, it is not feasible to keep together and separately a specific hatchery batch due to frequent gradings. Post-larvae growth performance was monitored for about two months.

	Gilthead seabream	Red seabream	European seabass
Larvae	↑ growth ↓ length variation = survival Digestive system: normal and responsive to DF ↓ skeletal deformities	↑ ↑ growth = length variation = survival Digestive system: normal and responsive to DF	= growth ↓ length variation = survival Digestive system: normal and responsive to DF ↓ skeletal deformities
Post-Larvae	↑ growth performance	↑ ↑ growth performance ↓ skeletal deformities	= growth performance

Figure 1. Summary of obtained results when larvae were co-fed live and dry feed from the onset of exogenous feeding (EFP) compared to TFP. Arrows indicate significant higher or lower values; equals indicate not significant differences; DF: dry feed.

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Results and discussion

For gilthead and red seabream the EFP promoted larvae and post-larvae performance (Figure 1). It is noted that growth enhancement in red seabream was highly remarkable. In the case of European seabass, no growth differences were observed between the two feeding protocols (EFP vs TFP). In all three species, survival of larvae co-fed live and dry feed from the onset of exogenous feeding was similar to those that followed the TFP, while no differences were observed regarding the percentage of larvae without swim bladder. Moreover, the functional development of the digestive system was normal in both treatments; in the case of EFP larvae digestive function (i.e. trypsin, chymotrypsin, amylase, lipase specific activities) was responsive to dry feed intake, especially during the first feeding days. In all three species, a common observation was the development of fewer skeletal deformities in the EFP.

Overall, present results suggest that the introduction of dry feed together with live feed in first-feeding gilthead seabream, red seabream and European seabass larvae does not compromise larvae and post-larvae growth performance and functional development of the digestive system. The incidence of fewer deformities in all species tested support the hypothesis that the ingestion and digestion of an appropriate dry feed may provide larvae with the necessary nutrients to form a healthy skeletal system. Nevertheless, species-specific differences are indicated, red seabream being the most responsive species while European seabass the least. However, in the latter case, there is a great variability of applied feeding protocols in commercial hatcheries, while in this study, only one of them was examined. In conclusion, co-feeding dry and live feed in first-feeding gilthead seabream, red seabream and European seabass larvae can be considered as a viable feeding protocol producing healthy fish with fewer deformities.

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THE EFFECT OF A SLOW-RELEASE AMINO ACIDS MIXTURE IN EUROPEAN SEABASS DIET ON FISH GROWTH PERFORMANCE AND AMINO ACIDS DIGESTIBILITY

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Introduction

The increasingly challenged fishmeal market together with sustainability concerns strongly dictate the constant search for alternative protein sources for aquafeeds. The product examined in the present study (NatuPro, Devenish Nutrition) is an alternative, nutritionally enhanced protein that due to a sugar-based carrier allows for a slow release of amino acids during the digestion phase resulting in a more efficient absorption and utilisation of amino acids by the animal. The product has been tested in growing pigs improving nitrogen retention and utilisation and reducing total and urinary nitrogen output (Kasserly et al. 2005). To our knowledge no literature data have been reported regarding its use in the diets of intensively reared fish species. The aim of the present study is to evaluate the inclusion of NatuPro in European seabass *Dicentrarchus labrax* diets on juvenile fish growth performance and amino acids digestibility. The evaluation was performed under the general scope of reducing the currently used dietary protein content by sparing high protein raw materials, fishmeal in particular.

Materials and methods

A feeding trial was designed that included three experimental diets: (a) a diet formulated according to current commercial diets for European seabass (Control diet, crude protein 46.9 %, fishmeal 15 %), (b) a negative control diet, i.e. a diet with 1 % lower crude protein level by 1.5 % lower fish meal level (C1, crude protein 45.7 %, fishmeal 13.5 %), and (c) a C1 diet with NatuPro included at 2 % (C1-N2, crude protein 46.0 %, fishmeal 13.5 %). Experimental diets were fed, for 140 days, to tetraplicated fish groups (mean initial body weight ± s.e.: 38.3 ± 0.08 g; fourteen fish per group; recirculating seawater system). Fish were fed a restricted ration according to body weight and water temperature. After the completion of the main rearing period and growth performance assessment, fish were fed the same diets including chromic oxide (1.0%) as a marker, once daily to satiation. Faeces were collected by stripping every two days. At the end of the digestibility trial all fish were sacrificed; liver and carcass were weighted to calculate hepato-somatic index (% body weight) and carcass yield (% body weight). Diets and lyophilized faeces were analyzed for chromium, protein and amino acid content. Data were analyzed by one-way ANOVA and tank was the experimental unit (n=4).

Results and discussion

No significant differences were detected among experimental diets for growth performance (i.e. final body weight, condition factor, specific growth rate, thermal growth coefficient, weight gain) and feed efficiency (i.e. feed conversion ratio, protein efficiency ratio, economic conversion ratio). Also, experimental diets did not affect hepato-somatic index and carcass yield. Protein digestibility was similar among experimental diets. However, values obtained were rather low probably due to the stripping method of faeces collection (Spyridakis et al. 1989; Shomorin et al. 2019) and the low water temperature (19.3 ± 0.6 ^{o}C) during the digestibility trial (Peres and Oliva-Teles 1999). Lysine (Lys) digestibility was significantly higher in C1-N2 diet compared to C1 and control diets (Table 1). Methionine (Met), glutamic acid (Glu) and glycine (Gly) digestibility was significantly reduced in C1 diet compared to Control diet; the inclusion of NatuPro (C1-N2 diet) highly improved Met and Glu digestibility in higher levels than those observed in Control diet, while Gly digestibility was back at Control diet levels.

Obtained results indicate that the inclusion of NatuPro in European seabass diets does not compromise fish performance and feed efficiency. The improvement of some essential and non-essential amino acids digestibility suggests the product's potential use in fish diets and agrees with previously reported results on other productive animals (Casserly et al. 2005). With the continuously increasing prices and scarcity of fishmeal, NatuPro is indicated as an interesting alternative protein source. However, since present experiment was conducted under lower than expected water temperature for the growth of European seabass, further research will be needed to better understand the mode of action under different rearing conditions, especially under the optimum water temperature range for the species (Yilmaz et al. 2020).

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Table 1. Amino acids apparent digestibility coefficients (ADC, %) of European seabass

	Diets			
	Control	**C1**	**C1-N2**	P
Essential amino acids				
Arginine	84.1 ± 0.73	84.2 ± 0.56	82.8 ± 0.38	ns
Histidine	82.6 ± 0.91	78.6 ± 1.91	78.4 ± 0.36	ns
Isoleucine	79.9 ± 0.27	79.9 ± 0.41	82.1 ± 0.63	ns
Leucine	76.1 ± 0.65	77.6 ± 1.57	75.0 ± 0.11	ns
Lysine	80.2 ± 0.16 a	79.8 ± 0.39 a	82.3 ± 0.19 b	*
Methionine	89.9 ± 0.87 b	83.9 ± 0.47 a	95.8 ± 0.63 c	**
Phenylananine	79.0 ± 0.12	77.8 ± 0.87	79.0 ± 0.27	ns
Threonine	82.2 ± 0.97	77.1 ± 1.59	81.7 ± 1.13	ns
Valine	80.8 ± 0.58	77.3 ± 1.43	81.3 ± 0.42	ns
Non-essential amino acids				
Alanine	84.0 ± 0.31	84.3 ± 0.42	84.5 ± 1.34	ns
Aspartic acid	74.7 ± 0.58	72.6 ± 1.35	68.9 ± 1.53	ns
Glutamic acid	88.9 ± 0.29 b	87.1 ± 0.03 a	90.2 ± 0.11 c	**
Glycine	85.1 ± 0.06 b	78.2 ± 1.74 a	86.9 ± 0.95 b	*
Proline	83.8 ± 0.32	84.2 ± 0.64	85.4 ± 0.36	ns
Serine	83.8 ± 0.01 ab	82.5 ± 0.62 a	84.9 ± 0.19 b	*
Tyrosine	82.9 ± 1.33	81.1 ± 0.92	81.8 ± 1.31	ns

Means with different letters are significantly different; ns: not significant; * $P<0.05$; ** $P<0.01$

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EFFECT OF FISHMEAL REPLACEMENT BY *Chlorella sorokiniana* ON GROWTH PERFORMANCE OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Given their high nutritional value and low environmental impact, microalgal biomass can serve for the partial replacement of dietary fishmeal (Glencross et al. 2020) towards more sustainable aquafeeds. *Chlorella vulgaris* is the most common commercial microalgal protein that has been successfully used for fishmeal replacement in aquafeeds (Karapanagiotidis et al. 2022). However, its rigid cell wall lowers its digestibility and nutrient availability to fish (Tibbetts et al. 2017). *Chlorella sorokiniana* is also a unicellular alga that can contain high levels of crude proteins and essential amino acids (Liu et al. 2022) and thus could also be a promising fishmeal substitute. However, studies on the use of *C. sorokiniana* meal for fishmeal replacement in aquafeeds are extremely limited. Thus, the aim of this study was to evaluate the dietary fishmeal replacement by graded levels of a *C. sorokiniana* meal on the growth performance and feed utilization of an important Mediterranean farmed species, the gilthead seabream (*Sparus aurata*).

Materials and Methods

A total number of 360 *S. aurata* juveniles of 2.6 g initial mean weight were obtained from a commercial fish hatchery, transferred to the aquaculture facilities of University of Thessaly and stocked at 12 tanks (125L) in a closed seawater recirculation system. Fish in triplicate groups (30 fish/tank, 3 tanks/dietary group) were fed to satiety, twice a day, four isonitrogenous (46%) and isoenergetic (21 MJ/Kg) diets differing in the source and the inclusion level of *C. sorokiniana* (CS) meal. CS0 diet used as the control one and contained fishmeal at an inclusion level of 200 g/Kg and zero levels of CS. A second diet (CSo20) was used containing a CS meal that was produced in the open ponds of University of Thessaly under photoautotrophic conditions, replacing the fishmeal protein of the CS0 diet at 20% corresponding to 54 g/Kg dietary inclusion level. The protein content of this CS meal was 48.8% (N to protein factor 4.95). In diets, CS20 and CS30 fishmeal protein of the control diet was replaced at 20% and 30%, respectively by a commercial CS meal corresponding to 70 g/Kg and 104 g/Kg dietary inclusion levels, respectively. The commercial CS meal was produced in closed photobioreactors (PBR) with a protein content of 37.7%. After 60 days of feeding, fish were weighted to measure growth and feed utilization parameters. Data were analysed using one-way ANOVA and differences between means were determined by Tukey's multiple-range test (P=0.05).

Results and Discussion

Survival was high and similar (P>0.05) among the groups (Table 1). All groups of fish promptly accepted the respective diets and feed intake was similar (P>0.05) among the groups, though it was a trend for higher consumption in fish fed the commercial CS meal-based diets. The latter two groups had higher, though not significantly (P>0.05), final weight, weight gain, specific growth rate (SGR), feed efficiency and protein efficiency ratio (PER) compared to the control group. Fish fed the CS meal produced in open ponds (CSo20 group) had also similar growth and feed utilization parameters compared to the control group, but their performance was significantly inferior compared to the fish that were fed with the commercial *C. sorokiniana* product. This performance differentiation maybe due to *C. sorokiniana* strain-specific nutritional features or to a possible higher biological contamination level of the CS biomass produced in open systems vs PBR. These results indicate that both *C. sorokiniana* meals can successfully replace part of fishmeal protein without impairing fish growth performance and feed utilization.

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Table 1. Growth performance and feed utilization of *S. aurata* fed with the experimental diets.

Parameters / dietary groups	CS0	CSo20	CS20	CS30
Survival (%)	100.0±0.0	95.6±5.0	97.8±1.9	100.0±0.0
Feed intake (g/fish)	12.18±0.57	12.06±1.02	12.76±0.52	13.48±0.40
Final weight (g/fish)	12.94±0.61[ab]	12.40±1.10[b]	14.79±1.23[a]	15.27±0.61[a]
Weight gain (g/fish)	10.35±0.61[ab]	9.80±1.10[b]	12.19±1.22[a]	12.66±0.61[a]
SGR (%/day)	2.68±0.08[ab]	2.60±0.15[b]	2.89±0.14[a]	2.95±0.07[a]
Feed efficiency	0.85±0.03[ab]	0.81±0.06[b]	0.95±0.06[a]	0.94±0.04[a]
PER	1.89±0.07[ab]	1.78±0.13[b]	2.08±0.13[a]	2.05±0.10[a]

Note. Values represent means ± standard deviation of triplicates. Values within each row not sharing a common superscript letter are significantly different ($P < 0.05$).

Studies on the use of *C. sorokiniana* meal for fishmeal replacement in aquafeeds are extremely limited. In *Oncorhynchus mykiss*, Chen et al. (2022) using a 5% dietary inclusion of CS meal, replacing fishmeal, significantly increased the feed intake by 19.3% and weight gain by 17.3% without impairing feed efficiency, while also improved the antioxidant capacity and immunity of fish. In the same species, Liu et al. (2022) reported a higher feed intake, growth performance and feed efficiency of fish fed a diet where fishmeal was totally replaced by CS. The authors attributed this to the higher digestibility and feed palatability of the used CS meal, its similar amino acid profile with fishmeal and the *Chlorella* growth factor that provides several bioactive compounds to fish nutrition. The present findings could help the aquaculture of gilthead seabream to further decrease dietary fishmeal levels by *C. sorokiniana* meal towards more sustainable aquafeeds.

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IN VITRO SCREENING OF POTENTIAL ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) COMBATING *Bacillus* STRAIN

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Introduction

A bottleneck to the growth of the aquaculture industry is disease outbreak. The most recent disease outbreak in shrimp farming systems, known as Acute Hepatopancreatic Necrosis Disease (AHPND), occurred in 2009 and resulted in enormous economic losses across the globe (FAO, 2022; Sarker et al., 2021; Mishra et al., 2017). The opportunistic pathogenic bacterium *Vibrio parahaemolyticus* (VP) produces plasmid encoded (pVA1) binary toxins $pirA^{VP}/pirB^{VP}$. The toxins cause up to 100% mortality by degenerating the epithelial cells of the hepatopancreases during the early stages of shrimp stocking (Lightner et al., 2012; Lai et al., 2015). *Bacillus* strains have been used widely in combating diseases in aquaculture. There is also evidence that *Bacillus* treatment improve brine shrimp survival against *V. parahaemolyticus* (Nguyen et al., 2021). Interestingly, the protection mechanism is still not clear. In this study, eight distinct *Bacillus* strains as potential AHPND disease controlling or mitigating agents were examined *in vitro* for their ability to degrade AHPND toxins and other phenotypes potentially contributing to their probiotic nature.

Materials and Methods:

Eight *Bacillus* strains have been used for the study. The *Bacillus* were collected from BCCM/LMG and DSMZ collection centre. The recombinant binary toxins ($rPirAB^{VP}$) were purified using His Spintrap column (Cytiva 28-4013-53, USA). In vitro degradation (over a period of 48h by 10^7 CFU/ml) of recombinant AHPND toxins were detected using SDS-PAGE followed by Western Blot techniques. Strains were tested for their capacity to degrade quorum sensing molecules (only acyl homoserine lactons; AHL) in an LB background over a period of 24 h. The flow cytometer was used to test their capacity to bind four fluorescently (FITC) labeled lectins (from plant origin). Biolog GENIII microplate (BiOLOG, Hayward) assays were used for metabolic profiling analyses and data were processed by PCA analysis. Extracellular proteins harvested from concentrated *Bacillus* culture were separated by SDS-PAGE and analysed using the BioNumerics 7 software (setting: 1% optimization of band migration) (Applied Maths, Kortrijk, Belgium).

Results and discussions:

Bacillus strain LMG9300, DSM1668 & DSM8785 exhibited strong AHL-degrading properties. Apart from AHL-degrading capacity, LMG9300 also showed significant rPirB toxin degradation overtime (Fig. 1). Each *Bacillus* strain produced different secreted proteins and dendrograms of secretome profiles from all *Bacillus* strains were constructed based on two strategies (namely based on presence/absence of bands or band intensity) (Fig.2). In both cases, 2 main clusters of strains could be observed, harboring the same *Bacillus* species. Yet, in the band based approach of clustering, LMG9300 was singled out. All *Bacillus* strains bind predominantly either the lectin WGA or the lectin ConA (Fig. 2). The grouping of the secreted protein profiles correlated to a large extent with the capacity to bind either the WGA or ConA. Metabolic profiles for carbon sources of each strain were examined using Biolog GENIII plates. The metabolic data were processed using heatmap and principal component analysis (PCA). Generally, three clusters were detected by heat map analysis. The heat map analysis for amino acids and peptides was not significantly different from heatmap analysis of carbon sources. Finally, the tested in vitro phenotypes (*in vitro* AHPND toxins degradation, secretome profile, metabolic profile and lectin binding profile) will be correlated with their *in vivo* capacity to control AHPND, hopefully facilitating establishing links with their in-vivo functionality.

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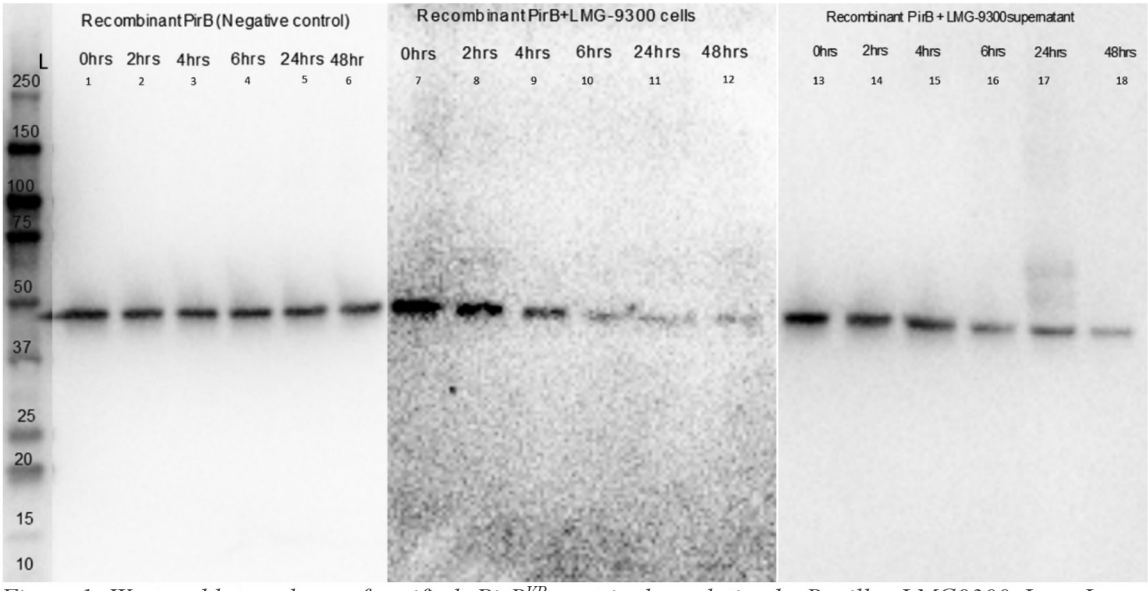

Figure 1: Western blot analyses of purified rPirB^{VP} protein degradation by Bacillus LMG9300. Lane L: Protein ladder (kDa). Lane 1–18: Protein samples taken at 0, 2, 4, 6, 24, and 48 h post incubation. Lane 1–6: Negative control (rPirB^{VP} and sterile PBS). Lane 7–12:results of incubation of rPirB^{VP} with 10^7 CFU/ml bacillus LMG9300 cells. Lan 13-18: Incubation of rPirB^{VP} with Bacillus LMG9300 cell-free supernatant.

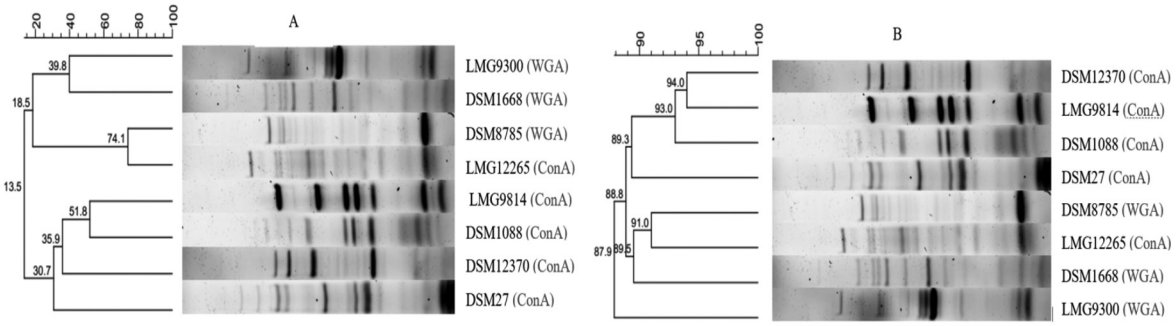

Figure 2: The dendrogram of secreted proteins profiles from all Bacillus spp. strains. (A) using Pearson correlation and (B) based on the number of different bands. Furthermore, the glycan architecture of strains was predominantly built either by the lectin WGA or ConA.

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BENEFITS OF KRILL MEAL INCLUSION TOWARDS BETTER UTILIZATION OF NUTRIENTS AND GROWTH IN GILHEAD SEABREAM JUVENILES

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Introduction

Feed is an essential part of aquaculture. Firstly, as the nutritional benefits and the general health and robustness of fish depends mainly on the nutrients supplied through aquafeeds. Secondly, as feed is the most expensive part of fish aquaculture and availability of a cost-effective feed remains as one of the bottlenecks to achieve an adequate aquaculture sector development. The feed attributes to around 60-70% of the production cost. Due to fluctuations in supply and prices of traditionally used marine ingredients such as fish meal (FM) and fish oil (FO), the aquaculture industry has inclined towards plant-based feeds. However, plant-based diets may result in deficient and imbalanced supply of essential nutrients, due to negative effects on palatability, and the presence of anti-nutritional factors on nutrient availability, which may also have other negative effects on fish health. Hence, to cover the nutritional requirements of fish, without compromising its health, and at the same time utilizing aquafeeds ingredients effectively, it is important to produce feeds with functional raw materials that could enhance the bioavailability and utilization of nutrients, which would lead to enhanced fish health and performance. In addition, it would enable the industry to save costs by improving feed efficiency. One such functional and sustainable marine ingredient is krill meal. The present trial was conducted to test the effect of krill meal inclusion towards enhancing the feed utilization and enhancing growth and nutritional in gilthead seabream (*Sparus aurata*) juveniles.

Methods

Juvenile gilthead seabream (8.4±0.04 g), were fed a practical diet with either a 15% FM/5.5%FO level of inclusion (KM0; control diet) or the same diet replacing FM by 20% (KM3; 30 g KM/kg diet), 33% (KM5; 50 g KM/kg diet) or 50% (KM7; 70 g KM/kg diet) Antarctic krill meal (KM) for 12 weeks in triplicates. At the end of the feeding trial, growth performance and feed efficiency were evaluated, and fish were subjected to a stress-challenge by confinement for 7 days. The omega-3 index (EPA+DHA% in RBC, which is used as an indicator of EPA+DHA% in the different tissues) was measured at day 0, 24 hours and after 7 days post stress.

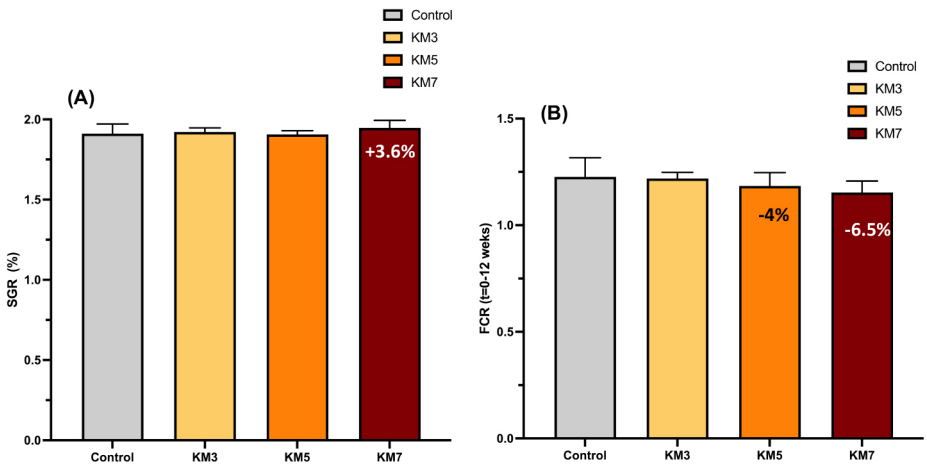

Figure 1. (A) Growth performance and (B) feed utilization of gilthead sea bream (*Sparus aurata)* juveniles fed diets with graded KM levels along the feeding trial. Values expressed in mean ± SD. Specific growth rate (SGR) = [(ln final weight - ln initial weight)/number of days] x 100; Food conversion ratio (FCR) = (total feed fed/total weight gained). KM = control diet; KM3 = krill meal 30 g/kg diet; KM5 = krill meal 50 g/kg diet; KM7 = krill meal 70 g/kg diet.

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Results

Fish fed with KM5 and KM7 presented an improvement in FCR when compared to fish fed on conventional control diet with FM and FO (15% FM/5.5%FO). In particular fish fed KM7 and KM5 diets presented an improvement in FCR by a 6.5% and 4%, respectively when compared to control group. Besides, a relatively higher growth performance (3.5%) was observed in fish fed with KM7 diet in comparison to control group (Fig. 1). Further, the lipid efficiency ratio (LER) was significantly enhanced with all the 3 doses of krill meal (9% higher LER for KM3 and KM5 and 15% higher LER for KM7, respectively, in comparison to control diet) and protein efficiency ratio (PER) was enhanced for KM5 and KM7 (3% and 7% higher PER for KM5 and KM7 diets, respectively) in comparison to control group. Omega-3 index was increased in all the three krill diets (4.3% increase in KM3, 4.7% increase in KM5 and 3.6% increase in KM7, respectively) in comparison to control group (1.2% increase) after 7 days post stress, indicating that nutrients in KM have the potential to possibly prevent the oxidation of vital polyunsaturated fatty acids, such as EPA and DHA and hence maintain the nutritional value of fish. These results demonstrate that krill meal inclusion leads to better utilization and retention of nutrients (fat and protein) in feed, and hence could significantly optimize feed efficiency, which provides economic benefits to farmers. Further, the inclusion or krill meal could enhance the nutritional value of fish as indicated through higher increase in EPA+DHA% (omega-3 index), and possible prevention of oxidation of EPA and DHA after oxidative stress, which would be beneficial for the consumers.

THE EFFECT OF SILVER NANOPARTICLES ON THE MORPHOLOGY AND PHYSIOLOGY OF THE DIGESTIVE SYSTEM OF THE BUTTERFLY SPLITFIN (*Ameca splendens*)

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Introduction

The aim of this study was to investigate the effects of silver nanoparticles (AgNP) on liver and intestine of butterfly splitfin (*Ameca splendens*), a potential model species. The focus was on analyzing the activity of digestive enzymes in the intestine and markers of oxidative stress in the liver and intestine. In addition, changes in the morphology of these two organs were demonstrated.

Materials and methods

This work was supported by the National Science Center, Poland Grant No. 2015/19/D/NZ8/03871. Fish of both sexes aged about 5 months were exposed to aqueous solutions of silver nanoparticles at concentrations of 0.01 mg/l, 0.1 mg/l and 1.0 mg/l for 42 days. Fish from the control group were maintained in water without AgNPs. Each experimental group was conducted in 3 replicates. On the last day of the experiment, the fish were euthanized, and their livers and anterior intestines were dissected. For enzyme activity analysis livers and intestines were frozen in liquid nitrogen. Then enzymatic analyses were performed to evaluate the enzymatic activity of the following enzymes in livers: alkaline phosphatase (ALP), acid phosphatase (ACP), superoxide dismutase (SOD) and glutathione peroxidase (GPX). In anterior intestine, the activities of alkaline phosphatase (ALP), acid phosphatase (ACP), amylase, trypsin, lipase, chymotrypsin and leucine aminopeptidase (LAP) were measured. Additionally, the intestines and livers underwent standard histological processing (H-E staining). Their morphology was then analyzed and the height of the intestinal folds, the height of the enterocytes, the supranuclear height of the enterocytes and the area of hepatocytes in the livers were measured.

Results

During the experiment, there was no mortality in any of the experimental groups and the control group. On the last day of the experiment, there were also no statistically significant differences in body weight, body length or Fulton's fitness index. Enzyme analyses showed that the tested nanoxenobiotic increased the activity of ACP, ALP and SOD in the livers of fish exposed to AgNP, while GPX activity was not statistically significantly different between groups. Exposure of butterfly splitfin to the tested nanoxenobiotic did not statistically significantly alter the activity of digestive enzymes in the anterior intestine (Table 1).

Analysis of anterior intestinal morphology showed that the histological structure of the anterior intestine revealed no significant histopathological changes. The AgNP 1.0 mg/l group showed the longest intestinal folds compared to the other groups. In addition, infiltrative cells (lymphocytes) and inflammatory cells were deposited in the intestinal submucosa in all groups exposed to AgNP solutions.

Table 1. Activity of digestive enzymes in liver and anterior intestine.

Parameters		Control	AgNP 0,01mg/L	AgNP 0,1mg/L	AgNP 1mg/L
Liver	ACP	0,0395 ± 0,01721[ab]	0,0301 ± 0,01584[a]	0,0509 ± 0,01915[ab]	0,0777 ± 0,03818[b]
	ALP	0,0737 ± 0,0319[a]	0,0912 ± 0,0613[ab]	0,1364 ± 0,094[ab]	0,2399 ± 0,1532[b]
	GPX	0,7543 ± 0,4347	0,5722 ± 0,3548	0,6822 ± 0,3843	0,7887 ± 0,2681
	SOD	0,0155 ± 0,0074[a]	0,0167 ± 0,0108[a]	0,0351 ± 0,0133[b]	0,0303 ± 0,01367[ab]
Anterior intestine	ACP	0,0018 ± 0,0010	0,0028 ± 0,0011	0,0019 ± 0,0009	0,0032 ± 0,0012
	ALP	0,1538 ± 0,0522	0,1929 ± 0,0803	0,1180 ± 0,0546	0,1292 ± 0,0413
	Amylase	0,2735 ± 0,1130	0,2595 ± 0,0944	0,2168 ± 0,1123	0,2568 ± 0,1461
	Lipase	0,0017 ± 0,0005	0,0016 ± 0,0004	0,0015 ± 0,0007	0,0020 ± 0,0006
	Trypsin	0,0053 ± 0,0030	0,0054 ± 0,0027	0,0047 ± 0,0024	0,0052 ± 0,0030

Means followed by different letters in the same row are significantly different ($P < 0.05$).

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Analysis of the liver parenchyma showed the formation of foci of increased accumulation of fatty vacuoles in the cytoplasm of hepatocytes. In fish from the control group, the level of *steatosis* was lower compared to fish from the AgNP-exposed groups. In the hepatocytes of fish from the control group, steatosis dominated in the form of single large fat vacuoles. The dominant type of steatosis was mircovesicular *steatosis*, with some individuals in the group of fish exposed to AgNP 1.0 mg/L also showing white adipose tissue in the exocrine pancreatic parenchyma adjacent to the liver. Histomorphometric analysis showed that hepatocytes in the AgNP 1.0 mg/L group had the largest surface area; however, there were no statistically significant differences in the value of this parameter between the groups.

Conclusion

The results indicate that exposure to AgNP can lead to disruption of liver homeostasis. Changes associated with hepatocyte vacuolization and the appearance of lymphoid cells in the liver have also been observed by other authors in zebrafish exposed to silver nanoparticles (Szudrowicz et al., 2022). In fish exposed to silver nanoparticles, increased activity of antioxidant enzymes in the liver was observed. Histopathological changes were found in the anterior intestine in groups exposed to AgNP. Similarly, necrosis of the intestinal folds was observed in the intestines of carp exposed to AgNP by Kakakhel et. al. (2021). On the other hand, no effect of the tested xenobiotic on the activity of digestive enzymes was observed in the studied fish.

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BEHAVIOURNAL MONITORING OF ATLANTIC SALMON (*Salmo salar*) REARRED IN EXPERIMENTAL TANKS USING DEEP LEARNING MODELS

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Introduction

In aquaculture, the monitoring and analysis of fish behaviour hold crucial insights into various aspects of fish welfare. Notably, operational welfare indicators (OWIs) and laboratory-based welfare indicators (LABWIs) have emerged as promising tools for welfare auditing, as highlighted by Noble et al. (2018). Recent advances in computer vision technology have opened up possibilities to improve the monitoring of fish behaviour, rendering it an invaluable resource for welfare evaluation. Such technology allows for non-invasive inspection in a variety of applications as it offers methods across diverse applications. Despite notable progress in computer vision particularly in object detection (Wen et al. 2021) a persistent challenge remains in high-density scenarios. This study explored the potential of using pose estimation (Jocher et al. 2023) in Atlantic salmon aquaculture to automatically assess swimming behaviour during feeding events in tanks.

Material and Methods

The video recordings captured using GoPro Hero4 Black cameras were used in this study to investigate the behaviour of Atlantic salmon (*S. salar*) reared in hexagonal tanks of 3300 litres, each holding approximately 90 fish (> 500g). The water current in the tanks was clockwise. Fig 1. gives an overview of the method. A key point annotation scheme, as shown in Fig. 1(a), was used to annotate the fish's snout, dorsal fin, and tail base. The training dataset was prepared by subsampling videos of four tanks using the CVAT annotation tool (CVAT 2023) in COCO key point format. Fig. 1(c) depicts the overall pipeline of the employed method. The annotations from COCO key points were converted into YOLOv8 format to train the pose model. The experimental/test videos were used to predict the fish pose. The predicted key points were used to calculate the orientation flows. The orientation flow is the angle of the fish's heading in relation to the opposite direction of the water current, as shown in Fig. 1(b). It is calculated by finding the angle between the tangential vector at the dorsal fin and the vector from the dorsal fin to the fish snout. As shown in Fig. 1(d), a spatio-temporal analysis is performed by visualizing the orientation flow over time. Our common observation is that the fish tend to swim facing the water current unless disturbed by some events. Finally, the results are obtained in the form of an orientation score, which is defined as the absolute value of the mean of the orientation flows over time, reflecting the spread of the distribution. The YOLOv8 pose model was trained on a workstation with an Intel(R) Core (TM) i9-10885H CPU with 64.0 GB RAM and an NVIDIA GPU Quadro RTC 4000 using python 3.9.

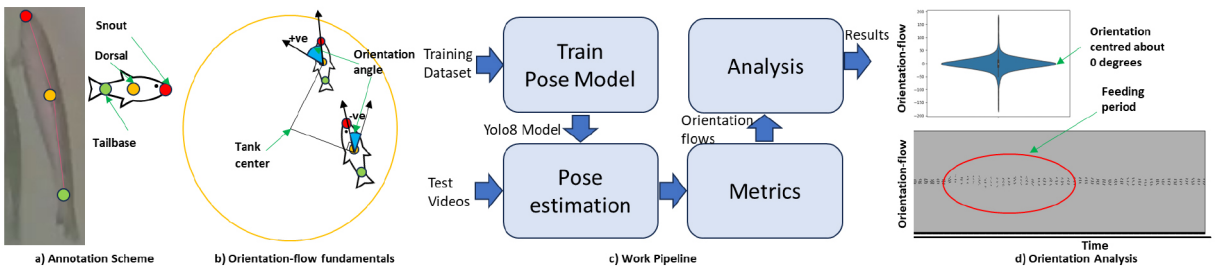

Fig. 1 Workflow overview: a) Annotation scheme. b) Calculation of swimming orientation based on key points and water current direction. c) AI Pipeline steps: pose model training with annotated frames, fish pose estimation in sampled videos for metric's extraction and metrics calculation and d) An example of a feeding event happening in a 60-second video clip. Top figure: most of the fish swim against the water as their anterior body forms an angle around 0° with regards to the water flow direction. *Bottom figure: violin plot showing irregular group swimming orientation caused by feeding, as the fish to start chasing pellets in random directions (encircled in red colour).*

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Frames	Train	Val	Test
# frames	1538	440	442

Precision	Recall	mAP50-95
0.973	0.887	0.937

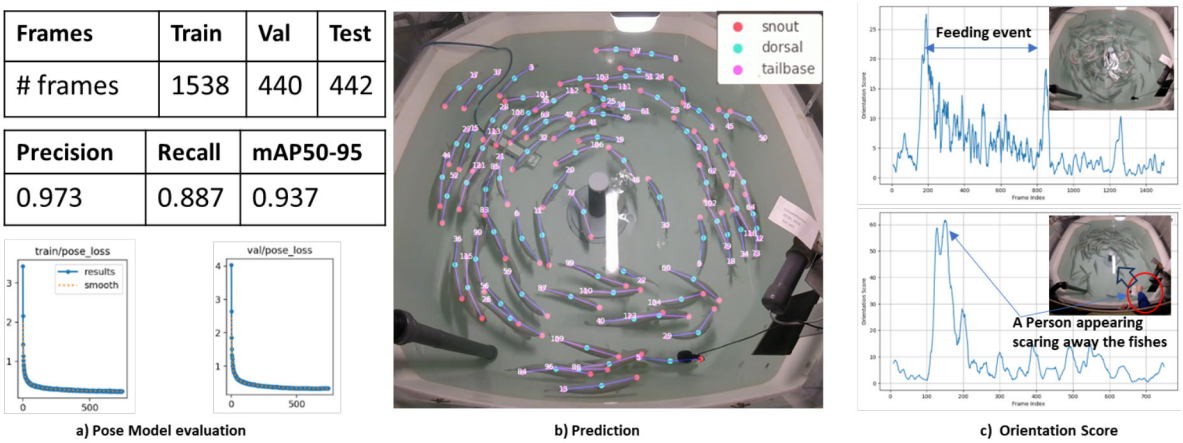

Fig. 2. a) The training parameters and evaluation metrics from the training to obtain pose model b) A snapshot of key points prediction. c) The top row represents how the orientation score changes during feeding while the bottom figure shows the score variation when a person appears suddenly on one side.

Results and discussion

A few results from analyses are presented here to illustrate the relevance of the tool for documenting and understanding behaviour. Fig. 2(a) shows the pose model evaluation metrics. A snapshot of the predicted key points is shown in Fig. 2(b). Fig. 2(c) demonstrates the orientation scores during two key events, feeding and disturbance by the presence of persons around the tanks. The study demonstrates that the proposed method can interpret the dispersion of fish using swimming orientation, and this can be a potential tool in welfare auditing in experimental studies. The results demonstrate that the tool has the potential to explore behaviour in different scenarios and settings. We intend to explore this aspect by applying it in different contexts and developing the tool further and auditing its utility for welfare documentation.

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Acknowledgements

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SUPPLEMENTING FREE AMINO ACIDS MIX ON FEED FORMULATION IMPROVES GROWTH PERFORMANCES, FEED CONVERSION RATIO, RESISTANCE TO ENVIRONMENTAL STRESS AND CUTICULAR FORMATION OF WHITELEG SHRIMP *Litopenaeus vannamei*

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Introduction

Mixes of free amino acids (MFAA) obtained from extensive hydrolysis of full protein chains are interesting candidates for aquaculture feeds. In addition to their specific amino acids profile, their low molecular weight ensure a fast and high level of assimilation with synergetic effects during the first development stages. Previous investigations on shrimp *(L.vannamei)* underline MFAA positive effects on zootechnical performances (Le Reste et al., 2019) and survival in case of bacteriological and viral challenges (Kersanté et al., 2021). The results presented here have been obtained from a study conducted in three different phases to investigate the effects of a mix of 17 amino acids obtained from extensive hydrolysis of poultry keratin, on plasma protein (PP), oxyhemocyanin (OxyHc), minute till surgical anesthesia for low salinity and dissolved oxygen stress test and their PP and OxyHc at surgical anesthesia, growth performance (GP), size variation (SV), feed conversion ratio (FCR), molting frequency (MF), survival rate (SR), ions and chitin in cuticle and cuticular structure of *L. vannamei.*

Protocol

To understand the specific consumed period of MFAA supplementary feed with 38.5% protein to sufficiently maintenance for *L. vannamei* physiology in terms of PP and OxyHc, shrimp were fed three feeds (control; two concentrations of MFAA as 5g/kg of feed (MFAA 0.5%) and 10g/kg of feed (MFAA 1%) for 16 days with three replications. The healthy *L.vannamei* juvenile with 14.12±0.35 g and 11.67±0.22 cm in size were held in 15 ppt medium at 100 ind/m^2 using 250L rectangular plastic tanks for 16 days. They were fed 4 times daily with 5% body wet weight. ***Exp I:*** Experimental shrimp at Do molt stage were collected the hemolymph for examining OxyHc and PP of day 4, 8, 12, 16. ***Exp II:*** It was operated as same at Exp I until 16 days of feeding prior to apply stress test with low dissolved oxygen (LO) and freshwater (FW). Both test checking the minute till surgical anesthesia (MA) the hemolymph of individual anesthetized shrimp for determination of OxyHc and PP was suddenly collected then transferred to 15 ppt aquaria with well oxygenation to recover and record the number of survivors. ***Exp III*:** The two feeds as control and MFAA 1% supplementary feed were operated. Specifications of experimental shrimp and tank including culturing methodology were identical to Exp. I with five replications for 60 days to determined GP, FCR, SR, MF and SV. After termination, shrimp carapace at Do molt stage was individually sampled for examining ions, cuticular thickness (CT) and number of layers (NL) and chitin content using Electron Microscopy and Energy Dispersive System.

Results

The results of Exp I found that shrimp raised with MFAA supplementary feed with both dosages for 16 days showed significant increase of OxyHc and PP. The results of Exp II for low dissolved oxygen stress test showed the significant longest period for shrimp fed with MFAA 1% supplementation ($p<0.05$) until the beginning time to surgical anesthesia (BTSA) (58.3 min) with the significant lowest concentration of OxyHc (1.93 mg/l) ($p<0.05$) versus with control (2.61 mg/l). In the same manner, PP values of MFAA 1.0% group (330.8 mg/ml) was significantly ($p<0.05$) lower than that of control (347.5 mg/ml). For freshwater stress test, shrimp fed on MFAA 1% feed showed the significant ($p<0.05$) longest period at BTSA (86.5 min) with the significant lowest ($p<0.05$) PP values (342.9 mg/ml) while OxyHc values among groups were not significantly different ($p>0.05$).

The results of Exp III showed higher significant % of weight gain, average daily gain and specific growth rate gain of MFAA 1% group ($p<0.05$) than those of control and FCR of MFAA 1% group was significantly lower ($p<0.05$) than that of control. % SR, MF, % CV of length and weight were not significantly ($p<0.05$) different between groups.

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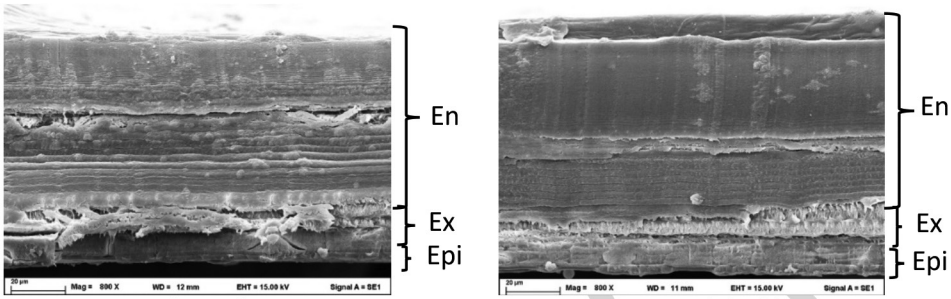

Control MFAA 1%

Fig. 1 The cuticle architecture of treatments and control at Do stage.
En= Endocuticle; Ex=Exocuticle; Epi=Epicuticle

Interestingly, the CT and NL of Do cuticle of MFAA 1% group was significantly higher than those of control ($p<0.05$). The cuticle architecture of MFAA 1% and control are shown in Fig 1. The endocuticle layer structure of MFAA 1% group was magnified.

The percentages of Ca and Mg of Do cuticle of MFAA 1% group were significantly higher than those of control ($p<0.05$) versus %Na and %Cl of control were significantly higher those of MFAA 1% ($p<0.05$). The chitin content of MFAA 1% group showed higher rate ($p<0.05$) than that of control.(+19.4%).

Discussion

This study underlined some particularly interesting effects of MFAA when applied on juvenile whiteleg shrimp, *Litopenaeus vannamei*. Firstly, regarding growth parameters with positive effects on biomass and feed utilization. Interestingly, we underlined strong influence of MFAA on hemolymph composition in term of oxyhemocyanin and plasma protein after the 16 days of feeding supplementation inducing better resistance to low dissolved oxygen and freshwater stress conditions. Monitoring of oxyhemocyanin and plasma protein in hemolymph at surgical anesthesia also disclosed the interest of MFAA as direct source of energy to balance the osmoregulation during freshwater stress and maintain life under shortage of oxygen. After 60 days of feeding period, the cuticule thickness and structure was also magnified in relation with higher examined concentrations of chitin, but also of calcium (Ca) and magnesium (Mg) linked with Ca and Mg carbonate crystals to construct the cuticle and positively influence the molting. These results confirm the interest of MFAA as a sustainable protein source converted into an efficient functional ingredient for shrimp nutrition.

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LACTIC ACID BACTERIA-BASED PROBIOTICS SUPPORT WHITELEG SHRIMP (*Litopenaeus vannamei)* GROWTH PERFORMANCE AND RESILIENCE TO DISEASE CHALLENGE

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Introduction

In shrimp farms, probiotics have been showed to promote growth, improve the efficiency of feed utilization, modulate the intestinal microbiota, and protect the host from disease by stimulating the immune system. In recent years, diseases caused by *Vibrio* spp. became the most serious threat to shrimp production, among them acute hepatopancreatic necrosis disease (AHPND) caused by *V. parahaemolyticus*, that affects shrimp in their early post-larvae growth stage. Among probiotic species, Lactic Acid Bacteria from different genera including *Lactobacillus*, *Enterococcus* and *Pediococcus*; and bacteria from *Bacillus* genus are commonly used for shrimp feed supplementation.

To elucidate the mode of action of a 4-species mixed probiotic product (AquaStar®, DSM Nutritional Products Ltd.) consisting of Lactic Acid Bacteria and *Bacillus subtilis*, the 4-species-mix as well as two individual components and their 2-strain mixture were fed to vannamei shrimp post-larvae in order to assess growth performance and resilience to *V. parahaemolyticus* challenge.

Material and Methods

Whiteleg shrimp (*Litopenaeus vannamei*) were imported as postlarvae (PL10) from Shrimp Improvement Systems (Florida, USA) and acclimated to lab conditions over one week. These shrimps are certified to be specific pathogen-free for the following pathogens: WSSV, YHV/GAV/LOV, TSV, IHHNV, BP, MBV, BMN, IMN, Microsporidians, Haplosporidians and NHP bacteria. The shrimp were randomly allocated to 30L tanks (100 PL/tank) equipped with individual biofilters and fed five different treatments in 5 replicates each: *Lactobacillus reuteri* (LR)*, Bacillus subtilis* viable cells (BS)*,* 2-strain mix LR+BS, 4-strain mix (*B. subtilis, E. faecium, P. acidilactici, L. reuteri* in equal proportions, AS), no probiotic control (CC). Shrimp were fed commercial crumbled diets with treatments at 1 x 10^7 cfu/g. Control feed was mock-treated with RO water. Growth was assessed by group weighing after 3 weeks. Shrimp were challenged after 3 weeks feeding (PL41) by immersion with 10^6 cfu/ml *Vibrio parahaemolyticus* MO904.

Results

Significant >20% improvements in final weight, body weight gain and specific growth rate were observed by treatments LR and AS (table 1). LR treated shrimp PLs had a significantly 22% reduced feed conversion ratio. PLs receiving BS had numeric improvements in final weight and a significant 17% improvement in specific growth rate. The survival rate during the feeding trial was above 90% in all tanks without any clear observable trend. A numeric improvement in survival to *V. parahaemolyticus* challenge was observed in shrimp treated with LR and the 4-strain mixed probiotic AS.

Discussion

The results obtained with shrimp post-larvae confirm previous experiences (Kesselring et al. 2019, Gruber et al 2023) with this 4-strain mixed probiotic product (AS; AquaStar® DSM ANH) in adult shrimp. *Bacillus subtilis* (BS) alone significantly improved the specific growth rate and led to numeric improvements of body weight gain and feed conversion ratio. A longer feeding period may have allowed the shrimp to reach a sufficient immune-competence to respond favorably to a potential *B. subtilis* related stimulation of their immune response as well as the nutritional effects of this strains' extracellular enzymes.

Lactobacillus reuteri (LR) supplementation led to significant improvements in final weight, body weight gain, feed conversion ratio and specific growth rate. LR supplemented shrimp PLs had numerically improved survival probability 48h after *V. parahaemolyticus* immersion challenge. This strain of *L. reuteri* has previously been described to have a pronounced ability to attach to gut epithelium *in vitro,* thereby preventing the attachment and invasion by pathogenic bacteria (Pillinger et al. 2022). It is indeed imaginable that these properties led to a more efficient energy use. Generally, bigger shrimp have a better defense against any type of challenge than smaller individuals.

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Table 1: Zootechnical growth performance of *L. vannamei* fed individual probiotic strains and a 4-strain probiotic mix similar to the commercial product AquaStar® (DSM Nutritional Products Ltd.) for three weeks. Mean and standard deviations are shown. Superscript letters indicate significant differences.

Parameters	CC	BS	LR	BS+LR	AS
Initial weight (mg)	**174.83±4.57**	**174.33±3.04**	**179.87±1.15**	**176.23±6.09**	**174.77±3.11**
Final weight (mg)	**723.62±47.75[a]**	**818.73±32.99[ab]**	**906.56±23.65[b]**	**786.29±46.70[ab]**	**887.85±32.71[b]**
Body weight gain (mg)	**548.79±45.02[a]**	**644.40±31.55[abc]**	**726.70±23.86[c]**	**610.06±43.47[ab]**	**713.08±32.20[bc]**
FCR	**1.56±0.13[a]**	**1.30±0.06[ab]**	**1.22±0.02[b]**	**1.37±0.09[ab]**	**1.31±0.09[ab]**
SGR (%/day)	**10.09±0.37[a]**	**11.03±0.26[bc]**	**11.54±0.20[bc]**	**10.65±0.34[ab]**	**11.59±0.27[c]**
Survival rate (%)	**97.33±1.13**	**97.67±1.25**	**97.33±1.35**	**94.67±2.13**	**95.33±2.32**
Survival VP challenge (%)	**12.5±6.7**	**16.7±7.6**	**37.5±9.9**	**20.0±8.3**	**33.3±9.6**

CC, control; BS, *Bacillus subtilis*; LR, *Lactobacillus reuteri*; BS+LR, combination of *B. subtilis* and *L. reuteri* 1:1; AS, 4-strain mixture of *B. subtilis*, *L. reuteri, E. faecium, P. acidilactici* 1:1:1:1

Conclusions

Probiotics with a high inclusion of different lactic acid bacteria demonstrated significant benefits over a *Bacillus subtilis* only supplementation in an early stage of shrimp production. The single component *Lactobacillus reuteri* was shown to be particularly effective in shrimp post-larvae due to its strength in competitive exclusion. The combination *L. reuteri* with *B. subtilis* could not realize the full potential of the single components, pointing out the adjuvant role of the *Enterococcus* and *Pediococcus* in the 4-species mix. This experiment presents an interesting pilot study furthering our understanding of probiotic efficacy in shrimp post-larvae.

EFFECT OF DIETARY TALL OIL FATTY ACIDS (TOFA) ON PERFORMANCE, IMMUNOLOGICAL STATUS AND PATHOGEN (*Aeromonas SPP.*) LOAD OF JUVENILE ASIAN SEABASS (*Lates calcarifer*)

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Introduction

Tall oil fatty acids with 9% natural coniferous resin acids (TOFA) is a novel feed ingredient which improves the production performance and intestinal microbiota of farm animals (*e.g.* Vienola *et al*., 2018, Uddin *et al*., 2021). Resin acids are anti-inflammatory and antibacterial secondary metabolites of coniferous trees (San Feliciano *et al*., 1993). The present study investigated the effects of dietary TOFA on the performance, immunological status and load of the pathogenic bacterium *Aeromonas spp.* in the intestinal tract and liver of juvenile Asian seabass (*Lates calcarifer*) in Thailand.

Materials and methods

Juvenile Asian sea bass were allocated into 20 freshwater cages of 2 m^3, 15 fish/cage, at a site with a known background challenge of *Aeromonas histolytica*. The water was aerated to maintain DO >5 mg/l. Feed was of commercial-type, with main ingredients of fishmeal, poultry meal, soybean meal and tapioca, and it was amended with 0 (Control), 0.35, 0.7, or 1.0 kg/ton of TOFA (Progres®, AB Vista, UK) for treatments T1-T4, respectively. The fish were fed 3 times/day at 3-5% of body weight for 16 weeks. Fish weight, weight gain, feed intake and feed conversion ratio (FCR) were determined every 2 wk, and the survival rate every 4 wk. Two fish/replicate were sampled for blood at 8- and 16-wk time points for determining the red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin and haematocrit, and the concentrations of total serum protein and immunoglobulin M (IgM). The same fish were sampled for liver and intestinal contents for determining the number of *Aeromonas* spp. by plate culturing, using *Aeromonas*-selective media. The study design was completely randomized. Data was analysed with one-way ANOVA, followed by Duncan's Multiple Range Test, using $P<0.05$ as a limit for statistical significance.

Results

Mean weight of the fish was 54.6 g, 111.8 g, and 170.0 g for wks 0, 2, and 4, respectively, without a significant difference between treatments. Dietary TOFA supplementation dose-dependently improved fish weight and weight gain from the 6-wk time point onwards ($p<0.05$). Table 1 presents fish weight on wks 8 and 16, and FCR for wks 0-8 and 0-16. Feed intake and fish survival rate was not affected by the treatments. At the end of the study, the survival rate was 89.3%, 92.0%, 93.3% and 96.0% for T1-T4, respectively. The treatments significantly affected FCR for the following time periods: wks 0-6, 0-8, 0-10, and 0-12 ($p<0.001$), with the best performance in T4.

Parameters analysed from blood and tissue samples are reported in Table 1. The TOFA supplementation significantly increased haematocrit (wk 8) and IgM (wks 8 and 16), and decreased *Aeromonas* spp. density in the liver and intestinal tract (wk 16).

Discussion and conclusions

In the present study, supplementing the diet of juvenile Asian seabass with TOFA promoted the growth performance, feed conversion and immunity of the fish, and at the same time improved their ability to control the growth of *Aeromonas* spp. in the liver and intestinal tract. Increased haematocrit and the trend towards higher serum protein levels in TOFA-supplemented groups may indicate positive effects on the physiological status of the fish. The effects were dose-dependent, and the treatment with TOFA at 1.0 kg/ton gave the highest responses. The results were in line with previous observations from studies with poultry and swine, in which dietary TOFA had positive effects on performance, gastrointestinal microbiota and disease resistance of animals (Vienola *et al*., 2018; Uddin *et al*., 2021). In conclusion, TOFA may function as a dietary strategy to improve the production performance and disease resistance of Asian seabass.

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Table 1. Effect of the treatments on parameters measured at 8 and 16 weeks, with statistical evaluation by ANOVA and Duncan's Multiple Range Test.

Item	Dietary treatments				
	T1[1]	T2[2]	T3[3]	T4[4]	*p*-value
Performance					
Fish weight, week 8 (g)	289.0[c]	306.7[b]	318.1[b]	336.7[a]	<0.001
Fish weight, week 16 (g)	540.0[b]	553.9[ab]	559.9[ab]	574.9[a]	0.020
FCR[5], week 0-8, (g/g)	2.00[a]	1.82[b]	1.70[bc]	1.59[c]	<0.001
FCR[5], week 0-16, (g/g)	2.67	2.53	2.45	2.32	0.056
Analyses, week 8					
RBC[6] count (*10^5 cell/ml)	4.96	5.36	5.72	6.00	0.091
WBC[7] count (*10^4 cell/ml)	0.50	0.60	0.50	0.60	0.963
Haematocrit (%)	30.20[b]	34.80[ab]	37.20[a]	40.40[a]	0.010
Immunoglobulin M (g/l)	0.26[b]	0.41[a]	0.41[a]	0.43[a]	<0.001
Serum protein (mg/dl)	5.44	5.69	5.81	6.01	0.081
Aeromonas, liver (Log cfu[8]/ml)	4.71	4.65	3.57	3.44	0.124
Aeromonas, gut (Log cfu[8]/ml)	6.15	6.10	5.48	5.27	0.079
Analyses, week 16					
RBC[6] count (*10^5 cell/ml)	4.12	4.46	4.46	4.60	0.717
WBC[7] count (*10^4 cell/ml)	1.30	1.40	1.50	1.50	0.888
Haematocrit (%)	34.60	36.40	37.60	39.00	0.539
Immunoglobulin M (g/l)	0.39[b]	0.47[ab]	0.48[a]	0.50[a]	0.048
Serum protein (mg/dl)	6.18	6.76	6.89	7.08	0.085
Aeromonas, liver (Log cfu[8]/ml)	4.60[a]	4.38[a]	3.28[b]	3.02[b]	<0.001
Aeromonas, gut (Log cfu[8]/ml)	6.08[a]	5.87[b]	5.27[c]	5.04[d]	<0.001

[1]TOFA 0 kg/ton, [2]TOFA 0.35 kg/ton, [3]TOFA 0.7 kg/ton, [4]TOFA 1.0 kg/ton, [5]feed conversion ratio, [6]red blood cell, [7]white blood cell, [8]colony forming units, [a, b, c] Different letters in the same row indicate significant differences between treatments ($p<0.05$).

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EFFECTS OF DIETARY TALL OIL FATTY ACIDS WITH RESIN ACIDS ON THE PERFORMANCE AND IMMUNITY OF JUVENILE WHITE SHRIMP (*Litopenaeus vannamei*) WITH AND WITHOUT AN INTENTIONAL *Vibrio parahemolyticus* CHALLENGE

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Introduction

Tall oil fatty acids with 9% natural coniferous resin acids (TOFA) is used as a feed ingredient for poultry and swine for improved production performance and beneficial effects on intestinal condition, but its potential for aquatic species has remained unexplored. The present experiment studied the effect of dietary TOFA on performance, immunity and disease resistance of white shrimp (*Litopenaus vannamei*) in an experimental model which included an 8-week period without intentional challenge factor, followed by a 1-week period with *Vibrio parahemolyticus* (*V.p.*) challenge.

Materials and methods

The experiment was carried out in 30 aquariums with 120 liters of 15 ppt saline water. Juvenile white shrimp were stocked at 200 shrimp/m^3, 25 individual/aquarium. The water in each aquarium was aerated to maintain DO >5 mg/l, and 20% of water was changed every 3 days. Commercial-type shrimp feed, based on soybean meal, wheat flour, fishmeal, poultry meal and corn gluten was amended with TOFA (Progres®, AB Vista, UK) at 0, 0.5, and 1.0 kg/ton for the dietary treatments T1-T3, respectively. Feed was applied to the shrimp 3 times/day at 3-5% of body weight. Uneaten feed was siphoned out 1 hour after feeding, dried in hot air and weighed. Shrimp weight, weight gain, feed intake and feed conversion ratio (FCR) were recorded every 2 weeks and survival rate every 4 weeks. At the 8-wk time point, 6 shrimp/tr. were sampled for haemolymph, hepatopancreas and intestinal contents. The experiment continued by allocating 3 replicate aquariums/tr. into a 7-day challenge period which started by a subcutaneous injection of the virulent EMS strain of *V.p.* ($6.9*10^7$ cfu/ml) to each shrimp. The survival rate was monitored daily. On day 7 post-challenge, the shrimps were sampled as described earlier. Haemolymph samples were analysed for parameters relating to immunity and oxidative status. Density of *Vibrio spp.* in samples was determined by plate culturing. The study was conducted in a completely randomize design. Data was analysed with one-way ANOVA, followed by Duncan's Multiple Range Test, using $p<0.05$ as a limit for statistical significance.

Results

Results are summarized in Table 1. On day 1, the mean shrimp weight was 2.46 g, with no difference between treatments. Compared to T1 (control) treatment, shrimp weight, weight gain and FCR were improved by T2 and T3 from the 4-wk time point onwards (p <0.05). Post-challenge survival rate was increased from 32.5% in the control group to 55.0 and 65.0% in T2 and T3, respectively (p <0.05). Compared to T1, the haemocyte count, phenoloxidase activity, lysozyme activity, superoxide dismutase activity and glutathione concentration in haemolymph were all significantly higher, while the density of *Vibrio* spp. in tissues was lower for T2 and T3 both before and after the challenge. Haemolymph protein was increased by TOFA only post-challenge.

Discussion and Conclusions

In the present study, dietary TOFA at 0.5 and 1.0 kg/ton improved the performance and survival rate of juvenile white shrimp, suggesting a better production potential for TOFA-fed shrimps, in comparison to the control group. The decreased *Vibrio* load in the tissues and the parameters analysed from haemolymph indicate that TOFA positively affected several immunological and antioxidative functions and the disease resistance of the shrimp. Previously, in-feed resin acids have been shown to reduce intestinal inflammatory processes (Aguirre *et al*. 2019) and to positively modulate gut microbiota (Vienola *et al*., 2018) in chicken. In conclusion, TOFA may become a dietary strategy for supporting the performance, immunity and disease resistance of white shrimp.

Table 1. Effect of the treatments on parameters measured at the 8-week time point (pre-challenge) and 7 days post-challenge, with statistical evaluation by ANOVA and Duncan's Multiple Range Test.

Item	Dietary treatments			
	T1[1]	T2[2]	T3[3]	*p*-value
Week 8 pre-challenge				
Shrimp weight (g)	12.79[b]	13.81[a]	13.95[a]	<0.001
FCR[4], weeks 0-8 (g/g)	1.46[a]	1.28[b]	1.20[b]	0.021
Survival rate (%)	78.67	81.33	85.33	0.435
Haemocyte count (*10^5 cell/ml)	23.17[c]	25.17[b]	28.33[a]	0.013
Haemolymph protein (g/dl)	5.02	5.23	5.34	0.309
Phenoloxidase activity (unit/min/mg protein)	32.60[b]	44.52[a]	48.33[a]	<0.001
Lysozyme activity (unit/ml)	162.00[c]	201.83[b]	223.00[a]	<0.001
Superoxide dismutase (unit/ml)	13.31[b]	16.22[a]	16.81[a]	0.004
Glutathione (nmol/ml)	19.97[b]	30.52[a]	38.88[a]	0.001
V.p.[5] Hepatopancreas (Log cfu[6]/ml)	3.95[a]	3.29[b]	3.03[c]	<0.001
V.p.[5] Intestine (Log cfu[6]/ml)	4.23[a]	3.97[b]	3.72[c]	0.002
Day 7 post-challenge				
Survival rate (%)	32.50[b]	55.00[a]	65.00[a]	0.016
Haemocyte count (*10^5 cell/ml)	11.50[b]	14.75[a]	16.25[a]	0.003
Haemolymph protein (g/dl)	3.50[c]	3.97[b]	4.70[a]	<0.001
Phenoloxidase activity (unit/min/mg protein)	44.47[b]	50.92[a]	52.67[a]	0.017
Lysozyme activity (unit/ml)	404.13[b]	590.50[a]	634.88[a]	0.009
Superoxide dismutase (unit/ml)	7.83[b]	10.26[a]	11.01[a]	0.006
Glutathione (nmol/ml)	76.12[b]	94.90[a]	99.58[a]	0.006
V.p.[5] Haemolymph (Log cfu[6]/ml)	2.18[a]	1.85[b]	1.30[c]	<0.001
V.p.[5] Hepatopancreas (Log cfu[6]/ml)	2.95[a]	1.80[b]	1.28[c]	<0.001
V.p.[5] Intestine (Log cfu[6]/ml)	2.79	2.35	2.39	0.051

[1]TOFA 0 kg/ton, [2]TOFA 0.5 kg/ton, [3]TOFA 1.0 kg/ton, [4]feed conversion ratio, [5]*Vibrio parahemolyticus*, [6]colony forming units, [a, b, c] Different letters in the same row indicate significant differences between treatments ($p<0.05$).

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GENETIC PARAMETERS FOR EARLY SEXUAL MATURATION IN ATLANTIC COD

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Introduction

Sexual maturation before farmed Atlantic cod reaches the harvest size is coupled with economic losses and potential ecological concerns. Reduced appetite and allocation of energy from somatic growth into gonads starts prior to and continues throughout the spawning season causing a significant, up to 50%, loss in body weight. Investment in gonadal growth and significant loss in body weight translate into resource wastage in terms of feed costs. Additionally, spawning and spread of fertilized eggs from sea-cages poses a threat of genetic introgression from domesticated cod into the wild.

Earlier genetic studies on early sexual maturation in cod have been either conducted in the infancy of cod aquaculture (Kolstad *et al.*, 2006) or struggled with maturation frequencies suitable for genetic studies ((Drangsholt *et al.*, 2014). Kolstad *et al.* (2006) estimated low to moderate heritability for sexual maturation registered at 2 years in two geographical locations: 0.16-0.29. Drangsholt *et al.* (2014) reported non-significant heritability for sexual maturation at 2 years from the same genetic material as presented here.

The assessment of selection potential against early sexual maturation in the National Cod Breeding Program after 5 generations of growth selection was prompted by the re-vitalisation of the Norwegian cod aquaculture.

Material and methods

Individually tagged Atlantic cod representing 104 full-sib families of the National Cod Breeding Program were tested for early sexual maturation at LetSea Research facilities (Dønna, Norway). Experiment was run on a semi-commercial scale from 29th of January 2020 until 23rd of March 2021 following standard production protocols, except from light manipulation regimes to avoid sexual maturation. At the end of the experiment, fish were euthanized and individually registered for round weight (WT), length (LENGTH), liver and gonads weight, and sex (male/female) and the status of sexual maturation (MAT, mature/immature) were determined by visual inspection after dissection. Gutted weight (GWT) was calculated by subtracting weights of liver and gonads from WT. Tissue samples were collected and 1272 individuals representing 67 families were genotyped with a newly developed 21K SNP-array (Illumina Infinium™ Array). Additionally, harvest weight (HWT) was recorded for 2011 sibs from 173 families in November 2021. Heritability for MAT was estimated with univariate sire-dam model both on the observed and underlying liability scale using ASReml (pedigree relationships) (Gilmour *et al.*, 2015), and with GCTA (genomic relationships, GWAS) (Yang *et al.*, 2011). Bivariate animal model was used for estimation of phenotypic and genetic correlations between growth traits (WT, GWT, LENGTH, HWT) and MAT.

Results and discussion

Out of the 1680 registered individuals, 814 were females and 866 males, and 87.4% of the fish were classified as sexually mature The frequency of sexually mature females was 83.7% whereas maturation frequency in males was 90.9%. Mature (and female) fish were significantly heavier than immature (and male) fish. These differences in round body weight were mainly due to differences in gonad and liver weight; there was only 30g and 20g difference in GWT between mature and immature fish and between females and males, respectively.

Table 1. Estimates of heritability for growth traits, and genetic and phenotypic correlations between growth and early sexual maturation.

Growth trait	h^2	Maturity	
		r_g	r_p
WT	0.33±0.04	-0.14±0.14	0.17±0.03
GWT	0.38±0.06	-0.27±0.14	-0.03±0.03
LENGTH	0.37±0.05	-0.21±0.14	0.07±0.03
HWT	0.29±0.05	-0.29±0.15	n.a.

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Estimated heritability for MAT using pedigree relationships was moderate to high: 0.33±0.06 on the observed scale and 0.51±0.10 on the underlying liability scale. Estimated heritability utilizing genomic relationships from a subsample of the material was somewhat lower: 0.23±0.04. Genetic correlations between MAT and growth traits were negative (favourable) and connected with high standard errors (Table 1), indicating that selection for rapid growth has not affected the frequency of sexually mature fish at 2-years of age. GWAS detected a suggestive QTL for early sexual maturation at chromosome 23 where a single SNP crossed chromosome-wide threshold. Additionally, earlier detected QTL for sex was validated in our breeding population.

The significant additive genetic variation, and insignificant genetic interrelationships with growth traits, indicate that selective breeding has potential as a part of a solution to solve problems connected with sexual maturation in Atlantic cod without adversely affecting the genetic gain in growth. The suggestive QTL for MAT should be confirmed from a larger data set.

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Acknowledgements

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HOW SECONDARY EDUCATION CAN HELP TO REDUCE THE SHORTAGE OF AQUACULTURE PROFESSIONALS?

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Introduction

In Hungary, the aquaculture sector is characterised by two main segments: pond fish farming and intensive (precision) fish production. The Hungarian aquaculture sector employs 1,462 permanent workers, of whom 645 have primary education, 598 have secondary education and 219 have tertiary education (AKI, 2022). During peak periods, casual workers help to carry out the tasks, worked 22,254 days in the sector (AKI, 2022).

Over the last 20 years, the aquaculture sector has evolved significantly: changes in methods, procedures, and technologies have created major challenges for workers and employers alike. In addition to this technological development, the working environment has also changed, but in this area, thanks to the close-to-nature production, it is only possible to improve working conditions in small steps. It is partly these factors that fundamentally determine a young person's career orientation and choice.

It is a sad fact that marine and freshwater aquaculture and fisheries, like other segments of agriculture, find it difficult to compete with the job opportunities and working conditions offered by other sectors of agriculture. In addition, the steadily increasing demand for aquaculture products requires enterprises in the sector to meet market needs by producing products tailored to consumer demands, but this requires skilled workers at all levels. However, it is important for companies to be able to define their expectations towards workers, which fundamentally determine the quality and depth of training they need (Padrós et al., 2023).

Basic training has disappeared from the palette of the Hungarian aquaculture sector's professional training for 5 years. The physical work is mainly done by semi-skilled workers who have little technical knowledge of the sector's work processes. The development of a National Aquaculture Training Strategy is a sectoral need, one of the first steps of which is to define the possibilities offered by different levels of education.

The basis for the supply of professionals at the secondary and tertiary level is the training in natural sciences and biology in secondary schools, which, integrated into the national educational framework, provides knowledge to students with this interest. The framework includes a so-called 'faculty', which provides students with an increased number of hours of study and learning in this area of science.

Materials and methods

A questionnaire survey was carried out to investigate the attitudes of teachers and the available infrastructure towards aquaculture in secondary schools (mainly high schools) with faculties offering natural science and biology courses.

Results

It can be concluded that the teachers working in these institutions responded positively to the sector's inquiry and were willing to fill out the questionnaire in a higher proportion than expected. However, it can also be said that the rigid system of education provides few opportunities to broaden knowledge of a particular agricultural sector, in this case aquaculture. On the other hand, there was a strong demand for practical training courses and study trips, and this is an area where the sector has the greatest potential for improvement.

The questionnaire also confirmed the earlier finding that one of the breakout and development areas of the Hungarian aquaculture sector is education and the development of the teaching methodology (Urbányi et al., 2023).

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A CRUSTACEAN OOCYTES DELIVERY TOOL FOR LARGE-SCALE GENE SILENCING

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Oviparous animals are characterized by an elaborated yolk production process and packaging in the oocytes before egg-laying. The major yolk protein (vitellin) is usually produced as vitellogenin outside the egg (Vg) and internalized into oocytes by receptor-mediated endocytosis (RME). Like many other crustacean species, *M. rosenbergii* vitellogenin is expressed in the hepatopancreas. *M. rosenbergii* vitellogenin possesses 2537 amino acids and shares at least 33% identity with other decapod crustacean vitellogenin, such as shrimps, crabs, and crayfishes. Vg contains several domains, including the lipid-binding domain involved in yolk-lipid vesicle formulation. Upon arrival at the oocytes, the Vg-receptor (VgR) extracellular domain interacts with a distinct amino acid sequence of the Vg and internalizes it to form yolk droplets. One distinctive characteristic of the VgR family is their role in the massive internalization and accumulation of lipoproteins. Vitellogenesis is recognized by an immense accumulation of the Vg in the oocyte that will serve the embryo's metabolic needs for development and growth. For that reason, we predicted that Vg endocytosis could be used as a valuable tool for oligonucleotides' high throughput delivery into the oocyte. Indeed, a specific Vg-derived peptide sequence (Vg24) was found capable of oocytes' specific entry by *in vitro* and *in vivo* means. However, a peptide with the same amino acid composition but scrambled order (scVg24) could not enter the oocytes. Vg24 synthesized with nine Lysine-Histidine repeats successfully induced dsRNA electrostatic interaction and piggybacked the bound dsRNA into *M. rosenbergii* oocytes. When *PAX6* (eye development transcription factor) dsRNA was piggybacked, that led to eye development retardation in embryos of the treated mothers. Alignment analysis of the *M. rosenbergii* Vg24 shared 85% identity with the corresponding Vg peptide sequences from other decapod species. The peptide similarity proposes cross-reactivity between the *M. rosenbergii* peptide and the VgR of other decapod species. The developed tool might serve to deliver other than dsRNA molecules into crustacean oocytes and might be a powerful asset for large-scale silencing or editing aquaculture-relevant genes of crustacea.

EXPLORING A NOVEL INSECT MEAL FOR THE DIET OF PACIFIC WHITE LEG SHRIMP (*Litopenaeus vannamei*)

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Introduction

Pacific white leg shrimp, *Litopenaeus vannamei*, is one of the most productive species in aquaculture (FAO, 2022). This species has high dietary protein demands, with a significant portion being fulfilled by fish meal (FM). The increased demand for feed supply necessitates the exploration of new protein sources. In recent times, insect meals, particularly black soldier fly larvae meal (BSFLM), have emerged as an alternative to FM due to its high protein levels and adequate essential amino acids profile (Richardson et al. 2021). In this context, the objective of this study was to determine the effect of a novel insect meal (NIM) on the diet of Pacific white leg shrimp. The insects used were obtained from a biocontrol production, with the surplus potentially being used for aquaculture feed purposes.

Materials and methods

Shrimps were initially acclimated for a period of five weeks in Halieutica research facilities (Angers, France). A total of 195 shrimps were distributed across 15 aquariums with a density of thirteen shrimps per aquariums (40l). Shrimps were reared in triplicate and fed at 5 % of their body weight for a period of four weeks. A control diet (CTRL; 7% FM and 37.3 % protein content) served as the basis of comparison for four additional experimental diets, in which 10% and 20% of the control diet were replaced by NIM or BSFLM. The experimental diets containing 10% and 20% of BSFLM were also used as a reference diet to evaluate the efficacy of NIM as an insect meal. A non-parametric Kruskal Wallis test was used to compare the mean growth performance of each treatment.

Results and discussion

Weight gains (WGs) and Feed conversion ratios (FCRs) did not exhibit significant differences among the treatments (Figure 1).

The comparable growth performance of the shrimps shows that NIM is a promising new alternative ingredient for replacing FM. Furthermore, since insect-based feeds (BSFLM and NIM) were directly integrated into the CTRL feed, there is still potential for enhanced performance through formulation refinement and adjustments to all raw materials to accommodate the inclusion of insect meal. In addition, a more comprehensive understanding of the feed's efficacy would require further investigations into aspects such as immunology and intestinal microbiota (Chen et al., 2021).

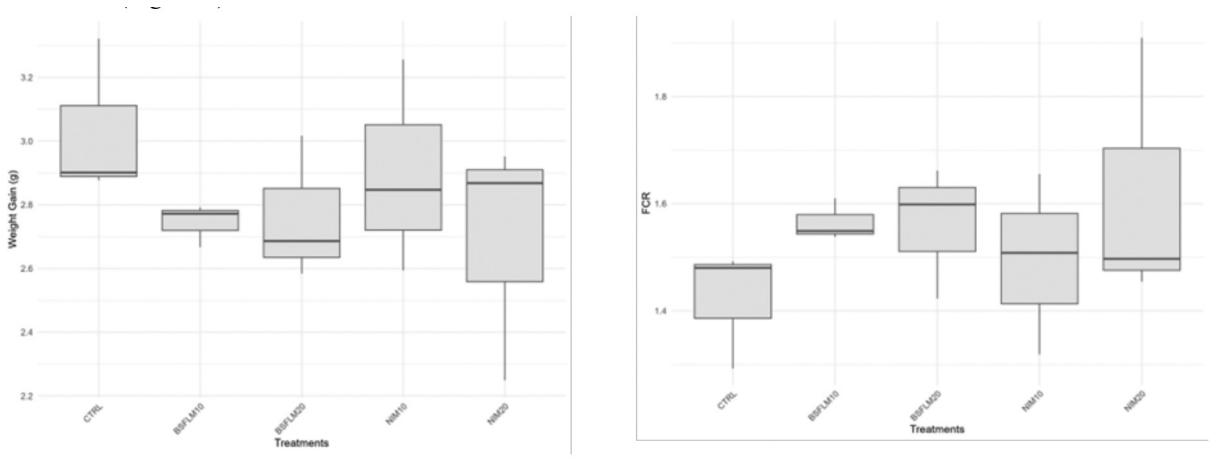

Figure 1. Comparison of WGs and FCRs among treatments.

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Conclusion

The growth performance results achieved with NIM are promising. Furthermore, the cost-effective production of this insect could potentially position it as a competitive alternative to other insect meals and fish meals.

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COMPARISON OF MUCOSAL IMMUNE TRANSCRIPT RESPONSE IN RAINBOW TROUT (*Oncorhynchus mykiss*) IN FRESH WATER AND SEA WATER AFTER VACCINATION

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Introduction:
Vaccination is known as the most efficient and adequate method to induce immune activation and thus protecting fish against pathogens since fishes live in an antigen-enriched environment (Tafalla et al., 2013). Beside the importance of the systemic immune system to protect fish against pathogens in the environment, mucosal-associated lymphoid tissues (MALTs) are also implicated in protection and play a crucial role in maintaining homeostasis (Parra et al., 2015; Khansari et al., 2018). There is also an extensive communication between the neuroendocrine and immune system where activation of neuroendocrine system elicits immune activation, thus either suppressing or enhancing the immune response. Most of our knowledge on the effect of vaccination in rainbow trout comes from studies in freshwater, but transfer of the fish to seawater after vaccination can be hypothesized to be a challenge for the fish that influences the immune response. In the present study, we aimed to compare how the mucosal immune system after long-term vaccination is affected by stress in both fresh- and seawater acclimated fish.

Material and Method:
Juvenile rainbow trout weighing on average 70 g was obtained from a local hatchery (Vänneåns fish farm AB, Knäred, Sweden). The fish were held in recirculating tanks at ca 10°*C* and fed daily with ≤2% of body weight. At the start of experiment, fish were immunized with Alpha Ject 3000 or injected with saline as controls. Starting 12 days post injection (dpi), salinity was gradually increased to 31 ppt over 10 days for half of the vaccinated and control fish, while the other half (vaccinated and non-vaccinated) remained in fresh water. To stress fish, they were chased for 5 min and after 48 hours of recovery, all fish were euthanized, length and weight measured, and skin mucus, blood and tissues skin, gills and gut were collected for further analysis.

Results and discussion:
Our results show an overall significant interaction between salinity groups (FW and SW) and treatments. The cortisol level in skin mucus were significantly higher in the stress and in vaccine+stress fish in both fresh water (FW) and sea water fish (SW) groups (Figure 1). In addition, cortisol was augmented in the vaccine+stress group in SW versus FW (Figure 1). This reflects a general stress response of animals to external stimuli that results in activation of the hypothalamic-pituitary-interrenal axis (HPI) axis to secretes cortisol by head kidney interrenal cells (Schreck and Tort, 2016). It is not yet clear if cortisol secretion at mucosal levels is delivered by the circulation or is secreted by local cells since cortisol secretion by immune cells has also been demonstrated. However, previous results showed that abiotic and biotic stressors are capable of inducing cortisol release in both systemic and mucosal system (Khansari et al., 2018; Ordóñez-Grande et al., 2020) and therefore cortisol has proven to be a reliable stress indicator.

Genes transcriptions were also analysed, and they were classified in three groups including stress related genes (*gr1*, *gr2*, *mr*, β-*ar*, *hsp70*), innate immune related genes (*il1β*, *il6*, *tnf*α, *il10*, *tgf*β1, *nadph oxidase*, *c3*, *enolase* and *lysozyme*) and adaptive immune related genes (*igm*, *igt* and *cd8*). In the gills of FW fish, stress and vaccine+stress enhanced the transcription level of *il1β*. The *il1β* transcript expression in stress and vaccine+stress was significantly lower in SW versus FW. In the skin, FW (stress and vaccine+stress) and SW (vaccine) fish, the mRNA level of *il1β* was decreased, whereas a significant increase was observed for the vaccine+stress group in SW. There was a significantly higher *il1β* expression in the skin of the stress and vaccine+stress groups in SW compared with FW. In the intestine, vaccine and vaccine+stress was found to induce *il1β* gene expression in FW while no alteration was registered in SW. The same expression pattern as gills was observed in intestine; a suppression of *il1β* transcript expression in the vaccine and vaccine+stress groups in SW (Figure 1). The results support that an inflammatory response was induced by the vaccine and stress in the peripheral tissues that is characterized by a first wave of expression of proinflammatory cytokines such as *il1β,* mainly produced by activated macrophages, that plays a crucial role in host defence (Stolte et al. 2008).

Overall, our results show that mucosal immune response at cellular level is triggered by intraperitoneal vaccine injection. Furthermore, the cellular immune response induced by vaccine/stress is significantly suppressed by salinity in rainbow trout. Thus, more studies should be devoted to investigating the effect of salinity on immune response elicited by vaccine in order how to mitigate the negative effect of hypersalinity within smoltification stage.

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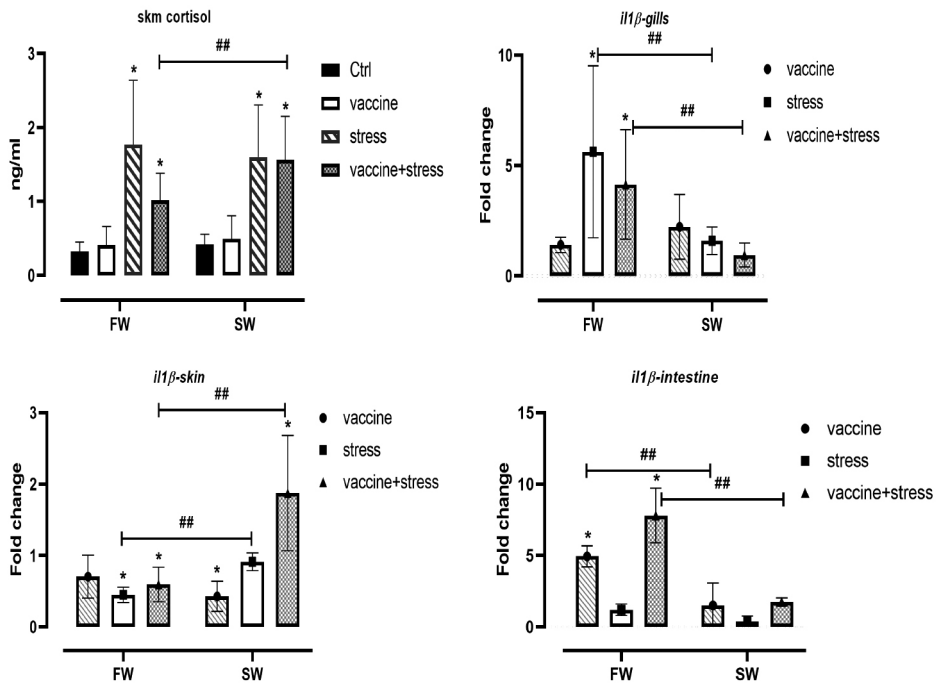

Figure1: Cortisol level (ng/mL) in the skin mucus and Fold change of *il1β* transcript expresion in gills, skin and gut. * shows significant difference of different treatments versus corresponding control and ## represents significant difference between FW and SW fish (vaccine, stress and vaccine+stress).

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HEALTH AND WELFARE OF SPOTTED WOLFFISH JUVENILE (*Anarhichas minor*) FED DIETS CONTAINING BLUE MUSSEL MEAL WITH OR WITHOUT SHELL

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Introduction

The emerging prominence of spotted wolffish (*Anarhichas minor*) as a potential candidate for cold water marine aquaculture in Norway and the Arctic region has garnered attention due to its promising attributes, including non-aggressive behavior, disease resistance, high fillet yield, and high market value (Moksness, 1994; Foss et al., 2004). Through the past 30 years of research and development, there has been notable progress in understanding and managing various fundamental aspects of spotted wolffish aquaculture and currently one fish farm in Norway is commercially producing spotted wolffish since 2013. However, scaling up the spotted wolffish production requires further studies and research. These include investigating reliable reproduction methods, comprehending nutritional needs across life stages for species-specific feed development, exploring sustainable feed options, conducting genetic studies and selective breeding for desired traits, and ensuring optimal health and welfare management in captive settings.

In this study, blue mussel meal was chosen as a potential alternative ingredient to assess its effects on the growth, health, and welfare of spotted wolffish. Blue mussel meal contains high protein and an amino acid profile similar to FM (Berge & Austreng, 1989) and been investigated as fishmeal replacement in the diet of poultry (Jönsson & Elwinger, 2009), rainbow trout (Árnason et al., 2015) and turbot (Weiss & Buck, 2017) with some promising positive results. The blue mussels used in meal production are derived from the waste stream of food production and are typically smaller than 5cm in size (Berge & Austreng, 1989). In mussel meal production, the shell is usually removed through deshelling process, which is both energy-intensive and costly. Consequently, processing the entire mussel, including the shell, is a more sustainable approach, despite the resulting higher ash content. For spotted wolffish, incorporating mussel meal with shells could be advantageous, given their natural diet of bottom-dwelling creatures like echinoderms, mollusks, and crustaceans (Albikovskaya, 1982, Foss et al., 2004). This study aims to understand how incorporating blue mussel meal, both with and without shells, into spotted wolffish diets affects their growth performance, stress biomarkers, plasma biochemistry and the occurrence of nephrocalcinosis (calcareous deposit) in kidney.

Materials and methods

The spotted wolffish (Anarhichas minor) were provided by Aminor AS (Halsa, Norway) and the feeding trial was conducted at Nord University research station, Mørkvedbukta (Bodø, Norway). 180 juveniles of approximately 500 – 800g (877.05g ± 18.88) were randomly distributed into six tanks with a total of 30 fish per tank. Two iso-calorific, iso-nitrogenous and iso-lipophilic diets were formulated and each was provided to three tanks of fish. The control diet contains mussel meal (CNT) and the experimental diet contains mussel meal with added shell (BMS). The experiment lasted 12 weeks and at week 0, 6 and 12, weight and length of the individual fish were measured for the calculation of weight gain (WG), specific growth rate (SGR) and condition factor (CF). At the end of the experiment, six fish from each tank were randomly chosen and sacrificed for sampling. Blood sample for analysis of plasma parameters was collected with a heparinized syringe (7.5g lithium heparin / 1ml Millipore water) from the caudal vein. Rest of the six fish from each tank were stored as whole body for identification of nephrocalcinosis through CT scan.

Results

In general, the growth parameters of juvenile spotted wolffish, including body weight, condition factor (CF), weight gain (WG), and specific growth rate (SGR), displayed notably higher values in the group of fish that were fed a diet containing shelled blue mussel meal (BMS), in contrast to those fed the control diet (CNT). At the 6-week mark (W6), the CF of the fish in the BMS group exhibited a significantly higher level compared to that of the fish in the control group ($p < 0.05$). Similarly, at the 12-week mark (W12), both WG and SGR of the fish were markedly higher in the BMS group than in the control group, and these differences were statistically significant ($p < 0.05$).

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Regarding plasma stress biomarkers, such as glucose, lactate, and cortisol levels, there were no significant differences observed between the fish groups fed diet CNT and diet BMS. The same lack of significant difference was observed for plasma osmolality, acid-base balance, and ion concentrations.

Furthermore, the incidence of nephrocalcinosis demonstrated a similar frequency of occurrence in both groups, with a ratio of 0.22% - equivalent to four out of the 18 fish observed in each group. The severity of nephrocalcinosis observed in both groups was characterized as minor in grade, and no significant differences were detected between the two groups.

Acknowledgments
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EFFECTS OF DIFFERENT LEVELS OF DIETARY CHOLINE WITH OR WITHOUT LECITHIN FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Introduction

Choline is one of the essential micronutrients for aquatic animals and plays an important role as a methyl donor and component of lecithin. This study was conducted to evaluate the effects of dietary different choline levels with or without on the growth, nonspecific immune response, antioxidant capacity and ammonia stress resistance of Pacific white shrimp *Litopenaeus vannamei*.

Material and methods

Experimental diets were prepared to contain choline levels of 2000, 4000, 6000 and 8000 mg/kg without lecithin supplementation (designated as C2000, C4000, C6000, C8000, respectively) and with lecithin of 2500 mg/kg (designated as CL2000, CL4000, CL6000 and CL8000 respectively). Juvenile shrimp (0.20 ± 0.00 g) were randomly distributed in acrylic tanks (240 L) with 25 shrimp per tank. Triplicate groups of shrimp were fed one of the experiment diets four times daily for 56 days. For the ammonia stress test, after the feeding trial, 18 shrimp per tank were randomly selected and distributed into 24 tanks (120 L) in triplicate groups per diet. The concentration of ammonia (NH_3) in tanks were kept 2.5 mg/L with ammonium Chloride (NH_4Cl). The mortality was monitored for 60 h.

Results

Growth performance results were significantly higher in shrimp fed diets containing over C4000 with or without lecithin than those in C2000 and CL2000 groups. Phenoloxidase, superoxide dismutase activity, glutathione peroxidase activity and catalase activity were significantly lower in shrimp fed C2000 and CL2000 diets compared to those of shrimp fed other diets. Digestive enzyme activity (trypsin, chymotrypsin, lipase and amylase) were significantly improved in shrimp fed diets containing over C4000 with or without lecithin than those of shrimp fed C2000 and CL2000 diets. Similar trends were found in relative expression of the insulin like growth factor-1, insulin like growth factor-binding protein, prophenoloxidase and heat shock protein 70 genes. During the ammonia stress challenge, C2000 and CL2000 groups showed the lowest survival than other groups. However, the two-way ANOVA showed that the supplementation of lecithin could increase the ammonia resistance of the shrimp.

Conclusion

Choline deficiency (≤2000 mg choline/kg diet) could resulted in negative effects on the growth performance, non-specific immune responses, antioxidant capacity, digestive enzyme activity and ammonia stress resistance in *L. vannamei*. Supplementation of lecithin in diet for *L. vannamei* had no effect on the determination of choline requirement from a growth performance. Broken-line regression analyses of weight gain indicated that the optimum dietary level of choline is 4609 without lecithine and 4594 mg/kg with lecithin supplementation in diet for *L. vannamei*.

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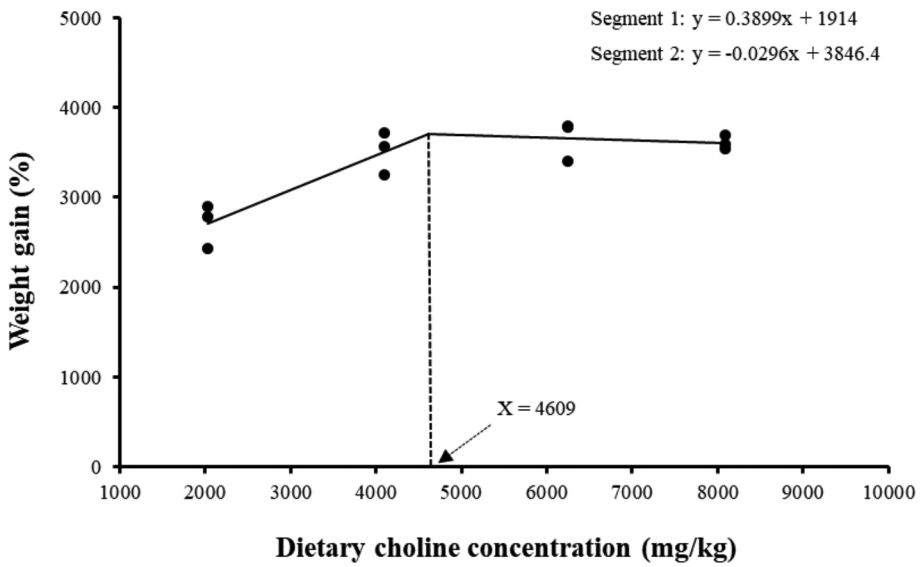

Fig. 1. Broken-line regression of weight gain for Pacific white shrimp *Litopenaeus vannamei* fed the experimental diets for 8 weeks.

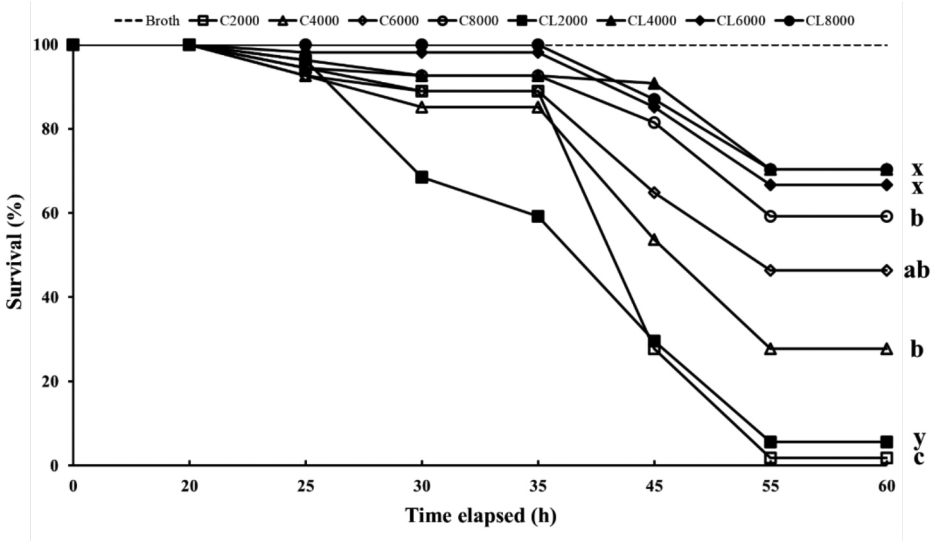

Fig. 2. Cumulative survival of Pacific white shrimp *Litopenaeus vannamei* during the ammonia challenge test. The shrimp were fed the experimental diets for 8 weeks before the challenge.

ELECTRICAL STUNNING AND FISH WELFARE IN TURKISH MARINE AQUACULTURE

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Introduction

World aquaculture production has broken the all-time production record with a total of 214 million tons, of which 178 million tons are aquatic organisms and 36 million tons are algae. Human consumption of seafood products has increased from 9.9 kg in the 1960s to 20.2 kg today. Considering the world population growth rate, aquaculture products contribute to human food as a great source of protein. Especially with increasing post-harvest food processes, income growth, urbanization, climate crises and changes in dietary trends, the average per capita consumption is expected to be 21.4 kg in 2030 (FAO 2022). Fish farming in Turkey was 79,031 tons in 2000, this amount reached 421,411 tons in 2020, an increase of more than 5 times. In Türkiye, 69.6% of this production, i.e. 293,175 tons, takes place as gilthead seabream (*S. aurata*) and European seabass (*D. labrax*) production. While the number of gilthead sea bream and European sea bass fry in Türkiye was 29 million in 2000, this figure was approximately 450-480 million fry of gilthead sea bream and European sea bass in 2022. Today, there are 265 licensed net cage enterprises engaged in marine fish farming in Türkiye. The number of enterprises with a capacity of 1000 tons is 93 (BSGM 2023).

The rapidly growing aquaculture sector in the world and in Türkiye has led to an increased awareness of environmental problems and fish welfare (Çoban et. al., 2020). Although there are many scientific studies on environmental problems, studies on animal welfare and fish suffering are very few. Although some improvements to fish welfare during harvesting are gradually being made, the vast majority of the world's farmed fish are currently harvested using inhumane methods. More welfare-friendly alternatives exist for some species, but much work remains to be done before they are widely adopted in the industry. Humane slaughter methods for other fish species are still under development and research should be prioritized. A report prepared for the European Commission in 2017 stated that member states are not fully complying with various directives on fish slaughter. The report also highlighted a major problem that stunning systems are not formally assessed and controlled in practice.

Percussive stunning or electric stunning is recommended for some species. These systems are important as long as they do not kill the fish. These systems should be followed by an appropriate killing method, such as gill cutting, decapitation or destruction of the brain, the important thing here is to ensure that the fish is killed before it regains consciousness (OIE, 2010).

Figure 1. Electric stunning systems (ESS) in the harvest.

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Retailers and consumers are undoubtedly the most important influencing forces in promoting and investing in fish welfare. Therefore, the present study examines producers' awareness and perspectives on fish welfare in Türkiye, the second - largest fish producer in continental Europe and the largest producer of gilthead sea bream and European sea bass in the world.

Material and Methods

In the study, a face-to-face/online survey was conducted with fish producers. The questionnaire consists of about 50 questions in two parts including production methods and approach to animal welfare. The survey questions were open-ended or based on a 4-point Likert scale. In the study, producer companies corresponding to 75% of the total production capacity were interviewed. The SPSS package program was used to evaluate the data.

Conclusion

All of the producer companies participating in the study attach importance to fish welfare and declared that they carry out studies (training, meetings, etc.) on this subject. They reported that they apply the most important elements of fish welfare such as stock density, feeding time and amount, cage maintenance etc. according to the fish species and geographical location. So much so that the survival rate during aquaculture in all net cages is between 10-15% and the FCR varies between 1:1 and 1:1.5 depending on the cultured fish species. The companies use either the ice slurry method or electric stunning systems (ESS) for the harvesting of gilthead sea bream, European sea bass and rainbow trout. All of the companies have an ESS (Figure 1). However, in line with the demand from the market, the preference for using ESS during harvesting varies. However, they also reported that the selling price of fish harvested using ESS is not higher than other harvesting methods both in the domestic and foreign markets. Of the companies using this impacting machine, 40% prefer domestic production. They stated that spare parts, maintenance-repair, etc. cannot be done in imported machines or that this process takes a lot of time. They reported that the most important problem faced by the companies during the ESS was the installation of this machine on the harvesting boat. Likewise, they also reported that when the wave height is high, sufficient amounts of fish from the fish pump cannot reach the electric impactor system sufficiently.

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MICROMORPHOLOGICAL CHANGES IN THE INTESTINE OF MARKET READY ATLANTIC SALMON FED ON DIFFERENT MICROALGAE BIOMASS

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Introduction

Atlantic salmon farming industry in Norway is engaged in carefully crafting feed formulations and employing innovative technologies to address the sustainability concerns. However, the evolution has been associated with challenges linked to welfare issues. To maintain a sustainable growth trajectory and to preserve the health of farmed fish, the industry must rely on feed ingredients that have beneficial implications beyond their intended growth promoting effects. Microalgae varieties are among the new ingredient sources being evaluated for salmon feeds. In the present study, we assessed the effects of meals from three differently processed microalgae on the growth, feed performance and intestinal health of Atlantic salmon reared in sea cages under commercial farming conditions.

Materials and Methods

Atlantic salmon of average weight 1.8 kg were employed in a 32-week feeding trial conducted at the pilot-scale farm site of Gildeskål Forskningsstasjon (GIFAS), Norway. The fish were fed one of the following five test feeds: control feed based on plant ingredients and 15% fishmeal and four other feeds containing 10% fish meal and different microalgal biomass – 7.5% of defatted *Desmodesmus* sp., extruded *Nannochloropsis oceanica*, bead milled *Phaeodactylum tricornutum,* and unprocessed *P. tricornutum*. Growth of salmon, feed conversion ratio, and histomorphological features of the distal intestine of the fish were compared at the end of the feeding term.

Results and Discussion

After 32 weeks in the sea cages, the fish had gained more than two times their initial body weight, reaching 4.09 kg on an average. Inclusion of different algal biomass in the feeds did not reveal any significant difference in weight gain. Nevertheless, the condition factor of fish on the *Desmodesmus* feed was significantly lower compared to those fed the control and the *Nannochloropsis* feeds. Feed conversion ratio for the latter fish group did not differ from that of the fish on the control feed, though the values of the other alga-fed groups were significantly lower compared to the control group. Furthermore, the fish on the *Nannochloropsis* feed had significantly higher values for villi height, submucosa thickness, muscle layer thickness, villi width, and height of enterocytes compared to the control and bead milled *Phaeodactylum* fed groups. The control group had wider lamina propria compared to the fish fed unprocessed *Phaeodactylum*. The latter fish group had fewer mucous cells compared to the control fish and those that received *Desmodesmus* and *Nannochloropsis* feeds. The fish fed *Desmodesmus* had more intraepithelial lymphocytes compared to the control fish and those fed *Phaeodactylum* (both types). The *Nannochloropsis* fed group had significantly higher number of supranuclear vacuoles in the enterocytes compared to the control fish and those fed *Desmodesmus*.

The principal component analysis of the distal intestine histomorphometry-based parameters pointed to the better gut health of the fish fed *Nannochloropsis* compared to the other diet groups, based on improved height and width of villi, and height of enterocytes. The bead milled *Phaeodactylum* negatively affected the villi features compared to those fed the unprocessed biomass.

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Conclusion

The studied microalgae affected the intestinal micromorphology differently; while the *Desmodesmus* sp. stimulated the barrier defence features, *N. oceanica* improved the villi features in the intestine of Atlantic salmon. Processed *Phaeodactylum* biomass was not effective in maintaining the normal morphological features of the intestine. Taken together, the microalgal products examined here can be considered as potential ingredients for salmon feeds, especially the biomass of *Nannochloropsis,*

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DEVELOPMENT OF A PROTOCOL FOR *IN VITRO* MATURATION OF PIKEPERCH (*Sander lucioperca L.*) POSTVITELLOGENIC OVARIAN FOLLICLES

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Introduction

Under *in vitro* conditions, isolated fish ovarian follicles retain the ability to resume meiosis and progress through the last stages of oogenesis when exposed to maturation-promoting stimuli. Assessing the dynamic response of follicles during *in vitro* maturation (IVM) can help estimate the reproductive state of the broodstock and gamete quality. To maintain viability and induce follicle development in culture, it is necessary to consider both effective hormonal stimulation and optimal media composition. In fish, gonadotropins and maturation-inducing hormones, such as 17α,20β-dihydroxy-4-pregnen-3-one (DHP), are commonly used to induce maturation of isolated ovarian follicles. Furthermore, both amino-acid rich culture media and additional incorporation of protein and lipid sources—such as serums and bovine serum albumin (BSA)—were found beneficial for maintaining follicular cell and oocyte viability, as well as promoting maturation and ovulation.

In pikeperch (*Sander lucioperca*), IVM was used to predict the maturational competence and latency time of female breeders (Ljubobratović et al., 2023). The aim of this study was to further optimize the established IVM protocol for immature, postvitellogenic ovarian follicles of this species. This was done by testing different culture media types and supplementation, as well as the effect of maturation-promoting hormones in a range of concentrations.

Materials and methods

Adult pikeperch females (TL: 37 ± 2 cm; W: 424 ± 63 g; GSI: 12 ± 1%) were sampled during the chilling period of the photo-thermal spawning induction (6 °C, 8 h light/16 h dark cycle at sampling moment), prior to any hormonal stimulation. Intact fully grown ovarian follicles were isolated manually and subsequently placed in culture media consisting either of 90% Leibovitz L-15 medium or full strength Cortland's medium. Both media types were supplemented with antibiotics and 15 mM HEPES, with pH adjusted to 7.5. Furthermore, the effect of additional culture media supplementation in the form of BSA (0-1%) or fetal bovine serum (FBS; 0-20%) was tested. Maturation was induced by adding either DHP (1-1000 ng/ml), human chorionic gonadotropin (hCG; 1-20 IU/ml) or a combination of the two, after which the follicles were incubated at 12 °C, under gentle agitation for 6 days. Resumption of meiosis and maturation progress was evaluated regularly, by scoring the percentage of follicles that underwent germinal vesicle breakdown (GVBD), ooplasm clearing and lipid droplet coalescence, compared to the control group with no hormonal stimulation. In addition, ovulation was monitored by checking the integrity of the follicular layer under light and fluorescent stereo microscope, after staining the follicular cells with SYBR Green.

Results and discussion

At the time of sampling, most of the fish contained follicles in postvitellogenic stage of development that have attained maturational competence (Ø 898 ± 15 µm). Both media types successfully maintained the viability and responsiveness of follicles throughout treatments; however, the rate of GVBD a 4-day stimulation with 100 ng/ml DHP was higher in Leibovitz L-15 medium (83 ± 11%), compared to ones in Cortland's medium (67 ± 9). The rate and speed of maturation were dependent on the concentration of DHP used. Furthermore, a high percentage of maturing follicles had issues with lipid droplet fragmentation. Treatment with only hCG resulted in comparable final GVBD rates (85 ± 13%); however, the length of incubation notably increased (up to 6 days), regardless of hormone concentration. Slower response during hCG stimulation enabled gradual and synchronized nuclear and cytoplasmic events, resulting in proper lipid droplet coalescence in all mature follicles, as well as evident hydration – increase in diameter was 33 ± 10 µm at the time of GVBD, as opposed to 14 ± 8 µm in the DHP-groups. The rate of ovulation was low after both DHP- (10 ± 8%) and hCG-induced maturation (9 ± 8%), with the majority of non-ovulated mature oocytes showing signs of activation and cell cleavage with longer incubation times. In hCG-groups, higher ovulation rates were achieved by subsequently including DHP (10-100 ng/ml) to the ongoing treatment, shortly before the follicles reached GVBD. Incorporation of supplements in the form of BSA (0.1-0.5%) and FBS (2-10%) to the 90% L-15 medium improved the hormone-induced GVBD of pikeperch follicles in

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culture. Moreover, it was noted that FBS in concentrations higher than 10% promotes maturation (>32 ± 6%), even with no additional stimulation with DHP or hCG. This outcome might be due to the complex biomolecular composition of FBS, which can include hormones and growth factors that can influence follicle development and induce oocyte maturation. However, although favorable for nuclear maturation, high concentrations of both BSA (≥1%) and FBS (≥15%) seemingly affected the lipid metabolism and cytoplasmic maturation of the oocyte, most often leading to disruption in the lipid droplet formation.

Conclusions

An *in vitro* maturation protocol was successfully developed for fully grown, postvitellogenic pikeperch ovarian follicles. The culture media consisting of 90% Leibovitz L-15 media (pH 7.5) with the addition of 0.5% BSA or 5% FBS maintains viability and responsiveness of follicles to hormones. The highest quality and rate of maturation was obtained after treatment with 2-5 IU/ml hCG after 6 days of incubation at 12 °C. Subsequent addition of DHP (10 ng/ml) to the culture media during an ongoing hCG treatment improves ovulation rates of matured oocytes, without notable changes in the GVBD rate and lipid droplet morphology. The protocol detailed here presents a useful tool for refining pikeperch spawning practices in the future, as it enables an *in vitro* assessment of the maturational competence of individual fish, as well as the prospective outcome of hormone-induced ovulation during artificial spawning.

Acknowledgements

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PHOSPHORUS UPTAKE AND REQUIREMENT FROM FEED AND WATER IN BY ATLANTIC SALMON (*Salmo salar*) JUVENILES KEPT IN A FRESHWATER SYSTEM

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Introduction

In recent years, aquaculture become one of the fastest developing food sectors of the world with an effect on environment. An increase of phosphorus rich effluents from culture system creates consequences on environment sustainability. Phosphorus (P) is present in fish diets, which are the essential source of P in aquaculture. Importance of P nutrition on bone development and energy kinetics in the cells are well studied (Strauch *et al*, 2018). Although, it is considered as biologically important for fish, it is as well established that excess phosphorus in fish feed can promote eutrophication of aquatic environments (Antony Jesu Prabhu *et al*, 2016).

The excretion of indigestible P leads to an accumulation of P in closed water systems, like recirculation aquaculture systems (RAS). Previous studies show that freshwater tilapia species can absorb P from the water through the gills and gastrointestinal tract (Al-Kholy et al., 1970) and in other species, it has even been shown that the accumulation of this mineral in water can be beneficial for growth and normal skeletal development (Strauch et al., 2019; Van Bussel, C et al., 2013). Normal developing skeleton is a prerequisite for sustainable production and animal welfare, but vertebral column deformities are a persistent concern for farmed Atlantic salmon (Fjelldal et al., 2012a).

The major objective of this study is to understand and quantify the differential impact of water-borne phosphorus and dietary phosphorus content on P retention juvenile Atlantic salmon. The final goal is to find an equivalence ratio between P-water and P-feed to reduce the dietary phosphorus content by recycling the P accumulated in water. Differences will be assessed by quantifying fish growth, P-accumulation in the whole body and opercula and comparing the skeletal development and the total fat content.

Materials and method

Atlantic salmon juveniles weighing on average 2.71 g were reared in this trial. This experiment includes three identical RAS systems with 48 freshwater tanks (each 50L). Three water treatments with different phosphorus level were applied in these systems: no phosphorus addition; supplemented to 30 mg P/litre and supplemented to 60 mg P/litre. Mono sodium phosphate was used to maintain phosphorus level in water. 8 different diets were tested in this experiment with different phosphorus levels in feed: 0.00%, 0.21%, 0.42% ,0.63%, 1.25%, 1.88%, 2.50% and 3.13%. Each diet was tested in triplicate groups at 120^0 C in freshwater. Growth performance of each tank was measured after 8 weeks. At the end of the trial, phosphorus content in whole body and opercula was analysed and skeletal changes were assessed.

Results

Growth performance was significantly impacted by P-water and P-diet. High water-P depressed growth performance. At the end of 8 weeks, phosphorus content in opercula and whole-body of fish has been analyzed . Analyses of skeletal abnormalities will support chemical analyses. A recommendation will be given how far water-borne P can replace dietary P in a sustainable manner.

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INTRODUCTION TO THE NEW RECIRCULATING AQUAPONICS SYSTEMS AT LEIBNIZ INSTITUTE OF FRESHWATER ECOLOGY AND INLAND FISHERIES – WITH SPECIAL FOCUS ON COST EFFECTIVE DESIGN

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Aim

In RAS and aquaponics, optimum system design is key to assure best results, defined by key performance indicators (e.g. removal of solid wastes, nitrification, NO_2 and CO_2 removal and O_2-supply). In this respect, a better understanding of the principles and performance of different functional components can improve robustness and performance of RAS.

The aquaponics team of Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB) has developed a modular, Recirculating Aquaponics System.

The aim of this presentation is, to show how to calculate, design and build a robust and price effective RAS for aquaculture and aquaponics.

Materials and Methods

The system set-up includes 16 identical RAS specifically designed for aquaponic research trials. Each system is equipped with one fish tank a polyethylene sedimentation tanks, moving-bed nitrification bioreactor and pH stabilization. The modular approach of the design allows for high flexibility, e.g., by "by-pass" to synchronize bio filters flow rate, feces collection and valves for fresh- and wastewater usage, if needed. Furthermore, it is possible to adapt the system design towards altering research questions (figure 1). New water use was 10% per day. pH was adjusted with NaOH by means of a medical drip applicator when needed and was kept at 7.8 ± 0.27 during the trial.

Furthermore, it is possible to customize the system for every new trial, if necessary. An outline of the system as it is in use at the moment can be seen in figure 1.

Results

Robustness and performance of the system was demonstrated during first year of operation. During this time, different research trials were carried out, with main focus on development of aquaponics feeds for different fish species [1, 2] These studies demonstrated that physico-chemical parameters (e.g. oxygen concentrations, temperature, pH, and levels of ammonium and nitrite), were within suitable ranges for fish tested.

During the trials oxygen and temperature could be kept constant in the defined limits of 7.11 ± 0.23 mg and 27.0 ± 0.3 °C. The values were measured once a day before feeding and water exchange. Solids were removed on a daily basis from each RAS from the sedimentation tanks.

Discussion and Conclusions

The authors believe, that the use of a low-budget but well-planned research system presents a chance to create something that opens up new possibilities for your future research and should be exploited more often. Through the above-mentioned study, it was possible to show that by controlled removal of waste water and aquaculture sludge the closing of nutrient streams between aquaculture and hydroponic can be supported. The ongoing development of aquaponics and the reuse of system-internal waste streams is becoming increasingly important.

We share our personal experiences on the design process -the planning, assembly, performance assessment and design improvement.

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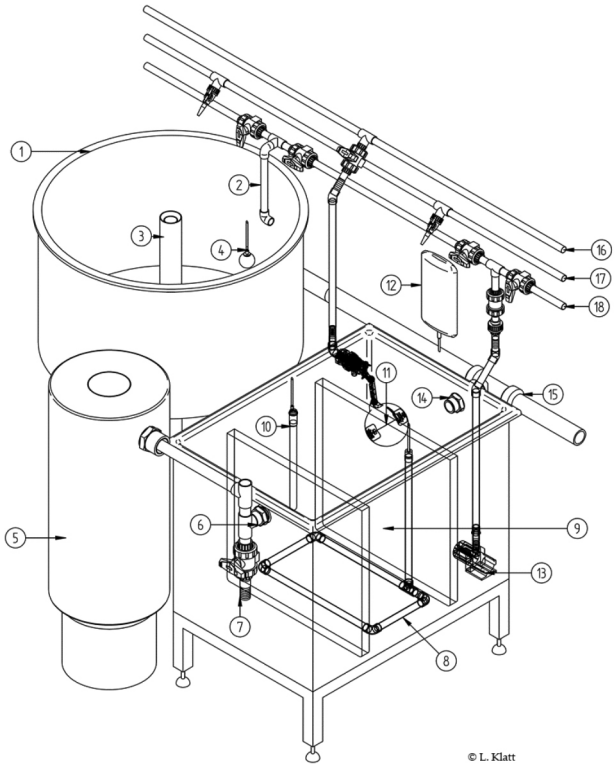

1	Fish tank
2	Inlet fish tank
3	Standpipe
4	Air stone
5	Settling tank / feces collector
6	Inlet biofilter
7	Outlet exchange water
8	Aeration biofilter
9	Moving-bed bioreactor (MBBR)
10	Heater
11	Float valve (fresh water inlet)
12	Drip-applicator (base)
13	Circulation pump
14	Connection to other MBBRs (closable)
15	Connection pipe MBBRs
16	Fresh water supply
17	Air supply
18	Circulating RAS water

Figure 1. Drawing of the individual RAS setup; individual components are numbered and specified.

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OTC MARICULTURE: STOCK AND ENVIRONMENTAL MONITORING FOR SUSTAINABLE MARICULTURE

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Introduction

The profitability of small and medium-sized fisheries in the inner and outer coastal waters of Mecklenburg-Western Pomerania is being made more and more difficult by the limitation of fish resources and regulatory measures such as catch quotas, seasonal fishing bans and area closures. In order to continuously ensure the regional demand for fresh fish in the face of declining yields, marine aquaculture is an alternative solution. The herewith presented project aims at three main points that currently still significantly restrict the spread of mariculture systems in Europe and especially in Germany:
1. The management of mariculture systems is significantly more complex than systems on land.
2. The animal welfare rating of the cultured fish is difficult to determine. Non-invasive evaluation is necessary for both ethical reasons and to increase yield.
3. The approval of new systems often fails because of bureaucratic hurdles and skepticism about risks of water pollution and negative environmental effects.

On the basis of data acquisition with comprehensive sensors - from e.g. image and video material, weather satellites, hydrodynamics (currents, waves), water temperature, salinity, pH, dissolved oxygen (O_2), redox potential (ORP) - the concept of a digital management tool for the aquaculture facility including its spatial environment, should be addressed via the project. By using methods from the field of artificial intelligence (AI) to evaluate the sensor data, a monitoring system for aquaculture systems with intelligent sensors is to be created. Digitalization in the form of mapping the value chain through real-time monitoring and the associated option for remote maintenance can greatly reduce the effort involved in managing a mariculture facility.

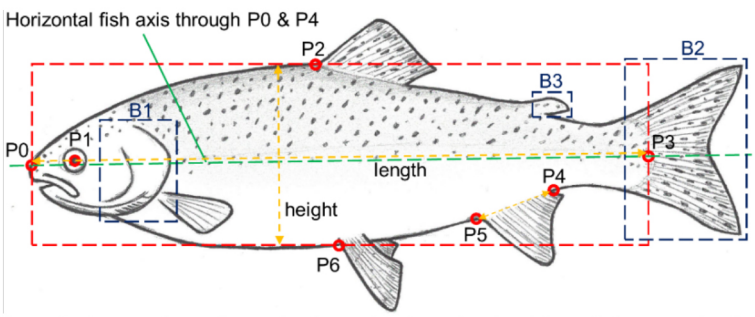

Figure 1: Annotation scheme for keypoint detection by AI- real time monitoring.

Table I. Isolated parasite species in the studied rainbow trout at the sampling on November, 26.th 2022; n=18. mA, mean Abundance; I, Intensity; mI, mean Intensity; P, Prevalence.

Parasite species	Stage	Site of Infection	P(%)	mI / I	mA
Digenea					
Brachyphallus crenatus	**Adult**	**Stomach**	**16.7**	**1.7 / 1-2**	**0.3**
***Diplostomum* sp.**	**Larvae**	**Eye lens**	**11.1**	**14.5 / 1-28**	**1.6**

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Material and Methods

For implemented studies, an offshore mariculture system in the Baltic Sea off Warnemünde/Rostock was chosen. Within the circular net cage (Vol. 70 m^3, PE) rainbow trout, *Oncorhynchus mykiss* were farmed within season of 2022. Real-time monitoring of animal welfare, animal behaviour and environmental parameters was done by comprehensive sensor technology and a camera system. Obtained video and photo material was processed and analysed using artificial intelligence. Additionally, health status of the fish was examined by means of parasitological and bacteriological investigations, following standardised methods (Bush et al. 1997, Unger & Palm 2021, Fuentes et al. 2023).

Results

For AI detection by software, fish keypoints and zones were defined on the rainbow trout, which are helpful in analysing fish behaviour (see Figure 1). For example, the relationship between the maximum height and length of individual fish can be used to determine the nutritional status. In addition, external injuries can provide clues to husbandry conditions and diseases, since these are mostly induced by stress and/or stocking density (Weirup et al. 2022). In addition, open wounds represent potential entry points for parasites and pathogens.

AI-assisted detection of damage to gill cover (operculum) and damage state of the caudal fin was successfully established for the trained video sequences. Statistical analysis and specifications of these markers are planned for season 2023.

The parasite fauna of rainbow trout was studied in April and November 2022. In April, only one specimen of eye fluke (*Diplostomum* sp.) parasite was found in the studied rainbow trout, originating from the freshwater hatchery. In November sample, beside these parasites, moderate prevalence of digenean parasite, *Brachyphallus crenatus* were obtained, which were transferred from the Baltic Sea environment, via intermediate hosts (see tab. 1).

Discussion and conclusion

The aim of the presented project is to develop a real-time monitoring system for improving the welfare and management of aquaculture systems. This is done by setting up digital image, which includes various animal welfare, animal behavior and environmental parameters. In addition, parasite and microbiome studies are used in this project to evaluate aspects of fish health and to be able to make comparisons between fish in aquaculture and fish outside of the net cage. With establishing the first markers and keypoints in the first season of project work, increasing the robustness of detection and a detection of the dietary status of the fish is in focus of the upcoming project work. This may include a test of our monitoring system and a comparison of different farming systems and conditions. Possibly enabling a wider field of application.

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VITAMIN C REQUIREMENT OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Introduction

Pacific white shrimp (*Litopenaeus vannamei*) is one of the most important species of shrimp in aquaculture with a global production of over 5 million tons per year. Vitamin C is an essential nutrient to maintain and enhance growth performance, immunity and antioxidant capacity of fish and shrimp. This study was conducted to determine the optimal level of vitamin C supplementation in diet for Pacific white shrimp.

Materials and methods

A vitamin C-free basal diet (C0) was formulated as a control to contain 200 g kg^{-1} fish meal, 360 g kg^{-1} soybean meal and 50 g kg^{-1} squid liver powder as main protein sources. Five other diets were prepared by adding vitamin C (L-ascorbic acid-2-phosphate, Stay C® 35) to the basal diet at 30, 60, 90, 120 and 150 mg kg^{-1} (designated as C30, C60, C90, C120 and C150, respectively). The vitamin C concentration in the experimental diets were determined to 5.99, 34.3, 63.1, 87.9, 131 and 158 mg kg^{-1}, respectively, for C0, C30, C60, C90, C120 and C150. Total 480 shrimp (0.70±0.00 g) were stocked into 24 acrylic tanks (120 L) in quadruplicates per dietary treatment.

Results

The survival of the shrimp was not significantly different among all the groups even though it was the lowest (93.8%) in C0 group. Final mean body weight was significantly higher in C90, C120 and C150 groups compared to that of C0 group. Phagocytic activity was significantly higher in shrimp fed C90 diet than in shrimp fed C0 diet. Phenoloxidase activity was significantly improved in shrimp fed C90 and C150 diets, while antiprotease and lysozyme activity were significantly increased in the vitamin C-supplemented groups than in shrimp fed C0 diet. Superoxide dismutase activity was significantly increased in C60, C90 and C120 groups in hemolymph, and significantly higher in C60, C90, C120 and C150 groups in hepatopancreas compared to that in C0 group. Catalase activity and total-antioxidant capacity were significantly increased in shrimp fed C90 diet compared to that of C0 group. Hepatopancreas ascorbic acid and, muscle hydroxyproline and collagen concentrations gradually increased with increasing dietary vitamin C levels (up to 63.1 mg/kg). Vitamin C supplemented groups had increased mRNA expression of hepatopancreas insulin-like growth factor-binding protein (C90, C120 and C150 groups), prophenoloxidase (C90, C120 and C150 groups), crustin (C30, C60, C120 and C150 groups) and HSP70 (C60, C90, C120 and C150 groups).

Table 1. Growth performance, feed utilization and insulin-like growth factors binding protein gene expression of *Litopenaeus vannamei* fed the experimental diets for 49 days.

	ITB[1]	FTB[2]	FMBW[3]	IGF-BP[4]	FCR[5]	Survival (%)
C0	14.0±0.21	191±5.08[b]	10.2±0.04[b]	1.00±0.35[c]	1.22±0.01	93.8±2.50
C30	14.0±0.07	202±9.62[ab]	10.3±0.08[ab]	1.67±0.23[abc]	1.22±0.04	98.0±5.00
C60	13.9±0.24	210±3.13[a]	10.5±0.16[ab]	1.59±0.12[bc]	1.19±0.03	100±0.00
C90	13.9±0.14	209±4.88[a]	10.7±0.10[a]	2.33±0.65[ab]	1.20±0.02	97.5±2.89
C120	14.0±0.37	208±5.01[a]	10.7±0.21[a]	2.52±0.49[a]	1.20±0.01	97.5±2.89
C150	14.0±0.07	211±2.93[a]	10.7±0.17[a]	2.28±0.41[ab]	1.19±0.02	98.8±2.50

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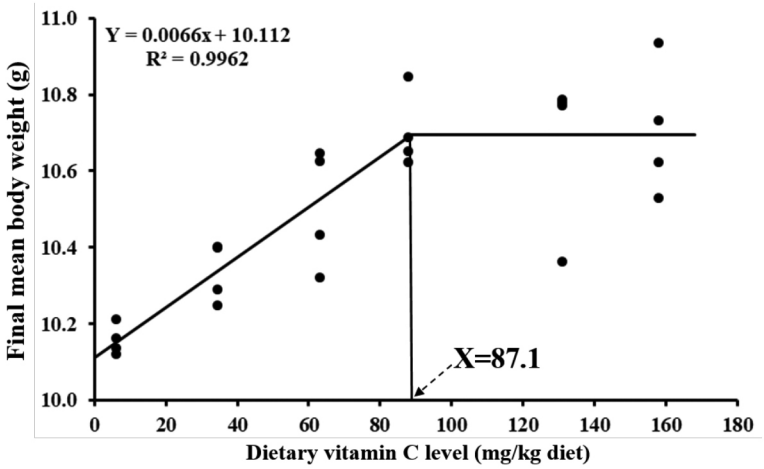

Fig. 1. Estimation of optimum dietary vitamin C requirement using broken-line regression analysis based on final body weight (g) of *Litopenaeus vannamei*

Conclusion

This study demonstrates that supplementation of vitamin C in diet for *L. vannamei* can enhance the antioxidant enzyme activity, which may contribute to improving their health and performance. Vitamin C supplementation in *L. vannamei* diet positively affects shrimp growth by increasing hydroxyproline and collagen synthesis. A broken-line regression analysis based on the growth of *L. vannamei* showed that the optimal dietary vitamin C requirement was 87.1 mg/kg.

THE EFFECT OF COMMON CARP MASS YIELD AND SUPPLEMENTAL FEEDING ON BASIC WATER QUALITY PARAMETERS UNDER SEMI-INTENSIVE POND MANAGEMENT IN CENTRAL EUROPE: IMPLICATIONS FOR SELECTIVE BREEDING IN NATURE-CLOSE CONDITIONS

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Introduction

Common carp is reared in man-made ponds, mostly under semi-intensive management in Central Europe, especially Czechia. Most of the ponds were built several centuries ago, spread freely in the landscape and resemble natural lakes, pools, and wetlands. Such ponds fulfil other important ecological and non-productive functions. This is the reason for never-ending public discussions about the present role of ponds and common carp culture. Fishery practices are often criticised as destructive to biodiversity and water quality (Pechar, 2000). There are no doubts about the effect of common carp stocks on pond ecosystems, but many other factors co-share the water quality in ponds. Still, when looking at the system of common carp culture in Czechia as on the animal production cycle, it is hard to find another comparable concerning the welfare of animals and carbon footprint (Roy et al., 2020). From a fish farmer's point of view, it is essential for the rentability of the common carp farming to maximize the fish mass yield per water area unit. The simultaneous study showed that carp stocks with higher performance potential better utilize the pond ecosystem productivity (Kocour et al., 2022). Here, we focused on relationships between production parameters, type of supplemental feed used and basic parameters of water quality to evaluate the level of possible negative effects of higher carp production in ponds on the environment.

Materials and methods

During 2019–2022, there were altogether 60 ponds (water area ranging from 0.8 – 11.7 ha) monitored for basic water quality parameters: Ammonia nitrogen ($N\text{-}NH_4^+$), Nitrate nitrogen ($N\text{-}NO_3^-$), Total inorganic nitrogen (TIN), Total phosphorus (TP), Chemical (COD_{Mn}) and Biochemical (BOD_5) oxygen demand. The water samplings were performed once per month from May to September (i.e. during the main growing season) close to the pond outlet and in the tributary (if any). Mean values of water quality parameters for each pond and year were calculated from the five values. Balances between water quality parameters from outlet and tributaries were calculated where applicable. Ponds were stocked with different age categories (AC) of common carp, with two mirror carp stocks of different performance potential (assigned as M2 and M2 x AL) and a scaly control group (for evaluating pond conditions). Fish were fed with two types of supplemental food (wheat grain or meal and a plant granule fodder). At harvest, the number and mean weight of the mirror and scaly carp survivals were recorded, and final carp mass and mass yields of the mirror and scaly carp stocks per 1 ha of pond water area were calculated. Amount of spent food and other factors (winter period, presence of wild ducks, excessive quantity of unwanted fish) were also considered. After excluding incorrect data, information from 41 ponds was included in the statistical analyses (one-way and multifactorial ANOVA, non-parametric Mann-Whitney U test, ANCOVA, multiple correlation matrix, PCA).

Results

Data showed significantly higher weight gain (WG) in M2 x AL stock in some age categories compared to M2. During the third growing season, the difference in WG was up to 33.6 % based on the type of analysis. Even survival was usually better in M2 x AL stocks, but the difference was statistically insignificant. It was also shown that unified mass yield (MY) and carp mass (CM) of M2 x AL stocks were higher than in M2 stocks, even if insignificantly. However, the share of M2 x AL stocks on total carp MY was significantly higher compared to M2 stocks. Unified carp MY in ponds ranged from 203 $kg*ha^{-1}$ to 1246 $kg*ha^{-1}$. Statistical analyses did not reveal powerful relationships between water quality and carp production parameters (MY, CM, the type of carp stock used, amount of supplemented food used and relative food conversion ratio). On the other hand, relationships among some water quality parameters were significant and moderate to high. Balances between quality of incoming and outcoming water indicated that the pond ecosystem did not change the water quality significantly, even if parameters of organic pollution may be moderately changed in relation to carp mass in ponds.

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Discussion and conclusion

It is supposed that ponds with higher CM have higher levels of TP, TIN, COD and BOD_5 in the water. In presented study, CM in ponds varied quite a lot, but no correlations of the CM with these parameters, except a moderate but significant correlation with COD, was observed. It seems that there may be more important factors responsible for the ecological status of the ponds than CM common in ponds of Czechia. Comparison of historical data and our observations for the level of TP in ponds of Czechia (Pechar, 2000) showed that from 1990' there has been no increase in the level of this main nutrient responsible for water eutrophication. Balances of water quality parameters in the tributary and outlet parts of ponds clearly showed that ponds in our study decreased in average the content of TIN (by 1.91 $mg*L^{-1}$), practically did not change the content of TP (-0.003 $mg*L^{-1}$) and only slightly increased the COD (2.95 $mg*L^{-1}$) and BOD_5 (5.21 $mg*L^{-1}$). Concerning organic pollution, the positive of ponds may be seen at least in the fact that they increased the ratio of organic matter that may be easily biologically mineralized (by 2.26 $mg*L^{-1}$). Moreover, the results indicate that the share of CM in the pond on the organic pollution increase is rather low. Based on the results, it can be concluded that a certain increase in average CM in ponds, an essential condition for implementing a selective breeding program, should not dramatically affect the basic water quality parameters.

Acknowledgements

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BIOAVAILABILITY AND PHARMACOKINETICS OF PRAZIQUANTEL IN GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Gilthead sea bream (*Sparus aurata*) farming is of great importance to European finfish mariculture accounting for 13% of its production value. A bottleneck to further increasing this species production is *Sparicotyle chrysophrii*, a gill-attaching blood-sucking monogenean, which is currently the most serious pathogen for gilthead seabream farming. To date, formalin bath treatments are the primary measure to battle sparicotylosis, however, bath applications in large cages are associated with several drawbacks.

Praziquantel (PZQ) is an anthelmintic drug widely used in veterinary medicine to control Platyhelminthes. The efficacy of the drug against a wide range of parasitic helminths in numerous farmed fish species has been recently reviewed (Bader et al. 2019; Norbury et al. 2022). Admittedly, drug pharmacokinetics (PKs) in the targeted organisms provide useful information optimizing treatment schedules. Nevertheless, to our knowledge, there is no literature examining the PKs of PZQ in gilthead seabream, thus, the present study attempts to investigate the kinetic profile and bioavailability (*F*) of dietary-administered PZQ in this species.

Materials and methods

Three hundred healthy gilthead seabream (52±3.7 g) were equally randomized in 3 groups of tanks; Low and High groups received an experimental diet supplemented with PZQ 75 mg/kg and 150 mg/kg, respectively, while the intravenous group received intravenously (i.v) the PZQ dosing of 75 mg/kg fish dissolved in DMSO (0.5 mL/kg BW). During the experimental procedure, fish were hand-fed once a day, assuring that the feed was consumed. Water temperature was maintained at 21±1°C. Ten fish/group were anesthetized (MS-222, 150 ppm) and blood samples were collected at 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 h post-treatment. Plasma was separated from blood by centrifugation (3000 *g* for 10 min at 4°C). Fish were then killed by an overdose of anaesthetic and gill samples were also collected. PZQ levels were analyzed after extraction by an HPLC-UV chromatographic apparatus.

Results and Discussion

Plasma PZQ concentrations after oral and i.v administration are shown in Figure 1. As expected, the double dosing resulted in significantly higher PZQ plasma levels although only an approximately 22% increase in C_{max} (6.7 μg/mL for the low group vs 8.2 μg/mL for the high group) was observed. PZQ elimination from fish circulation following dietary administrations was found to be relatively sharp for both examined dosing regimens albeit distinguished profiles were evident. Particularly, the elimination half-life ($t_{1/2\beta}$) was shorter at high compared to the low PZQ dosing, revealing values of 14.4 and 25.7 h, respectively. The *F* of PZQ was calculated to be 49%, confirming a high absorption in fish circulation. In agreement with this, a high *F* of PZQ (51%) has also been reported in yellowtail kingfish (*S. lalandi*) (Tubbs & Tingle 2006).

Gill PZQ concentrations in gilthead seabream after two single oral doses are shown in Figure 2. The maximum concentrations of PZQ in fish fed 75 and 150 mg/kg were found 20.7 and 39.1 μg/g, respectively, 4 h post-feeding. Additionally, a dose-dependent effect in relative exposure to PZQ was observed. Furthermore, the $t_{1/2\beta}$ of PZQ in fish fed the low dose was found at 8.9 h while in the high-dose group, the corresponding value was calculated to be 11.7 h, indicating faster elimination for the low dosing. The aforementioned results clearly indicate that among examined tissues, distinct kinetic profiles of PZQ exist. Since a route of xenobiotics elimination may be via the gills (Hansen et al. 2001), the discrepancies in drug PKs between plasma and gills found herein could be attributed to this assumption.

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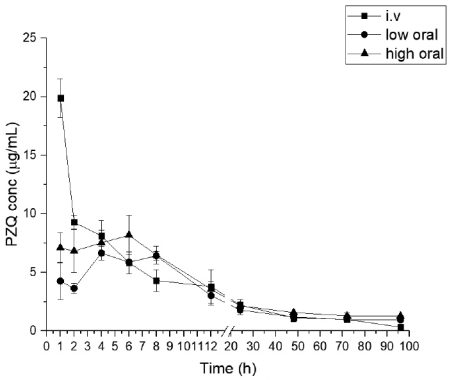

Figure 1. PZQ concentration (μg/mL) in gilthead seabream plasma after intravenous injection and two single oral doses (low=75 mg/kg, high=150 mg/kg). Values shown are mean±stdev (N=10).

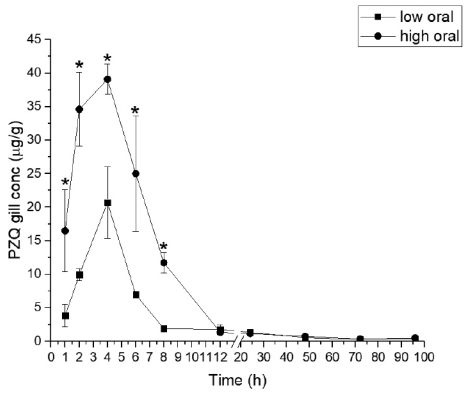

Figure 2. PZQ concentration (μg/g) in gilthead seabream gills after two single oral doses (low=75 mg/kg, high=150 mg/kg). Values shown are mean±stdev (N=5). ✻ indicates a statistically significant difference.

Conclusions

Based on the information obtained from the PZQ analysis in the gilthead seabream body compartment, the 150 mg/kg dosing regimen is superior to the 75 mg/kg, as confirmed by the significantly higher drug levels in all tested tissues. Since PZQ concentration trends to decrease sharply and the elimination time of the drug in plasma was shorter in fish that received the 150 mg/kg dosing regimen, the daily dosing should be divided into two medicated meals, to ensure adequate drug circulatory levels during treatments. The efficacy of the proposed medicated scheme remains to be evaluated in field trials against *S. chrysophrii*.

Acknowledgment

The project is co-funded by Greece and the European Union under the Fisheries and Maritime Operational Program 2014-2020 (75% EMFF contribution, 25% National Contribution). https://praziquantel.gr/

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ORGANIC MINERALS SUPPLEMENTATION AFFECT FILLET QUALITY OF FARMED GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Over the last decade, due to the continuous growth of the aquaculture sector, marine-origin ingredients, i.e. fish meal (FM) and fish oil (FO), have been partly substituted with sustainably produced plant raw materials. The aforementioned changes in fish feeds composition affect the micronutrients balance resulting into lower levels of bioavailable minerals in aquatic feeds compared to fish natural diet, thus inorganic mineral supplements are commonly used to meet fish requirements.

Gilthead sea bream (*Sparus aurata*) farming is of great importance to European finfish mariculture accounting for the 13% of its production value. This species is marketed mainly as whole fish and more recently as fillets. Unfortunately, gilthead seabream fillets suffer from gaping, a texture deterioration phenomenon which leads to economic loss, because of the rejection by consumers due to its unappealing appearance. Factors that have proven to be strongly associated with the fish propensity to gap, include the species, harvest or slaughter history, temperature during storage (Lavety et al., 1988; Sheehan et al., 1996; Robb et al., 2000) and diet (Kousoulaki et al., 2016).

Taking all the aforementioned into account, this study aimed to evaluate the impact of organic mineral feed inclusion on growth performance and fillet quality in terms of gaping occurrence, in gilthead seabream.

Materials and methods

Three isoproteic, isolipidic and isoenergetic diets were formulated: a control diet (Diet1) containing high FM (30%), a plant-based diet (Diet2) containing low FM (12.5%) and a plant-based diet supplemented with organic minerals (0.18%) (Diet3). These finishing diets were fed to triplicate groups of gilthead seabream (253g initial body weight) for a 92-day period. Water temperature maintained at 19°C in an open flow water system. Fish were fed by hand to apparent satiation with the three diets. At the end of the trial, fish were slaughtered by the commercial method (ice-killing), individually weighted, packed with ice (0°C), and shipped to a commercial fish processing unit. After mechanical scale removal by drum, fish were machine filleted, weighted, ice-packed and transferred (within two hours) to the Hellenic Centre for Marine Research (HCMR). Individual fillets were assessed for their gaping according to a recently published gaping scale for Mediterranean fish species (Kogiannou et al., 2022), pH, water holding capacity (WHC), total composition, fatty acid profile, chemical freshness (K value), and total and soluble collagen content. The SPSS version 26.0 was used for the statistical analysis. Growth, physical and chemical parameters are presented as mean±st.dev. and comparisons among means were made using one-way analysis of variance (ANOVA). The non-parametric χ^2 test was applied to find statistical differences in the frequency of gap occurrence between the diets. Differences were considered significant at the level of $P<0.05$.

Results

Fish growth parameters and feed intake were similar for all treatments, while organic minerals inclusion had beneficial effect on fish fillet quality. Specifically, as shown in Figure 1. distribution of gaping scores frequencies were found to be statistically different among diets. In both Diet1 and 2, increased high gaping scores incidence were observed while flesh integrity remained quite intact in fish received Diet3. Additionally, fish fillet liquid losses and pH were not different in gilthead seabream fed the organic minerals supplemented diet compared to Diet1 and 2. No statistically significant differences were found also in the fillet composition of the fish fed the experimental diets however, increased levels of n6 fatty acids were observed in both plant-based diets (Diet2 and 3). Finally, the total collagen content and collagen solubility in gilthead seabream muscle, calculated based on the hydroxyproline content, were found to be significantly affected by the diet composition and the organic mineral supplementation.

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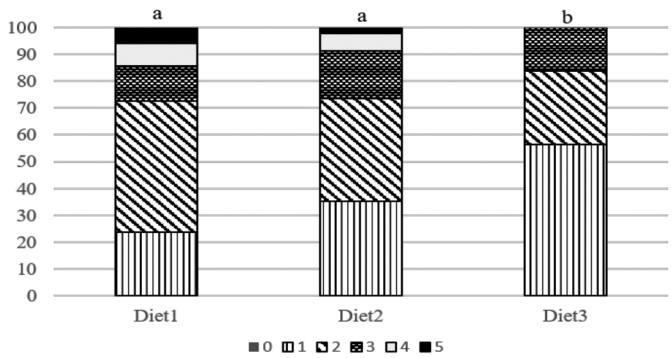

Figure 1. Distribution of gaping scores frequencies (%) of gilthead seabream fed the three experimental diets. Significance differences are indicated with different letters (P <0.05).

Conclusions

Dietary organic minerals included in low fishmeal diet for gilthead seabream significantly improved fillet quality in terms of achieving lower gaping incidence probably due to changes occurred in collagen content and solubility. The exact mechanism responsible for these observations remains to be identified.

Acknowledgement

The project is co-funded by Greece and the European Union under the Fisheries and Maritime Operational Program 2014-2020 (75% EMFF contribution, 25% National Contribution). Project title: "Improving of produced gilthead seabream and pagrus fillets by reduction of the gaping problem (PERFILLET)" (2019–2022).

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DIETARY NON-STARCH POLYSACCHARIDES AND ENZYME ADDITION AFFECT THE MICROBIOTA COMPOSITION AND METABOLITES IN THE GUT OF NILE TILAPIA

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Introduction

With the current aquafeed formulations including high levels of plant-based ingredients, there is a higher carbohydrate inclusion, including non-starch polysaccharides (NSP), which are less digestible by fish (Kokou and Fountoulaki, 2018). To improve the NSP digestibility and thus feed efficiency, exogenous enzymes of microbial origin such as xylanases can be added (Maas et al., 2021). When such enzymes are included in the feeds, they can assist in the degradation of the NSP into less polymerised compounds, making them more available for fermentation by microbiota, resulting in the production of volatile fatty acids. Understanding how the dietary NSP levels and the enzyme addition may affect microbial composition and metabolite production in the gut, and how this relates to nutrient digestibility can assist in optimization of dietary formulations with aim to enhance fish performance and digestibility, especially when raw materials high in NSP are used. In this study, we explored the impact of two different non-starch polysaccharide and ezyme levels on the microbial composition and microbial metabolites in the gut of Nile tilapia (*Oreochromis niloticus*). Nile tilapia has omnivorous dietary habits and the potential for fibre fermentation (Amirkolaie et al., 2005), thus making it an interesting species to understand non-starch polysaccharide effects on the gut microbiota.

Materials and Methods

A 2 x 2 factorial design was used to test the effect of enzymes and dietary NSP levels. The enzyme supplementation included phytase (1000 FTU/kg, Danisco Animal Nutrition) and xylanase (6000 U/kg, Danisco Animal Nutrition). The NSP levels were included at 122 g NSP/kg DM diet (moderate) and at 311 g NSP/kg DM diet (high). The feeding trial lasted 6 weeks, with four replicate tanks per diet. At the end of the trial, digesta samples were analyzed for microbiota composition, volatile fatty acid and NSP content. For the microbiota analysis, DNA was extracted with commercial kits and the V4 variable region of the 16S rRNA gene was sequenced, using Illumina MiSeq. Taxonomic composition was analyzed according to Kokou et al., 2020. For diversity analysis, the '*microeco'* R package was used. Volatile fatty acid concentrations were measured as described in Maas et al. (2021). The NSP content were measured as described by Blakeney et al. (1983). Network analysis using *sparcc* as correlation method was performed to explore associations between the different microbial taxa, including the environmental variables (nutrient digestibility, NSP content, VFA) as explanatory variables (Kurtz et al., 2015).

Results

The results showed that both NSP level and enzyme inclusion can affect the gut microbiota composition. An increased microbial richness and diversity was observed with high NSP or no enzyme addition compared to the enzyme addition in lower NSP levels. Such changes were attributes to a change of the Fusobacteria (Figure 1A), and specifically an increase in the abundance of *Cetobacterium*, in the gut of Nile tilapia, fed with the MEDENZ diets. This was in accordance with an increase in VFA content in the gut, which was higher in the specific diet (Figure 1B). The digesta VFA content and the nutrient digestibility could explain the variation in the microbiota composition, as indicated by redundancy analysis. Network analysis indicated that NSP levels and enzyme addition may interfere with microbial associations, by altering the clustering co-efficiency. Our previous study showed that enzyme addition to high NSP diets may enhance the microbial associations in the distal gut of Nile tilapia, by increasing the network complexity (clustering co-efficiency and density), which was hypothesized to benefit the gut microbiota homeostasis (Maas et al., 2021).

Conclusion

These findings suggest that both NSP level and enzyme addition can have a significant effect on the gut microbiota diversity and composition, with the latter being the major factor in this study. Volatile fatty acid content and composition were consistently altered by the enzyme addition in the diets. The interpretation of such results is important in order to better understand the microbial dynamics in the gut and how such dynamics may be altered by dietary formulations such as the presence of NSP or by enzyme addition, ensuring an optimal fish performance.

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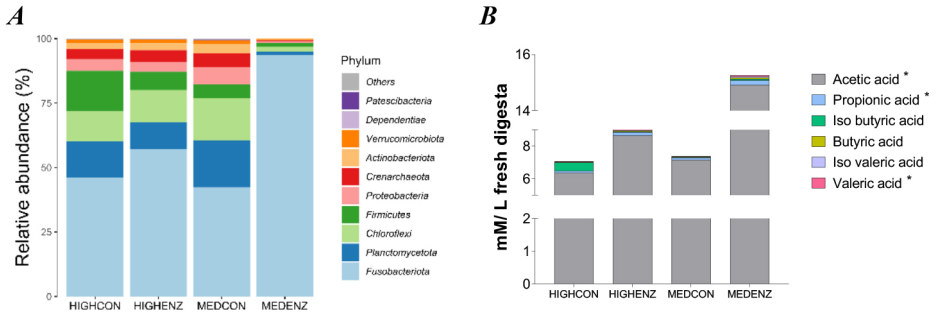

Figure 1. *A. Phylum composition in the distal gut digesta. B. Volatile fatty acid (VFA) composition in the distal gut digesta. Asterisk indicate significant differences between the groups (P<0.05). Two-way anova indicated a significant effect of enzyme addition in the total VFA content and specifically of acetic, proprionic and valeric acid (indicated with *).*

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ARE THE EFFECTS OF DEOXYNIVALENOL (DON) ON PERFORMANCE, LIVER AND GASTROINTESTINAL TRACT HEALTH OF RAINBOW TROUT (*Oncorhynchus mykiss*) INFLUENCED BY DIETARY COMPOSITION?

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Introduction

Ever since mycotoxins have been described as emerging feed contaminants for European aquaculture, it has become evident that deoxynivalenol (DON) is the most prevalent mycotoxin in aquafeeds (Koletsi et al., 2021). Different fish species display different sensitivities to DON. Compared to other fish species, rainbow trout (*Oncorhynchus mykiss*) is very sensitive to DON (Hooft et al., 2011; Koletsi et al., 2021). Experimentally, studies on the effects of DON have generally targeted the concentration of the mycotoxin instead of focusing on the composition of the diet. Studies with DON in carnivorous fish have hitherto been performed against a background of optimal quality marine-based diets. Yet, as argued above, ingredient composition of aquafeeds has diversified rapidly to include more plant-based ingredients. This not only introduces the risk of feeding mycotoxin contaminated ingredients but also introduces a risk that the negative effects of DON can be amplified by sub-optimal diets. Information on the interaction between DON and diet composition is generally lacking. Therefore, this study investigated if dietary composition influences the effects of DON on the health and performance of rainbow trout (*Oncorhynchus mykiss*).

Material and methods

Four experimental diets (2x2 factorial design) were formulated which differed in 1) the type of protein source; fishmeal (FM) versus soybean meal-based (SBM) and 2) the DON content of wheat; clean versus naturally contaminated wheat. Triplicate groups of n=30 fish were assigned to each diet: (1) CON-FM; DON= 0 µg/kg feed; (2) DON-FM; DON=1200 µg/kg feed; (3) CON-SBM; DON= 46 µg/kg feed; (4) DON-SBM; DON= 1300 µg/kg feed. The 8 week experiment was divided into two feeding periods: after 6 weeks of restrictive feeding, fish were fed *ad libitum* **for 2 weeks**. **Influences on performance were evaluated by determining growth,** protein and energy gain metrics, and on health parameters through the determination of histopathological changes in the liver and gastrointestinal tract (GIT).

Results

Restrictive feeding showed negative effects of DON and dietary composition on performance but did not show an interaction between DON and diet composition. Similarly, subsequent *ad libitum* feeding showed effects of DON and/or diet composition on growth, feed efficiency and body biometrics, but no interaction effects. These data confirmed the challenging nature of the SBM-based diet and confirm previously noted negative effects of DON on performance. Neither DON, nor diet composition indicated significant effects on liver health, nor was there an interaction effect. We discuss that the combination of DON and a sub-optimal diet based on SBM could accentuate the effects of DON, in particular concerning GIT functioning. Indeed, the histopathological assessment of mucosal fold width, enterocyte width and goblet cell density indicated significant interaction effects between DON and diet composition in the midgut. Yet, the differences were generally small and interaction effects were restricted to the midgut. Overall, this study suggests that DON is harmful to rainbow trout regardless of diet quality.

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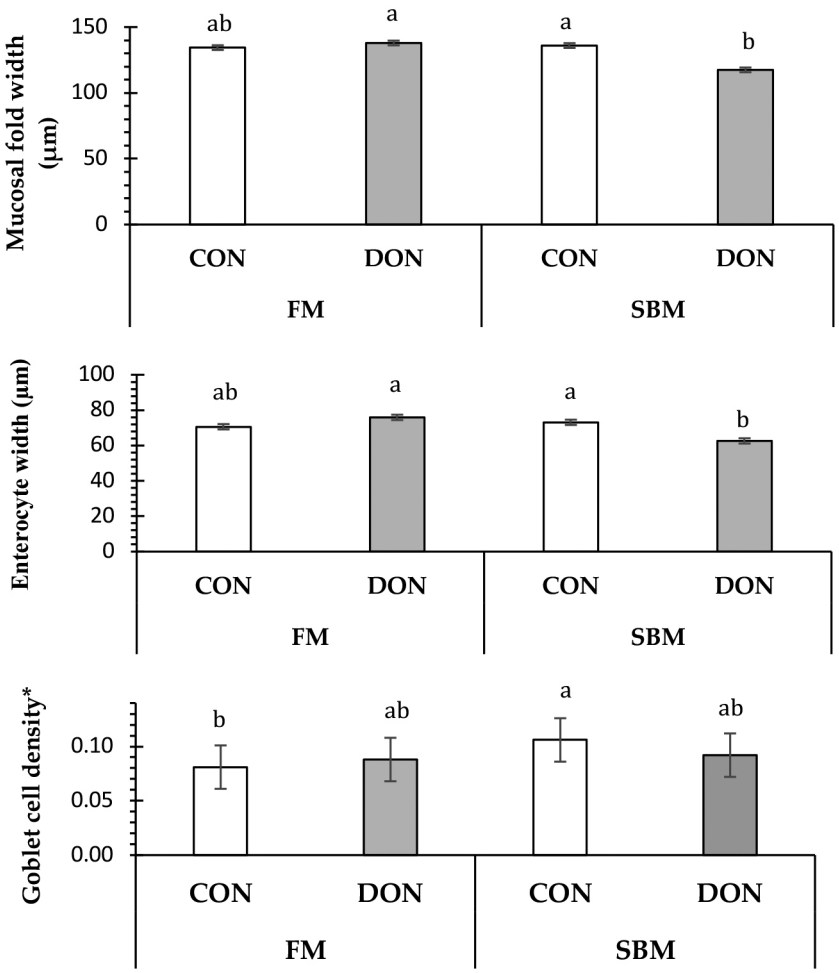

Figure 1. Interaction effects between dietary DON (CON versus DON) and diet quality (FM versus SBM) on mucosal fold width ($p \leq 0.05$), enterocyte width ($p \leq 0.05$) and goblet cell density ($p \leq 0.05$) in the midgut of rainbow trout fed the experimental diets: CON-FM, DON-FM, CONSBM and DON-SBM restrictively for 6 days (week 1) and 40 days (week 6) and ad libitum exposure for 15 days (week 8). *Goblet cell density was calculated as the number of cells per µm fold height. Error bars indicate standard error of means. Treatments lacking a common letter are statistically different ($p \leq 0.05$) according to Tukey's multiple comparison test

COMPARISON OF METHODS FOR ASSESSING FIN CONDITIONS

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Introduction

Fin condition is one of the most frequently used welfare parameter for farmed fish. Several methods for actually evaluating and calculating fin conditions have been used in the past like the Kindschi index which correlate the longest fin ray vs. the body length of a fish. For this study a new method is presented which uses the calculation of the area index (AI) and relating this to the total body length or the standard body length (improved AI). In order to compare the suitability of the methods mentioned below, the present study thus investigated the fish indices based on different methods for two different species - rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio L.*), aiming at recommending the most useful fin condition assessment method for fish farming.

Material and methods

Data from 4 different fish farms in Switzerland (2x trout, 2x carp) have been collected for more than 19 months. At each sampling up to 15 fish have been sampled belonging to maximum three size classes (<100g, 100-250g, >250g). Different Fin lengths and area were determined in Adobe Photoshop CS. For the estimation of the fin condition a visual classification of the fin appearance by using 5 different categories was done. The following parameters have been calculated from the fin measurements and were compared to the visual estimation of the fin condition:

· longest fin ray vs. total / standard body length (Kindschi 1987, Ellis et al. 2009)
· fin basis (wide) vs. fin length
· longest fin ray vs. length of caudal fin (Good et al. 2011)
· fin area vs. total / standard body length

Results and discussion

The two trout farms show the same pattern of correlations of the fin indices with the visual fin scoring. Moreover, the correlations can be strong. This clearly shows that fin index assessment is a reliable parameter for this fish species, and especially the area-based calculation and Kindschi methods result in highly significant correlations with the visual fin scoring method. With the Kindschi method, care must be taken that even with very badly damaged fins the longest fin ray may be intact. This could falsify the result, especially in the case of fin damage from aggressive encounters between fishes.

THE EFFECT OF INCREASING CONCENTRATIONS OF A NOVEL ULTRA-HIGH PROTEIN SOYA AND REGULAR HIGH PROTEIN SOYA MEAL ON THE PERFORMANCE OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction:
Soybean meal is widely used in aquafeeds and, due to the favourable amino acid profile and competitive pricing, usually one of the main ingredients of fish feeds. However, high inclusion rates of soybean meal have been reported to have adverse effect on fish performance and to result in soy-induced enteritis (Knudsen et al., 2008). This has been attributed to different antinutritional factors present in soybeans, such as saponins and oligosaccharides (Krogdahl et al., 2010). Whereas to date most of the work studying the impact of soybean meal on fish performance has been carried out in Atlantic salmon, there is indication that high inclusion rates of regular soybean meal may also affect the performance and gut histology of other species, such as the gilthead seabream (*Sparus aurata*, Perera and Yúfera, 2017). Novel soy-products, containing very high amounts of protein (UHP-soya) and low amounts of oligosaccharide (Roe et al., 2019), could, therefore, offer significant advantages over the use of regular soybean meal when formulating feeds for Mediterranean species such as the gilthead sea bream. The present study sets out to compare the effect of increasing concentrations of regular soybean meal and UHP-soya on the growth and performance of gilthead seabream. By gradually replacing the protein fraction of a soy-free reference diet with regular high-protein soya (Hipro-soya) and UHP-soya, the present trial determines the effect of increasing concentrations of soy and different soy-products on the performance of gilthead seabream.

Materials and Methods:
The five experimental diets were formulated be iso-proteic and iso-energetic and to average a crude protein content of 48% and a level of digestible energy of 18 MJ/kg. The reference diet contained 15% of fish meal and corn gluten, wheat gluten, feather meal and poultry meal as main protein sources. With the exception of fish meal, which was kept constantly at a level of 15%, other raw materials were replaced by two substitution rates of either UHP-soya and Hipro-soya. The relative contribution of protein by both soybean meal was kept constant, resulting in total substitution rates of 15 and 30% for diets containing regular soybean meal and 13.1 and 26.3% for diets containing UHP-soya. This design should allow to assess the true protein value of both soybean meal in an experimental setup using a raw material basket representative for current commercial formulations for Mediterranean species.

The experiment was carried out at the Laboratory of Aquaculture, Department of Veterinary Medical Sciences of the University of Bologna, Cesenatico, Italy. Gilthead seabream juveniles were obtained from an Italian hatchery. At the beginning of the trial, 50 fish (initial average weight approx 40 g) per tank were randomly distributed into 18 square tanks with a capacity of 800 L. Each diet was administered to triplicate groups, with random assignment. Tanks were provided with natural seawater and connected to a closed recirculation system (overall water volume: 20 m^3). The rearing system consisted of a mechanical sand filter (PTK 1200, Astralpool, Barcelona, Spain), ultraviolet lights (PE 25 mJ/cm^2: $32m^3$ h^1, Blaufish, Barcelona, Spain) and a biofilter (PTK 1200, Astralpool, Barcelona, Spain). The water exchange rate was 100% every hour, while the overall water renewal amount in the system was 5% daily. During the trial, the temperature was kept at 24 ± 0.5 ◦C and the photoperiod was maintained at 12 h light and 12 h dark through artificial light. The oxygen level was kept constant (8.0 ± 1.0 mg L^{-1}) by means of a liquid oxygen system regulated by a software programme (B&G Sinergia snc, Chioggia, Italy). Each day, ammonia (total ammonia nitrogen ≤0.1 mg L^{-1}) and nitrite (≤ 0.2 mg L^{-1}) were monitored by spectrophotometer (Spectroquant Nova 60, Merck, Lab business, Darmstadt, Germany) and salinity (25 g L^{-1}) was measured by a refractometer (106 ATC, Giorgio Bormac S.r.l., Carpi, Italy). Sodium bicarbonate was added if needed to keep pH constant at 7.8–8.0. Animals were fed to satiation with automatic feeders, twice a day, six days a week, set to release pellets gradually for one and a half hours. The uneaten pellets of each tank were collected, dried overnight at 105 ◦C, and weighed for feed intake (FI) calculation.

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At the beginning and at the end of the experiment, all animals in each tank were anaesthetised by MS222 at 100 mg L^{-1} and weighed. Specific growth rate (SGR) and feed conversion rate (FCR) were calculated. Proximate composition of the carcasses was determined on a pooled sample of 10 fish per tank at the beginning and on a pooled sample of 5 fish per tank at the end of the trial.

Results:

The present study performed a detailed analysis on performance of gilthead seabream in response to increasing concentrations of UHP- and Hipro-soya. Growth performance, feed efficiency, carcass composition as well as gut histological parameters were affected by the treatments. The results of the study show a differential response of *Sparus aurata* to increasing concentrations of UHP-soya and Hipro-soybean meal.

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PROTEIN VALUE AND AMINO ACID DIGESTIBILTY OF VARIOUS RAW MATERIALS FOR FISH DIETS AS EVALUATED SEPARATELY IN TWO IN VITRO AND TWO IN VIVO MODEL SYSTEMS

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Introduction

Variation in nutrient digestibility is among the most critical points of variation in feed quality and constitutes a key parameter when evaluating new feed raw materials for fish. Nutrient digestibility is traditionally assessed *in vivo* by animal feeding trials, but recent EU obligations to put the 3Rs concept into practice have facilitated research initiatives to develop complementary *in vitro* methods. One of the current project initiatives addressing this topic is the H2020-FETOPEN project Fish-AI, which aims to develop an *in vitro* screening platform based on rainbow trout (*Oncorhynchus mykiss*) digestive enzymes and intestinal cells to evaluate nutritional and health values of novel aquafeeds. As part of this project, we have developed a static *in vitro* digestion (IVD) model for simulating the *in vivo* feed digestion in rainbow trout. In the present study, the performance of the Fish-AI IVD model was assessed by comparisons with two independent *in vivo* feeding trials using juvenile rainbow trout and mink (*Neovison vison*) as experimental animals. In addition, the Fish-AI model was compared against the standardised human *in vitro* model INFOGEST[1].

Materials and methods

A total of seven diets based on commercially relevant protein raw materials with assumed contrasting effects on protein digestibility were formulated. Six test feeds contained the challenging raw materials guar meal and soybean meal at relatively high inclusion levels, feather meal at low and high inclusions, and the combinations guar/feather and soybean/feather. A diet high in fish meal was included as a reference. The study in rainbow trout comprised a 5-week feeding trial using fish with an average start weight of 55 grams. Fish were fed the seven experimental feeds in duplicate tanks each. Fecal collection was conducted by stripping. The mink trial was carried out using adult male mink with a mean body weight of 2.8 kg. Four healthy mink were assigned for each of the seven experimental diets. The animals were kept in individual cages equipped for controlled feeding and quantitative faecal collection. The experiment lasted for 14 days. Feed and faeces samples were analyzed by established procedures[2]. The Fish-AI IVD procedure was based on the static INFOGEST model with several modifications, including the use of enzyme extracts and bile obtained from rainbow trout, whereas the INFOGEST protocol was performed as described elsewhere[1]. The IVD products from both *in vitro* procedures were analyzed using size exclusion chromatography (SEC) and quantification of soluble nitrogen with Dumas for estimation of the proportion of small soluble peptides potentially available for uptake[3]. Difference among diet groups was analyzed for statistical significance using a one-way ANOVA followed by Fischer's LSD test. The level of significance was set to $p<0.05$.

Results and discussion

Figure 1 illustrates the results regarding apparent protein digestibility for the seven experimental diets as tested in the four separate model systems. Significant effects of diet were evident. Rainbow trout fed either the control, guar-high, or soya-high diet generally showed high (>95%) protein digestibilities, whereas inclusion of feather meal in the diet, singly or in combination with guar or soya, clearly reduced protein digestibility. The results obtained with mink were in overall accordance with the results obtained from the study in rainbow trout and strengthen the observation that the biological value of the feather meal protein was low. The results from the two *in vitro* model systems displayed relatively similar protein digestibility profiles among the experimental diets as the two *in vivo* models, thus demonstrating that the low protein value of the diets containing feather meal also could be detected *in vitro*. The absolute values of protein digestibility were higher for the INFOGEST than for the Fish-AI IVD procedure (70-86 vs. 43-62%) and indicate that there is still room for improving the Fish-AI IVD efficiency. These studies are currently ongoing and will be presented and discussed.

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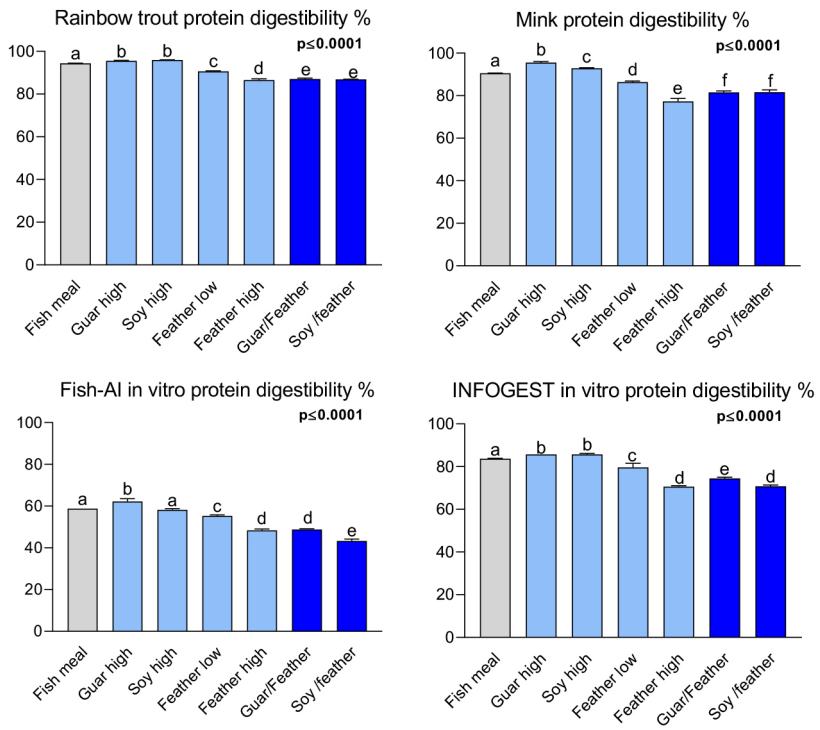

Figure 1. Estimated protein digestibility of the 7 experimental diets. Data from rainbow trout and mink are apparent digestibility coefficients of the sum of individual amino acids. In vitro protein digestibilities are calculated as % of small soluble peptides from SEC and Dumas analyses.

Acknowledgements

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EVALUATION OF TOXICITY OF PRAZIQUANTEL AGAINST FISH CELL LINE AND NON-TARGET MARINE ORGANISMS

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Introduction

Praziquantel (PZQ) is a synthetic broad-spectrum anthelmintic, widely used in veterinary and human helminthiasis. In farmed fish, PZQ has been demonstrated to be highly effective against a range of internal and external parasites. Thus, PZQ constitutes a good candidate substance based on its proven efficacy against flatworms such as *Sparicotyle chrysophrii*, a microcotylid blood-sucking monogenean, which is currently the most serious pathogen for gilthead sea bream (Sitjà-Bobadilla et al., 2010, Rigos et al., 2021). PZQ is generally considered safe in treated animals (Norbury et al., 2022). Little work has been done examining the effects of PZQ in the surrounding marine environment, albeit assessment of the effects on non-target organisms is one of the most important components of environmental risk assessment. Therefore, the objective of this study is the evaluation of the toxicity of PZQ on cell culture and selected non-target marine organisms.

Materials and methods

Amphibalanus amphitrite toxicity assay: The barnacle-rearing method was carried out according to Kotsiri et al. (2018). Assays were conducted by adding 10 nauplii into individual wells of a 24-well plate with 2 ml of artificial seawater (25‰) and various concentrations of PZQ. Nauplii have incubated for 48 h in the presence of PZQ at 25°C and the plates were examined after 24 and 48 h. Each animal was inspected under a stereomicroscope and its condition was recorded. The LC_{50} was determined as the concentration of PZQ that resulted in 50% mortality of the nauplii.

Marine bacteria growth assay: The ability of PZQ to inhibit the growth of the bacteria *Vibrio. alginolyticus* was assessed by the disk diffusion test. Sterile filter paper discs (4 mm) were loaded with samples of serial dilutions of PZQ, allowed to dry at room temperature and then were placed on agar plates, which were seeded with the strain of bacteria. Plates were incubated for 24 h at 35°C. Marine bacteria growth inhibition was determined by measuring the zone of inhibition in mm around the filter paper disc. As a positive and negative control, standard discs were loaded with 0.5 M penicillin G or solvent, respectively.

Cytotoxicity assays: The cellular toxicity of PZQ was evaluated in epithelial cell line cultures, RTgill-W1. The cytotoxicity of PZQ was evaluated in 96 well plate cultures applying the neutral red (NR) assay to assess the ability of viable cells to incorporate and bind the supravital dye neutral red in the lysosomes. In a flat bottom plate, 5×10^4 cells ml^{-1} were placed and incubated at 19°C for 24 h until adherence. Next, the medium was changed and PZQ was added to the culture in different concentrations. After 24 h, NR medium was added, the plate was incubated, washed, the dye was extracted and the absorbance was measured at 540 nm.

Statistical analysis: For statistical comparisons between groups, one-way ANOVA followed by Dunnett's multiple comparisons test at $P<0.05$ was used where appropriate using GraphPad Prism v.9. For LC_{50} all data were fitted to a three-parameter logistic curve according to the following model: $(Y=Bottom + (Top - Bottom)/(1+10^{((X - LogIC_{50}))})$.

Results & Discussion

Based on ecotoxicity tests against *A. amphitrite* (24 h: LC_{50}= 47.5 mg/L), also confirmed by marine bacteria growth assays, PZQ induced no effect on the growth of the tested bacterial strain, the toxicity of PZQ was found to be low (Fig. 1a, c). This conclusion is in agreement with previous findings in acute and long-term experiments (Cioli et al., 2003; Norbury et al., 2022). Also, the results indicated that very low and low concentrations (≤ 1 mg/L) of PZQ induced no effect on RTgill-W1 cells viability after 24 h of exposure, while medium and high concentrations of PZQ exerted cytotoxic effects (Fig. 1b).

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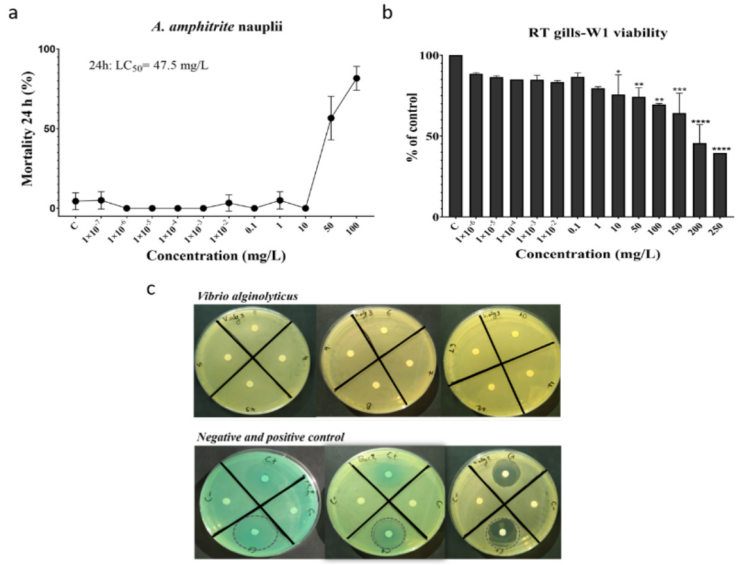

Fig.1: a. Results of *A. amphitrite* toxicity assays. Values are expressed as the percentage of animals that did not react to light mechanical stimuli from the total number of animals placed in a well of a 24-well tissue culture plate. The cumulative results from 2 independent experiments with 3 replicates each is shown **b.** Results of NR assays on RTgill-W1 cells. Results are expressed as percentage (mean ± SD) of PZQ-treated cells compared to untreated cells. * indicates a statistically significant difference. The cumulative results from 5 independent experiments with 3 replicates each are shown **c.** Results of marine bacteria growth assay in the presence of PZQ. TSA agar plates were split into four sections (each corresponding to a serial dilution (1×10^{-7} to 1×10^{3} mg/L). Indicative plates from the experiment with 3 replicates each are shown.

While the environmental concentrations are likely dependent upon dose and stocking density, there is no evidence that high concentrations of PZQ are being discharged into the environment after a treatment regimen. Data from a previous study have shown that PZQ was detected in very small concentrations (0.003 mg/L) in water during PZQ administration and was not detected in the sediment (Ido et al., 2019).

In agreement with existing limited knowledge, this trial suggests that PZQ use in aquaculture is relatively safe for the treated animal and the environment. Nevertheless, there is a need to investigate the fate of this anthelminthic in aquaculture systems in order to more accurately assess concerns about ecological impacts.

Acknowledgements
The project is co-funded by Greece and the European Union under the Fisheries and Maritime Operational Program 2014-2020 (75% EMFF contribution, 25% National Contribution). website: https://praziquantel.gr/

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EVALUATION OF FERMENTED SOYBEAN MEAL AS A SUSTAINABLE REPLACER OF FISHMEAL IN THE DIETS OF EUROPEAN SEA BASS JUVENILES (*Dicentrarchus labrax*): EFFECTS ON GROWTH PERFORMANCE

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Introduction

Aquaculture production relies heavily on the availability of sustainable and viable compound feeds. For many decades fishmeal has been considered a superior ingredient and the primary protein source of choice for the aquafeed industry due to its desirable nutritional characteristics. However, due to the increasingly limited supply of fishmeal, sustainable alternatives need to be thoroughly evaluated for the production of aquafeeds. Fermented soybean meal (FSBM) has gained much attention over the past decades due to its improved nutritional value, lower ANFs content, increased digestibility, and health benefits (Kumar et al., 2020; Kari et al. 2022). Thus, the aim of this study was to investigate the potential of FSBM to replace fishmeal as measured by growth performance, feed utilization, and health of European seabass (*Dicentrarchus labrax*).

Materials and Methods

A 12-week feeding trial was conducted to compare different inclusions of fermented soybean meal (FSBM, SOYAMAX BIO PLUS, INOLASA, COSTA RICA) as fishmeal (FM) replacements in the diets of European sea bass juveniles (*Dicentrarchus labrax*) in terms of growth performance. Six isonitrogenous (46.5% crude protein) and isolipidic (16% crude lipid) diets were formulated: a fish meal-based diet containing 30% FM which was served as a control diet and five experimental diets replacing FM by 15, 30, 50, 70, and 80% with FSBM. The formulation strategy aimed to replace the 30% FM (67% protein content) with the FSBM (48.5% protein content) while keeping concurrently similar nutrient levels. A reasonable amount of wheat flour was added to the control diet which was reduced gradually in the rest of the diets. Simultaneously, the blood meal and corn gluten dietary inclusion levels were slightly increased to compensate for the lower crude protein level in the FSBM relative to the FM. SPC levels were kept to a minimum in all diets. The corresponding replacement of FM protein with FSBM protein in the different diets was 11, 21, 36, 50, and 57%, respectively. All diets (3.5 mm pellets) were produced by cooking extrusion.

European sea bass juveniles were obtained from a commercial fish farm located in Agios Serafim (Phthiotis region) owned by PHILOSOFISH SA and transferred to the Hellenic Center for Marine Research (HCMR) facility located in the campus of Agricultural University of Athens, Greece. The fish were distributed among 18 cylindroconical experimental tanks of 1,000L, 35 fish per tank, with 3 replicates for each feed. The average sea bass initial weight among the tanks was 30.48 ± 0.14g (SD). Fish were hand-fed to apparent satiation, twice per day, and uneaten dry feed that was collected in each tank's waste collection system was monitored and taken into account before calculating the daily feed consumption. Similarly, feces were also collected and stored at -20 ^{0}C pending digestibility analysis. The average seawater temperature was 20.2 ± 5.6 ^{o}C.

Results

Our findings revealed that sea bass consuming the FSBM 15% and FSBM 30% diets exhibited the highest overall growth performance and better feed utilization, showing a numerically higher average final body weight (75.11 g and 73,24 g, respectively) compared to the other groups (71.92 g, 70.96 g and 69.92 for Control, FSBM 50%, and FSBM 80%, respectively). However, those differences were not statistically significant ($P>0.05$). The final body weight on the FSBM 70% (67.8 g) was significantly lower compared to the FSBM 15% diet ($P<0.05$). However, no significant differences were found compared to other dietary groups. The same trend was observed in weight gain (WG), thermal growth coefficient (TGC), daily growth index (DGI), and protein efficiency ratio (PER) for FSBM 15% and FSBM 30% in comparison to the fish fed the other diets. Furthermore, the replacement of FM by FSBM did not affect the palatability of the diets since no significant differences were recorded in feed intake among the dietary treatments ($P>0.05$). Feed conversion (FCR) was also similar among the diets ($P>0.05$) except for the observed significant lower value for fish on the FSBM 70%. The replacement of FM by FSBM had no effect on the somatometric indices in either of these dietary treatments.

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Overall, this study demonstrated that the tested FSBM allowed at least a replacement of up to 50 % of FM in the diets of European sea bass without impairing growth performance and diet utilization, provided fish nutrient requirements are met. The results clearly demonstrate the high nutritional value of FSBM for European sea bass. Depending on the potential economic considerations, higher replacements of FM by FSBM (70-80%) are possible and may be considered. Additional analyses should demonstrate the physiological basis for the potential of FSBM to replace FM.

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EFFECT OF LARVAL REARING PRACTICES ON THE DEVELOPMENT OF SKELETAL DEFORMITIES IN ZEBRAFISH *Danio rerio* (HAMILTON 1822)

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Introduction

Skeletal abnormalities develop frequently in aquaculture and laboratory fish. They consist of either post-metamorphic deviations of initially normally developed bones (e.g., Printzi et al., 2022), or deviations from the species developmental pattern (e.g., Kourkouta et al., 2022; Printzi et al., 2021, 2023). A variety of biotic and abiotic factors, including feeding practices and nutrition, has been shown as critical for fish skeletal development (Boglione et al., 2013; Printzi et al., 2021, 2023). In addition to serving as an important quality index, skeletal abnormalities are also considered a valuable welfare indicator that reflects how well fish husbandry conditions align with fish preferences (Printzi et al., 2021). In the present study we examined whether different feeding practices during the larval stage affect the development of skeletal deformities in zebrafish.

Materials and Methods

Larval rearing was performed under five different feeding regimes, following the results of Printzi et al. (2021), using Artemia nauplii and dry microdiet. The control group (A) was based on a long period of co-feeding, while the rest of the regimes included less frequent meals (group B), abrupt shift from live to inert feed (group C), application of two-days fasting period (group D), or exclusive feeding on dry feed (group E) (Fig. 1).
Embryos were obtained from a common broodstock (AB strain), under standard for the species conditions. Random samples of individuals were double stained with Alizarin Red and Alcian Blue for the skeletal analysis at the end of the trials. Experiments were performed in three independent replicates.

Results and Discussion

Detected abnormalities consisted mainly of irregularly ossified vertebral centra (Fig. 2a, 2b), hemirays separation (Fig. 2c), bending of the neural processes (Fig. 2d) and the branchiostegal rays. Less frequent abnormalities included fin deformities, pre-haemal kyphosis, and caudal-peduncle scoliosis.

Feeding practices during the larval period had a significant effect on the rates of skeletal abnormalities ($p<0.05$, G-test). Compared with the control group (A), groups B, C and E presented significantly higher abnormality rates (Fig. 2e). In agreement with the results of Printzi et al. (2021), decreased usage of Artemia nauplii (groups C and E) resulted in a general increase of the abnormality rates (Fig. 2e). The decrease in the number of daily feedings (group B) resulted in comparatively increased rates of bended neural processes, and to a less extent of vertebral ossification irregularities and branchiostegal deformities. Finally, the effects of fasting (group D) were limited to a significant elevation of the rate of abnormal hemirays (Fig. 2e).

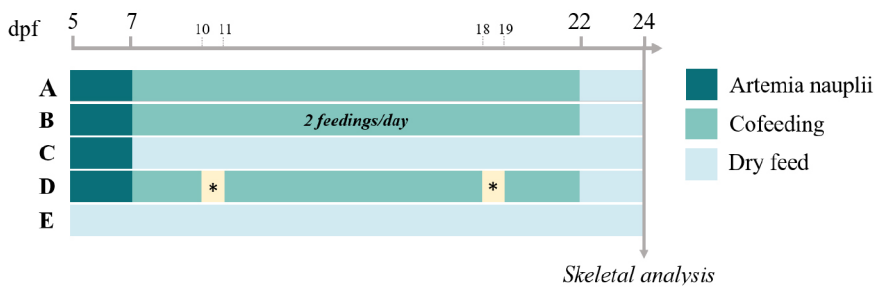

Fig. 1. Applied rearing practices. A, control group with both *Artemia* and dry feed; B, as in control group, but with fewer daily feedings (2); C, abrupt transition from *Artemia* to dry microdiet; D, as in control group, but with two fasting periods (*) of two days each; E, exclusive provision of dry feed. In all except B groups, feed was provided to the larvae five times daily.

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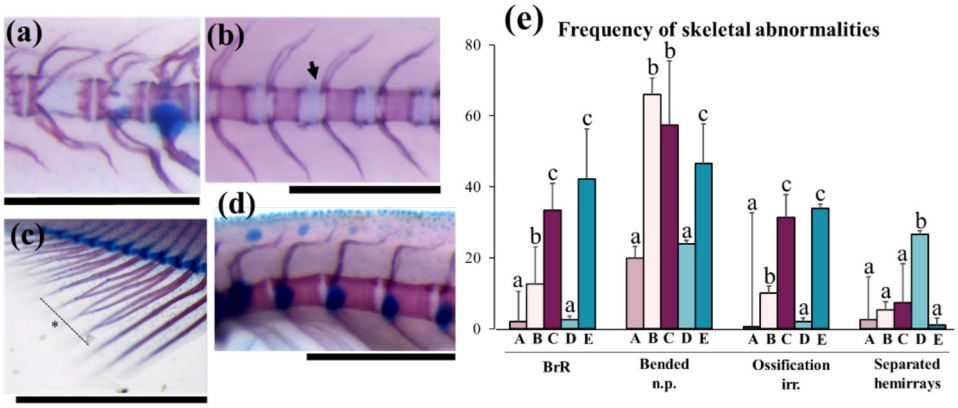

Fig. 2. Representative cases (A-D) and frequency of the main abnormality types in the different experimental groups (E). A, B, irregularities of vertebral ossification; C, hemirays separation; D, bended neural processes. Scale bars equal to 0.5 mm.

Our results demonstrate elevated rates of deformities at the conditions with a relatively higher use of dry feed. Although more research is needed to support these findings in respect to the causative factors, gene expression analysis of specific genes related to skeletogenesis, could provide some basic insights. An interesting approach would be to test a possibly altered osteoblast-osteoclast balance as a potential cause of osteoporotic conditions (Bergen et al., 2019).

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PHENOTYPIC ALTERATIONS OF JAW ABNORMALITIES IN GILTHEAD SEABREAM DURING THE ON-GROWING PERIOD

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Introduction

The aquaculture industry is significantly concerned about skeletal abnormalities, which mainly occur during the larval and early juvenile stages due to various factors (Boglione et al., 2013). To minimize their negative impacts on fish quality and welfare, these abnormalities are culled from the reared populations before the on-growing period. Recent studies suggest that some types of abnormalities, such as haemal lordosis, have a high recovery potential during fish growth in net pens (Fragkoulis et al., 2019; 2022). This raises questions about the early cull off practice's appropriateness. In this study, we examined the recovery potential of jaw abnormalities (two types of pugheadness and the shortening of the lower jaw) in Gilthead seabream.

Material and Methods

At 162 dph (ca 14.7±3.7 g W) 158 fish with short upper jaw, 94 fish with short lower jaw and 275 normal fish were anaesthetized, weighted, individually photographed and pit-tagged (FDX-B, Trovan Ltd., USA). They were then transferred to an experimental net-pen for on-growing. At 514 dph (ca 351±56 g) all fish were anesthetized, photographed, and tag-identified for further morphological examination and geometric morphometric analysis (as described in Fragkoulis et al., 2019). All fish originated from a common larval population that was reared under standard conditions.

Results & Discussion

Short-upper jaw abnormalities were categorized as type-I (Par) or type-II (Kub) based on the absence or presence, respectively, of gross malformations on the premaxillary and maxillary bones (Fig. 1). At the end of the trial, 40% of the initially pit-tagged fish were not found due to mortality or tag loss. The group with the short lower jaw presented a significantly higher rate of tag non-recovery than the normal fish ($p<0.05$, G-test) (Fig. 1).

Given that the tag was always implanted in the same area, differences in the frequency of unidentified fish should be ascribed to differences in the mortality rates between LoJ and the rest phenotypic groups.

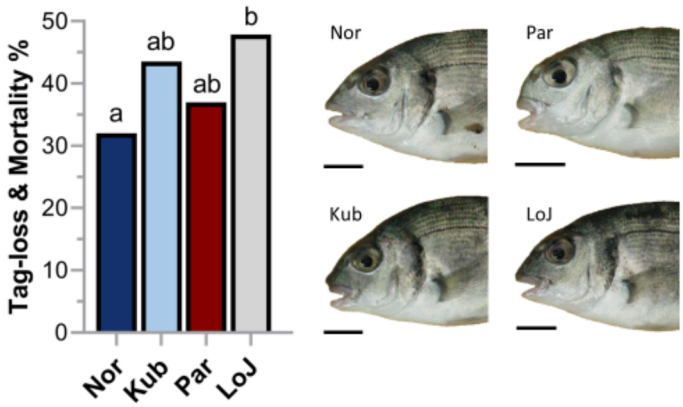

Fig. 1. Tag-loss and mortality rates of the different phenotypic categories during the on-growing period. Frequencies marked with different letters are significantly different ($p<0.05$, G-test). Nor, normal. Kub, type II upper jaw abnormality. Par, type I upper jaw abnormality. LoJ, short lower jaw.

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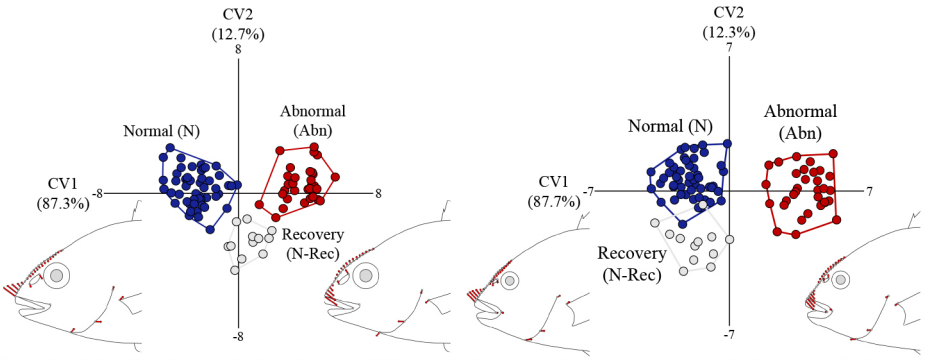

Fig. 2. Distribution of the three different phenotypic categories of the Par deformity (normal, recovered, abnormal) along the first two axes of canonical variate analysis (CV1, CV2) at 162 (left) and 514 (right) dph. The percentage of shape variance that each canonical axis explains is shown in brackets. Spline diagrams demonstrate the components of shape changes that are relative to the extreme values of CV1 axis.

Following fish morphological examination, 30% (14 out of 46) of the initially Par fish presented a normal phenotype by the end of the on-growing period (514 dph). In contrary, no recovery from LoJ or Kub abnormality was found. The partial recovery from Par abnormality was furthermore supported by the results of geometric morphometric analysis, with the recovered individuals presenting no significant differences in skull shape from the normal fish ($p>0.05$, Canonical Variate Analysis, Fig. 2). Together with the haemal lordosis (Fragkoulis et al., 2019; 2022), vertebral fusions (Witten et al., 2006) and light gill-cover abnormalities (Beraldo & Canavese, 2011; Amoroso et al., 2016), this is the fourth abnormality type that recovers during the on-growing period.

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GROWTH PERFORMANCE OF SELECTED AFRICAN CATFISH (*Clarias gariepinus*, BURCHELL, 1822) LINES USING DIFFERENT DIETS

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Introduction

Feed cost is one of the highest expenses in aquaculture production, mainly due to the provision of sufficient protein content. Nowadays, protein supply is covered by fish meal among others, although fish meal production is gradually decreasing while its price is increasing. Several studies have been performed to investigate different alternative protein sources; however, most of them revealed inadequate growth performance in fish with alternative protein feeds. Nevertheless, better economic indexes could be achieved in some fish species with positive selection for feed utilization. African catfish is an important species in aquaculture production in numerous African, Asian, and some European countries. Our objective was to investigate the growth performance of F3 and F4 generations of an African catfish (*Clarias gariepinus*, Burchell, 1822) line selected for utilisation of low fish meal feed in a half-industrial experiment and to test feeding with alternative protein sources. We also tested the early growth performance of a Vietnamese line as a possible alternative genotype of our lines.

Materials and methods

African catfish was selected for efficient utilisation of low fish meal feed through three generations. Two performance tests were conducted on the F3 and three in the F4 generations. The selection, fish rearing, and investigations were performed on a half-industrial scale in a flow-through system using 2 m^3 tanks at the Bajcshal Ltd. site, with a water temperature of 23-24°C. All generations were produced with 4 multifactorial crosses of 5 male and 5 female individuals. To avoid indirect selection in the control group (CG), the big and the small specimens were used for crossing, and reared on the control diet (CD) containing 8.1% fish meal. Parallel with that, positively selected groups were created in triplicates (PS1-PS3) by crossing the biggest individuals fed with the experimental diet (ED) containing only 6% fish meal. All fish were fed with the same pellet size according to their age and size and all feed had the same crude protein (42%) and row fat (12%) content. During selection fish were reared until market size and body weight (g) was measured in the F1-F3 generations (F1 n=1683; F2 n=1783, F3 n= 2983). In the F3 generation the control group was created from the group previously fed with the control feed and two experimental groups were created from two of the positively selected groups (PS1, PS2), then all groups were divided into two subgroups and fed with the experimental and the control diet to accomplish a performance test in flow-through and RAS system. In the F4 generation, the control and all three positively selected groups were used in flow-through and RAS systems for performance tests with the same conditions as in F3, while an additional test was performed in flow-through using a 5% microchloropsis algae-containing diet (AD). The feed contained a low amount of fish meal (6%), 22% soybean meal and 5% extruded soybean meal, 42% crude protein, and 12% raw fat, while the control diet (AC) contained 24% soybean meal and 8% extruded soybean meal. The tests performed on the F4 generation lasted for 6 weeks.

An additional larvae-rearing test was performed to analyse the early growth rate of a Vietnamese African catfish line. The larvae were reared in RAS, for 30 days at 25°C, using CD feed from 0.5mm to 1.5mm. The Data analysis was performed in Excel with a $p<0.05$ significance level.

Results

The selected lines showed a higher growth rate compared with the control genotype with both diets in the flow-through system. The direct selection gain for the body mass was 11% in the F3 and 21% in the F4 generations fed with the experimental diet (ED) and 14% and 26% fed with the control diet (CD). While in RAS the average selection gain was 32% and 33% in F3 and F4 fed with the experimental diet (ED), and 12% fed with the control diet (CD) in both generations, respectively. There was no significant difference between the CD and ED diets. In the RAS system, a feed-specific selection gain of 21% was also present in both F3 and F4 generations. In this case, the feed-specific differences were significant. Using algae-containing feed the selection gain was 21,3 and 19,3 % in the case of AD and AC feeds. Algae complementation had a minimal effect on the meat colour in the yellow range. In the case of larvae rearing the Vietnamese line's specific growth rate was the highest (14.5%).

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Discussion and conclusion

We can conclude that the selected lines (PS1-PS3) performed better with all tests conducted on the F3 and F4 generations than the control genotype (CG). However, the effect of selection for low fish meal feed could be detected only in the RAS system in both F3 and F4 generations. All groups fed with microchloropsis algae-containing feed had higher growth rates. The average body weight was 5.3% higher than the control.

The effect of the selection for feed could not be detected in both the F3 and F4 generations, thus the effect of selection for higher body mass was significant, suggesting that there is a good potential for achieving better performance by selection in African catfish. However, finding out if the Vietnamese genotypes can be used in long-term breeding programs requires further investigation.

Acknowledgement

The work was supported by the National Research Development and Innovation Office (NKFIH) Hungary, grant number 2017-2.3.3-TÉT-VN-2017-00004, the Thematic Excellence Programme 2020, National Challenges Subprogramme (TKP2020-NKA-16) projects and by iFishIENCi project (European Union's Horizon 2020 research and innovation program under grant agreement No 818036). The project is co-financed by the European Union and the European Social Fund.

EXTENSIVE TESTING OF A FEMALE-SPECIFIC MARKER ON THE HUNGARIAN EX-SITU LIVE GENE BANK STERLET (*Acipenser ruthenus)* BROODSTOCK

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Introduction

Sturgeon caviar is an incredibly profitable product, making females more valuable in aquaculture. However, the application of sex control has not been widely implemented. All species of sturgeons are gonochoristic, but none of them exhibit clear external sexual dimorphism, nor do they possess heterochromatic sex chromosomes (Wuertz et al., 2006). Identification of females in current practice can only be achieved through examination of differentiated gonads, typically at the age of 3-5 years, depending on the species. Consequently, males can only be removed from production after an extended rearing period (Keyvanshokooh & Gharaei, 2010). This has prompted the search for early sex identification and methods for sex control in sturgeons (Havelka & Arai, 2018). Currently, this involves rather costly and cumbersome hormone level measurements or ultrasound examinations, which are still uncertain during the early stages of development. However, a recently isolated sex-specific genetic marker shows a new potential of use (Kuhl et al., 2021), though it has not been widely tested in practice yet. In this study, our goal is to initially assess the practical functionality of the sex marker on the ex-situ sterlet broodstock of Hungarian University of Agriculture and Life Sciences (MATE), Institute of Aquaculture and Environmental Safety (AKI), Research Center of Fisheries and Aquaculture (HAKI).

Materials and methods

Sterlets used in this study are part of the Live Sturgeon Gene Bank of MATE AKI HAKI. This sterlet stock consists of 202 matured individuals and approximately 600 juveniles. All matured fish in the broodstock are tagged with Passive Integrated Transponder and their sex is known through experience from reproductions. We collected fin clip samples from the broodstock which were preserved in 96% ethanol until the DNA isolation process. DNA isolation was performed using the E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek, Inc.) following the producer's instructions. The DNA were qualified and quantified by NanoDropTM 2000 spectrophotometer (Thermo Scientific™). Based on the DNA concentrations, the extracted DNA was diluted to 100 ng/µl. During this study, we used the AllWSex2 sex-specific marker (Kuhl et al., 2021), which was amplified by a PCR from the diluted template DNA. The final volume of the reactions was 15 µl thereof, 1,5 µl of 10X DreamTaq Buffer (Thermo Scientific™), 0,3 µl of dNTP (10mM, Thermo Scientific™), 7,7 mM of each primer, 1 u of DreamTaq DNA polymerase (Thermo Scientific™), 100 ng µl of template DNA and 10,5 µl of nuclease-free water. The first step of the PCR (Kyratec SuperCycler Trinity Revision 2.0.0) was a 2 min. denaturation at 95°C, followed by 45 cycles of 1 min. at 94°C, 45 sec. at 56°C and 45 sec. at 72°C, and final extension 5-min. at 72°C. PCR products were inspected on 1,5% agarose gel in TBE buffer for 45 min at 70V. The phenotypic sex was determined based on obtained gametes or by biopsy.

Results

The samples involved in this study were sourced from 119 females, 72 males and 11 hermaphrodite breeding individuals. Among the 119 females, we identified 106 genetically positive females (89,1%), while 13 individuals (10,9%) produced negative PCR results even after being tested three times. From the group of 72 males, we detected 60 as genetically males (83,3%) and 12 were identified as genetically females (16,7%). The sex distribution among hermaphrodites unfolded as follows: 7 individuals were genetically determined as females (63,6%) and 4 individuals were found to be genetically males (36,4%).

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Discussion and conclusion

Overall, it can be concluded that the AllWSex2 female-specific marker performs very well in sex determination, even in cases of a large sample size. We have identified 13 naturally feminized individuals in the female group where the female specific marker could not be detected. We have found 12 naturally masculinized individuals among the male specimens that are genetically females. From these 12 males, 4 produced large quantity and high-quality sperm during the artificial propagations in recent years. The misallocated results could stem by natural sex reversal or mutation on the binding site of the used sex specific primers. To investigate these phenomena, further studies are required in genetic verification of sex by applying other appropriate sex marker(s) or examining the effects of possible mutations on sex development as well as the genetic background of intersex individuals.

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MECHANISMS OF RESISTANCE TO SALMON LOUSE *Lepeophtheirus salmonis* IN ATLANTIC AND PACIFIC SALMONS: CHEMICAL SIGNALING BETWEEN PARASITE AND HOST

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The crustacean ectoparasite *L. salmonis* is the biggest problem in Atlantic salmon commercial aquaculture. In addition to financial losses, it severely affects fish welfare and public perception of the industry. The available antiparasitic measures are costly, stressful and traumatic for the fish and do not provide a satisfactory solution to the problem. A large-scale international project funded by the Norwegian Seafood Research Fund (FHF) is working to elucidate mechanisms underlying host resistance to the parasite. This work is expected to contribute to the development of effective antiparasite measures. Susceptibility to the parasite is markedly different between species in the *Salmo* vs. *Oncorhynchus* genera. The project is using state-of-the-art methods to study differences in chemical signaling, molecular and cellular responses in differentially susceptible salmon species. The use of CRISPR Cas gene silencing provides a powerful experimental approach to the search for genes responsible for lice resistance.

The success of lice infestation is determined by the ability of free-swimming copepodid stages to find and contact salmon, attach themselves and resist immune and cellular responses directed against the parasite. The project investigates both phases of parasite-host interactions: before and after attachment. Here we present chemical signaling research. Chemical cues (semiochemicals or kairomones) play a key role in host detection. Water conditioned with Atlantic salmon but not with non-salmonid sea fish species attracts copepods. Given that lice attracting kairomones are not produced by the majority of fish species, blocking their biosynthesis is unlikely to cause any negative effect. The identification of genes involved in this pathway may open a practical way to improve resistance to the parasite. Semiochemical studies include three parts: water chemistry, lice behavior assays, and genomics. Water conditioned with Atlantic salmon, Pacific salmon and other marine species was collected from several farms and research stations in Norway and Canada, processed with solid phase extraction (SPE), and analyzed by GC-MS and LC-MS. Putative semiochemical compounds were selected for testing on the free-swimming life stages of the parasite. Lice behavior studies use two complementary approaches. The immediate response is assessed by observing and recording directional movement towards the stimulus in an arena chamber. Another method evaluates response to a light flash following exposure to water containing test material. Lice copepods consistently show strong reactions to Atlantic salmon conditioned water but not to water conditioned with non-salmonid fish species. Comparison of lice behavioral responses to water conditioned with Atlantic and three Pacific salmon species is underway. Filtration through SPE columns removes semiochemicals and the response of lice to various sorbents has been evaluated. Recovery of the chemical stimuli in eluates from the columns was confirmed. A suite of putative semiochemicals was selected and tests of the lice behavioral response to them are underway.

The most ambitious goal of the project is termination of semiochemical production by gene silencing. It is important to find out in which tissues semiochemicals are produced and how they are released into water. The directional assay suggests that skin is a stronger attractant than other tissues and biological materials (blood plasma, mucus, and feces). Another assay indicates a dose-dependent effect of skin extract and the possible presence of semiochemicals in other tissues and mucus. In search for candidate genes, priority was given to cytochromes P450 (CYP). These enzymes play the key part in various pathways of secondary metabolism. In addition to the conserved CYP present in all vertebrates, some genes are specific to certain taxonomic groups. Their substrate specificity and physiological roles are unknown. Comparison of the genomes of salmons and other marine fish species has found several salmon-specific *cyp* with high expression in the skin. CRISPR Cas silencing of *cyp2m1* was performed in Atlantic salmon.

Studies conducted by scientists from five countries combined parasitology and fish health, marine biology and invertebrate behavior, chemistry, genomics, and molecular biology. Research is ongoing and has reached a level where synergy is expected from pooled competency.

HEART FOR AQUACULTURE – SPONTANEOUS CONTRACTING HEART CELL CULTURES AS A TOOL FOR MEASURING THE EFFECT OF VIRUSES AND ENVIRONMENTAL POLLUTANTS *IN VITRO*

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Introduction:
Circulatory disorders in the heart are an increasing challenge to the sustainable development of salmonid aquaculture worldwide. The main pathogens causing these problems are piscine orthoreoviruses (PRVs), which often cause Heart and Skeletal Muscle Inflammation (HSMI). In addition to pathogens, acute or chronic toxic exposures to nano- and micrometre-sized plastics and oil spills are a significant risk to aquatic life. The aim of the present study was to develop novel cardiac cell cultures from salmonids to reduce and replace the use of animals to investigate the effects of viral and environmental stressors on cardiac cells. In addition, gene expression signatures in these cardiac primary cultures of salmonids (SCPCs) after infection with PRV-1, PRV-3, piscine myocarditis virus (PMCV) and salmonid alphavirus 3 (SAV-3) were investigated to determine whether innate immune responses could be used as markers to confirm virus replication.

Materials and methods:
A novel method was used to culture SCPCs, which are constantly beating for up to eight weeks. Three-week-old SCPCs from Atlantic salmon, brown trout and rainbow trout were infected with the viruses and then cultured at 8°C and 15°C for a further four weeks. The virus-infected cultures were sampled immediately after infection and at 3, 7, 14, 21 and 28 days post infection. In addition, SCPCs and salmonid larvae were exposed to nano- and microplastics or crude oil. The number of contractions of the cultures was counted and samples of the cells were taken. Cell and media samples were used for virus detection, and cell samples were used to measure 10 genes involved in antiviral and pro-inflammatory responses by RT-qPCR.

Results:
The heart cell cultures were more sensitive to the effects of environmental stressors than heart *in vivo*. While the *in vivo* studies did not show any change in heart rate, the *in vitro* studies showed an increase in the number of contractions after exposure. Preliminary results from the virus exposures show that antiviral genes were upregulated when cardiac cells were actively replicating viruses and that these genes are likely to play an important role in the cardiac immune response.

Conclusion:
SCPCs may therefore be a valuable tool for monitoring host-pathogen or host-environment interactions involving cardiac cells of fish species used in aquaculture.

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TRANSCRIPTOME OF A LOCAL GERMAN RAINBOW TROUT STRAIN RESISTANT TO VHSV PROVIDES INSIGHT INTO IMMUNE RESPONSE LEADING TO DIFFERENT DISEASE OUTCOMES

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Introduction:
Viral haemorrhagic septicaemia virus (VHSV) is a highly contagious pathogen affecting salmonid fish populations. Recent evidence suggests that host genetics play a key role in susceptibility to the virus, with differences in innate antiviral immune responses underlying VHSV resistance in rainbow trout strains. The aim of the present study was to investigate gene expression signatures in a VHSV-resistant local German rainbow trout strain (R7) under VHSV infection and to compare them with expression signatures in a highly susceptible commercial strain (R9).

Materials and methods:
Fin, gill, gut, kidney and spleen tissues were collected from susceptible and resistant adult rainbow trout at 2 and 4 days post infection with VHSV. RNA-seq was performed from kidney samples at 2 dpi. In addition, gene expression of 27 selected genes was determined by RT-qPCR in all samples collected. The results were compared with gene expression in primary cell cultures from fins, scales and kidneys of fish of the same strains infected with native and inactivated virus.

Results:
It was found that the genes involved in the interferon response of rainbow trout showed clear differences in gene expression levels between the two origins. Especially on day 2 after infection with VHSV, a large number of genes differed significantly ($p < 0.05$), especially in spleen and kidney. Particularly striking were the gene expressions of pro-inflammatory cytokines and type II interferons. While gene expression in tissues of resistant origin increased steadily over the observation period, there was a rapid increase (day 2) followed by a decrease in gene expression (day 4) in samples of susceptible origin. The *in vitro* experiments confirmed the *in vivo* results that VHSV induces a stronger immune response in cells derived from the R9 strain.

Conclusions:
Our results could not confirm that an increased antiviral response is the main factor for increased resistance. Furthermore, it can be concluded that trout from the susceptible R9 origin died because of a dysregulated inflammatory response and cytokine storm.

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INCORPORATION OF WHOLE-GENOME SEQUENCING AND FUNCTIONAL INFORMATION TO REFINE QUANTITATIVE TRAIT LOCI AND IMPROVE PREDICTION ACCURACY FOR CONTROLLING SCUTICOCILIATOSIS (*Philasterides dicentrarchi*) IN TURBOT (*Scophthalmus maximus*)

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Introduction

Infectious diseases represent a major threat to farmed animals, having a large impact on animal health and welfare, production, and compromising human food security. Genetic disease control strategies that focus on enhancing host response to infectious pathogens have gained attention in recent years. Disease resilience, the ability of an animal to cope and survive an infectious disease, has emerged as a desirable breeding goal. However, resilience is a complex trait that involves various underlying mechanisms, including host resistance, endurance and infectivity, which are not easily disentangled.

In this study, we used whole-genome sequencing and imputation to genotype all the genetic variants, including structural variants, in a turbot breeding population challenged with the parasite *Philasterides dicentrarchi,* a ciliate that causes scuticociliatosis resulting in high mortality in farmed flatfish. The design of this challenge allows disentangling the different components of resilience[1], and the goal of this study is to evaluate whether whole-genome sequencing combined with genome functional annotation might i) improve our ability to accurate measure these traits, ii) reveal their genetic architecture and iii) increase the accuracy of selection.

Materials and methods

The turbot population consisted of ~1,400 animals from 36 full-sib families, challenged with *P. dicentrarchi* using a complex semi-factorial design involving donor and recipient families (full design in Anacleto et al. 2019), to enable decomposing the resilience trait into resistance (propensity to become infected), endurance (propensity to survive the infection) and infectivity (propensity to transmit the disease). For this project, we sequenced the whole genome of the 54 turbot parents of the challenged families using a Novaseq 6000 150 PE sequencing. After quality control, filtered reads were aligned with BWA-MEM to the new turbot chromosome level genome assembly[2]a group frequently showing limited sex chromosome differentiation and high SD evolutionary turnover. Turbot (Scophthalmus maximus (GCA_013347765.1). Single nucleotide polymorphisms (SNPs) were called with BCFtools and structural variants with Smoove, following a custom pipeline[3]but remain challenging to accurately type and are hence poorly characterized in most species. We present an approach for reliable SV discovery in non-model species using whole genome sequencing and report 15,483 high-confidence SVs in 492 Atlantic salmon (Salmo salar L.. The genotypes of the 54 whole-genome sequenced (WGS) parents were used as the reference population to impute the 2b-RAD SNPs sequenced genotypes of their offspring using FImpute v.3. After quality control, the resulting imputed dataset was used to estimate genetic parameters of resilience traits with ASREML v.4.2, and conduct genome-wide association study (GWAS) for each trait using GCTA v.1.24.7. The functional annotation of the genome including regulatory regions, obtained from the AQUA-FAANG project, was intersected with the genetic variants, and used to test if their prioritization led to improved accuracy of genomic selection using BayesRCO.

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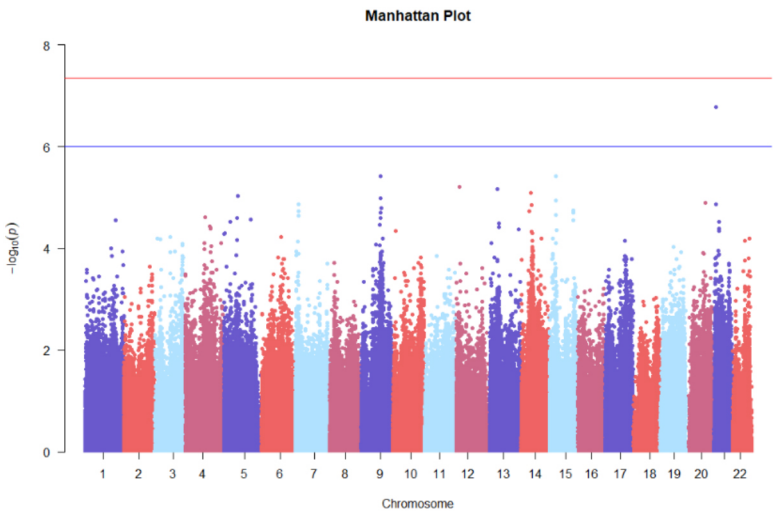

Figure 1. Manhattan plot of the GWAS with GCTA software for the trait of resilience. The values on the y-axis represent the −log10 of the P value and the x-axis the positions on the chromosomes. The red line is the 5% genome-wide significance threshold and the blue line is the 5% chromosome-wide significance threshold (Bonferroni correction).

Results

The average sequencing coverage across the 54 whole genome sequenced samples was ~14 ± 8.6. After quality control, 2,825,587 SNPs and 8,722 high-confidence SVs remained for imputation. There were 10,301 common SNPs between the WGS and the 2b-RAD of the offspring, which were used as anchorage for imputation of the whole population to whole-genome genotypes. After imputation, 1,100,299 SNPs and 1,390 offspring passed filtering and quality control. The heritability estimate for the composite resilience trait time to death was 0.15 ± 0.04, similar to that previously reported[4]disease resilience, the ability of a host to survive or cope with infectious challenge, has become a highly desirable breeding goal. However, resilience is a complex trait composed of two different host defence mechanisms, namely resistance (the ability of a host to avoid becoming infected or diseased, and GWAS using the imputed data identified a polygenic genetic architecture of the trait, with no major SNP surpassing the genome-wide significance threshold (Fig.1).

We are now estimating the genetic parameters and performing GWAS for resistance, endurance and infectivity to understand the genetic makeup of resilience to *P. dicentrarchi* in this turbot population. The study also aims to investigate the accuracy of genomic prediction using preselected variants integrating genome annotation information from the AQUA-FAANG project to enrich the dataset with functional information and improve the predictive performance of the models. The incorporation of biological knowledge into genomic prediction models can provide insights into the underlying biological processes of disease-related traits and be useful for future functional studies.

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BIOAEROSOLS AS A POTENTIAL TRANSMITTER OF FISH PATHOGENS IN AN ATLANTIC SALMON (*Salmo salar*) RECIRCULATED AQUACULTURE SYSTEM (RAS) SMOLT FARM

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Introduction

Pathogen transmission in aquaculture is mainly through two modes, vertically via eggs and horizontally via water (Oidtmann et al., 2013). Aerosols represent another potential mode of transmission. Although it is indisputable that human and animal pathogens are disseminated frequently via aerosols, there is little knowledge of aerosol transmission in aquaculture (Wang et al. 2021). The Faroe Islands are known for their high-quality Atlantic Salmon produced in closed containment systems called Recirculated Aquaculture Systems (RAS), wherein the water gets constantly cycled through various compartments. The system also involves the constant import of large volumes of air for degassing purposes, and recent studies have shown that indoor systems generate pathogen-laden aerosols from water (Roberts-Thomson et al., 2006, Gołaś et al., 2022). The sea surface microlayer (SML) has been identified as a source of aerosols that could transmit bacteria and viruses that can stay infective and transmit through long distances (Sharoni et al. 2015, Reche et al. 2018). In our study, we will focus on the aerosolization potential of five fish pathogens in a commercial Atlantic Salmon RAS smolt farm: Salmon Gill Pox Virus (SGPV), Infectious Salmon Anaemia Virus (ISAV), Infectious Pancreatic Necrosis Virus (IPNV), Piscine Orthoreo Virus (PRV), and *Flavobacterium psychrophilum*.

Materials and Methods

Aerosol samples were collected using the Coriolis Micro and Coriolis Compact (Bertin Instruments, France) wet and dry impaction-based cyclonic air samplers, respectively. The Coriolis Micro has a variable flow rate of 50-300L/min, while the Compact has a fixed rate of 50L/min. The location choice for the sample collection included targeted areas of high aerosol formation burden based on preliminary results from a pilot study using the Coriolis Compact. Coriolis Micro employed sampling cones containing 15ml 1X PBS (Corning, USA) with 0.1% Ecosurf (Dow Inc.) surfactant in the sampling cone, while 1ml of 1X PBS+0.1% Ecosurf reconstituted the samples from the Compact. Aliquots of 1ml of the neat samples were made for viral propagation in cell lines and bacteriological cultures and stored at -80°C. Due to the higher sample volume of about 15ml obtained from the Coriolis micro, Amicon Ultra 50kDa will concentrate samples to attain better detection limits. Simultaneous sampling of fish and water was also done to assess the infection dynamics of the pathogens in the RAS tanks. All the samples were stored at -20°C until further processing. RNA extraction was performed using the Kingfisher semiautomated nucleic acid extraction machine per the manufacturer's instructions under standard laboratory conditions. The extracted RNA samples will be screened for the above five pathogens using RT-qPCR on a Quantstudio RT-qPCR system(Applied Biosciences, USA)

Results

Preliminary results from the pilot study have demonstrated the presence of all the pathogens of interest, SGPV, ISAV, IPNV, PRV, and *F. psychrophilum* in the aerosol at varying Ct-values between 26-36. An interesting observation from the RT-qPCR results indicates differential pathogen levels in the air and varied over the sampling period, with higher Ct during peak infection. The pathogen levels in the water seem to influence the levels in the air directly; however, significantly lower.

Conclusions

Based on the pilot study, the pathogens get aerosolized at differential rates depending on their load in the water. Several other factors, such as the location of sampling, proximity to air vents, and machinery, influence the aerosolization of the circulating water. This underlines the importance of a high biosecurity level in closed containment systems (CCS) to prevent the uncontrolled spread of pathogens between different RAS systems in a smolt farm. The results derived from the fish and water samples will help determine the aerosolization potential of the pathogens.

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IS THE CHOICE OF WEANING STRATEGY OF EURASIAN PERCH (*Perca fluviatilis* L.) LARVAE DEPENDS ON DOMESTICATION LEVEL OF SPAWNERS?

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Introduction

Information on how domestication affects early life stages in fishes is limited. One of the recent studies showed that domestication has an impact on the digestive capacity of Eurasian perch larvae that coincided with a higher growth rate recorded for domesticated ones (Palińska-Żarska et al. 2020). Weaning is a critical step for cultured percid species as the transition from live food to commercial feed often results in high mortality. Further studies are required for the optimization of the weaning protocol at the earliest possible stage of perch larvae e.g., is full substitution of live food by commercial diet should be preceded by co-feeding period or not? (Król and Zieliński 2015). Therefore, the aim of the study was to determine the effect of a different weaning strategy (sudden switch vs co-feeding) of perch larvae originated from domesticated or wild spawners on the results of their subsequent rearing parameters.

Material and methods

Eurasian perch larvae from wild and domesticated spawners were obtained followed by separate reproductive protocols described by Żarski et al. (2019). Incubation of eggs and larvae rearing procedures, except feeding scheme, were the same for domesticated and wild stocks and conducted according to standardized protocols described by Palińska-Żarska et al. (2020). Four experimental groups (in triplicate each), differed by origin of the larvae (domesticated or wild) and two weaning strategies (sudden switch or co-feeding), were reared in RAS system during subsequent 34 days. From 4 dph until 17 dph, two groups of larvae (domesticated and wild) were fed exclusively by *Artemia* nauplii and the others two groups (also domesticated and wild) were co-fed using *Artemia* nauplii and commercial diet. Afterwards, fish were switched entirely to commercial feed. At the end of the experiment all survivors (34 dph) per tank were counted, weighed, and measured. Other rearing parameters were assessed according to the scheme described by Król and Zieliński (2015). A two-way ANOVA was used to test the effects of origin of the perch larvae (domesticated or wild), weaning strategy (sudden switch or co-feeding) and the interactions of both these factors on subsequent rearing parameters of perch larvae. Differences were considered significant at $P<0.05$.

Results

At the end of experiment, domesticated and co-fed (*Artemia* nauplii and commercial feed) perch larvae characterized by better growth, lower mortality caused by type II cannibalism and final bigger biomass, whereas these wild perch larvae which were fed only by *Artemia* nauplii, until were switched entirely to commercial feed, had better final survival, lower mortality other than cannibalism and final bigger biomass. Based on two-way ANOVA, origin of perch larvae (domesticated or wild) affected individual body weight, mortality caused by type II cannibalism and mortality other than cannibalism. Weaning strategy significantly effected on individual body weight, mortality caused by type I cannibalism and final survival of the fish. None of the factors had a significant impact on the final fish biomass. However, significant interactions between origin of the larvae and the weaning strategy were found in all tested rearing parameters (Table 1).

Discussion and conclusion

Our study supports the hypothesis that the type of food is the main challenging factor in intensive Eurasian perch larviculture conditions affecting its final efficiency. Recent study suggested that the production of digestive enzymes does not matter when easily digestible high-quality food, such as *Artemia*, is offered, although digestion capability was higher in wild than in domesticated perch larvae (Palińska-Żarska et al. 2020). However, in the presented study, significant changes in growth between perch larvae originated from wild and domesticated spawners were observed even before *Artemia* was completely replaced with commercial feed using co-feeding scheme. This suggests that other processes than only the production and activity of digestive enzymes are modified by progressive domestication. In conclusion, our results indicate, that choice of the weaning strategy in Eurasian perch larvae may depend on the domestication level of their parents and could significantly affect their subsequent rearing parameters.

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Table 1. F-values and P-values (* P<0.05) from the two-way ANOVA analysis used to study the effects of origin of perch larvae (domesticated or wild), weaning strategy (sudden switch or co-feeding) and the interaction of these two predictors on dependent rearing parameters.

Predictor **Dependent**	**Origin**		**Weaning strategy**		**Origin x Weaning strategy**	
	F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
body weight	7,59	0,02*	6,43	0,03*	11,72	0,01*
fish biomass	1,68	0,23	0,31	0,59	15,22	0,01*
survival	0,10	0,76	8,50	0,02*	6,11	0,04*
cannibalism type I	3,13	0,11	11,91	0,01*	15,85	0,01*
cannibalism type II	15,53	0,01*	3,26	0,11	16,72	0,01*
other type mortality	12,59	0,01*	2,60	0,14	6,90	0,03*

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USE OF DIFFERENT PHOTOPERIODS OF ARTIFICIAL GREEN LIGHTING (LED) IN BIOFLOC SYSTEM ON THE GROWTH AND OXIDATIVE STRESS OF *Litopenaeus vannamei*

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Introduction

Several parameters influence the establishment of a BFT system and farmed animals' performance. One of these parameters is light, which is an abiotic factor of paramount importance for organisms that live in the aquatic environment. Aquaculture production systems with shrimp exposed to plenty of natural light may perform better than systems with low light levels due to the growth of photosynthetic microorganisms. Added to this, the presence of light also positively influences the abundance of microorganisms, reflecting in the better performance of *Litopenaeus. vannamei*. This research aimed to assess the impact of various green light photoperiods from green LED lamps on water quality, microorganism community, antioxidant capacity (ACAP), lipid peroxidation (TBARS), and growth performance of the Pacific white shrimp *L. vannamei* in the BFT system.

Material and Methods

The study was conducted in the Shrimp Production Laboratory of the Institute of Oceanography of the Federal University of Rio Grande – FURG, Brazil. Trial was performed in 150L indoors tanks, using *L. vannamei* larvae with an initial weight of 0.48 g at a stocking density of 500 shrimp m^{-3}. The experiment lasting 61 days. The experiment was designed with four treatments and four replicates each, with different photoperiods using LED green light: **1)** 16h LI/8h DA, **2)** 12h LI/12h DA (control), **3)** 8h LI/16h DA and **4)** 4h LI /20h DA.

Results

No significant differences were found in the water quality parameters following the completion of the trial ($p > 0.05$). However, there were significant differences in the bacterial abundance of free coccoids, free filamentous, attached filamentous, vibrios, and bacilli ($p > 0.05$) and in protozoa, such as flagellates, ciliates, rotifers, nematodes, and amoebae ($p < 0.05$, Fig 1). There were also significant differences in lipid peroxidation (TBARS) with lower lipid peroxidation in the 12h LI/12h DA, 8h LI/16h DA, and 4h LI/20h DA treatments and higher antioxidant capacity (ACAP) in the hepatopancreas and muscle tissues in the 8h LI/16h treatment DA ($p < 0.05$). In addition, shrimp from treatment 8h LI/16h DA showed a higher final weight than the control treatment 12h LI/12h DA ($p < 0.05$).

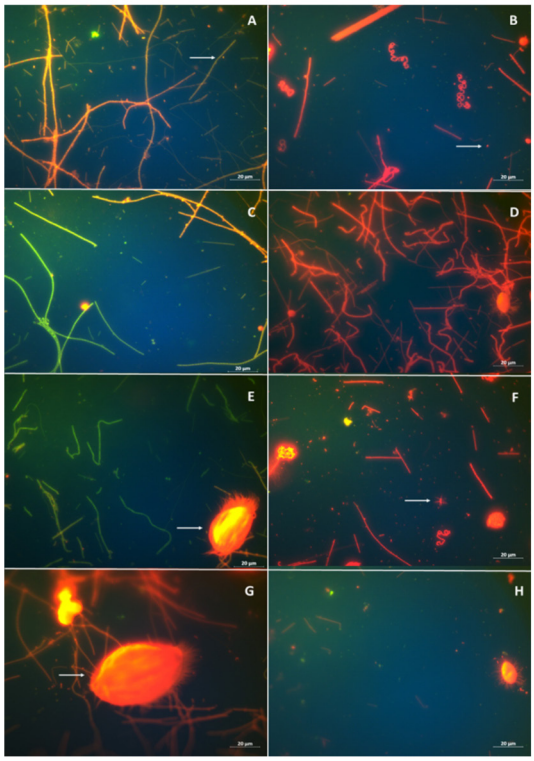

Fig. 1. Abundance of bacteria in the microbial floc Magnification of 1000x by epifluorescence microscope (image: Wellica G. Reis).

Conclusion

Using green LED light in different photoperiods positively influenced the antioxidant capacity and oxidative damage (lipid peroxidation) of *L. vannamei* and zootechnical performance. Therefore, results suggest that the photoperiod of 8 hours of green light and 16 hours of darkness can be recommended for rearing *L. vannamei* in biofloc system.

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Acknowledgements
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A DECISION SUPPORT TOOL FOR ECOSYSTEM-BASED MANAGEMENT AND BIODIVERSITY CONSERVATION IN TRADITIONAL CENTRAL AND EASTERN EUROPEAN CARP FISHPOND SYSTEMS USING ECOPATH WITH ECOSIM

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Introduction

Promotion of ecologically beneficial forms of aquaculture has emerged as a policy priority in recent years (European Commission, 2021), with promotion of culture techniques that closely resemble natural systems. While still managed, traditional carp ponds in Central and Eastern Europe are an example of a sustainable aquaculture technique that has been shown to contribute to the biodiversity and maintain regulatory functions of ecosystems, comparable to natural wetlands (Popp et al., 2019). However, there may be varying management practices between farms, with differing intensities of production. Traditional carp ponds are thus complex socio-ecological systems, and to make sure they are sustainably managed and regulated, environmental interactions must be understood. Ecological assessments for regulatory and management use should be simple, practicable in terms of farming operations, and cost-effective, in addition to making sure that human benefits from the system are not heavily compromised. The goal of this study was thus to develop a rapid decision support tool to determine the ecological outcomes of different management intensities.

Materials and methods

Aquatic biodiversity data of selected taxonomic groups (Amphibia, Gastropoda, Bivalvia, Insecta, Aves) and aquatic vegetation was sampled from 6 carp ponds of varying management practices from June – September 2023. Environmental parameters were also recorded. Species abundance data was used in biodiversity analyses, including the calculation of multiple diversity indices expressed through Renyi diversity profiles, and multivariate analyses of ecological community composition. Collected biomass data was used to parametrise an Ecopath with Ecosim (EwE) model for each production intensity. EwE models were used to determine ecosystem health indicators through ecological network analysis (Aubin et al., 2019).

Results and Discussion

The preliminary biodiversity analyses showed differences in biodiversity between different pond management regimes are evident, with more intensively managed systems varying more from the natural state. Diversity profiles showed that the natural pond had the highest biodiversity across all indices, whereas semi-intensively managed ponds showed higher species richness but lower Shannon-Weiner, Simpsons and Berger-Parker diversity than intensively managed ponds. Non-metric multi-dimensional scaling showed that semi-intensively managed and natural ponds tend to be more similar in terms of community composition. Further analysis of data collected in September 2023 is ingoing and will be included in further multivariate analyses to determine the interactions of environmental variables and community composition and to carry out the ecological network analysis with EwE.

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THE EFFECT OF SINKING AND FLOATING FEEDS ON THE EFFICIENCY OF INTENSIVE PIKEPERCH (*Sander luciperca*) CULTURE

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Introduction

Pikeperch is successfully used for the diversification of inland aquaculture due to its high-quality flesh, fast growth, and overall market profitability (Overton et al., 2015). Fully grown and marketable sized pikeperch is highly demanded by gastronomy industry and angling community. During the last decade this species was subjected to an intense scientific study in both Central and Western Europe. To establish pikeperch as a permanent part of European inland intensive aquaculture, the formulation of new feed is necessary to adequately address the specific nutritional needs of pikeperch. This issue was not yet solved. Also, the characteristics of the pellets itself, whether they float on the surface or sink to the bottom, may influence the growing rate and nutrient utilization of cultured and fed juveniles. The present study is focused to determine the influence of the different pellets characteristics on the efficiency of pikeperch intensive production in RAS (Recirculating Aquaculture System) during grow out phase.

Material and methods

Experimental fish were stocked into 8 experimental tanks (1.5 m³ each) in one production RAS of Faculty of Fisheries and Protection of the Waters in Vodňany, Czech Republic. Juveniles in number of 8000 individuals (1000 juveniles per tank, mean IBW (Initial Body Weight) 21.38 ± 7.32 g, biomass of 13.42 kg. m^{-3}, were cultured) for 112 days with control period of 28 days. After 14 days of adaptation experimental fish under new conditions, the experiment was started. Fish were fed during 16 hours per day using two commercial dry feed diets set at DFR (Daily Feeding Ratio) 1.2 % of fish biomass. The first group was fed with floating pellets Skretting Europa 15F (3.5 and 5mm). The second group was fed with sinking pellets Biomar Effico Sigma (3 and 4.5mm) Both types of pellets contain the same level of protein (55%) and of fat (16%). At the beginning and the end of experiment, 50 randomly selected experimental fish from each tank were measured. These fish were anesthetized in solution of clove oil (0.04 $ml.l^{-1}$). From this data following production markers were determined - IBW= Initial Body Weight; FBW= Final Body Weight, WG= Weight Gain, SGR= Specific Growth Ratio, TL= Total Length; FCR= Food Conversion Ration, CF= Condition Factor. Also, the growth heterogenity was assessed.

Results and discussion

An achieved final body weight of 80 g was observed in the more rapidly growing group fed by sinking feed, compared to 72 g in the group fed by floating feed. Group fed by sinking feed also exhibited significantly higher WG and TL indicating higher growth of fish fed by sinking feed. Higher feed utilization was found in group fed by sinking feed as well as evidenced by lower FCR of 0.88 $g.g^{-1}$ and a higher SGR of 1.24 $\%.d^{-1}$ compared to group fed by floating pellets which managed to reach FCR 0,93 $g.g^{-1}$ and SGR 1.13 $\%.d^{-1}$. Both groups exhibited no statistical difference in survival rate (92.13 and 94.45 %). These results would suggest that sinking feed is a preferable choice. However, there were different results in growth heterogenity and Condition Factor. Group fed by floating pellets reached statistically higher CF (1.16) compared to the other group (1.09) probably because of increased physiological exercise resulting from periodical surfacing for floating pellets. Additionally, group fed by floating pellets showed lower growth heterogenity with fewer cases of cannibalistic and growth deprived individuals.

Acknowledgements

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Table 1 – Summary of production parameters after 16 weeks of breeding using 2 different types of feed.

	Group F	Group S	T-test, p–value
IBW (g)	20.36 ± 5.91	19.93 ± 5.53	p= 0.451
FBW (g)	72.12 ± 36.5 [a]	80.01 ± 40.97 [b]	p= 0.043
WG (%)	254.7 ± 12.1 [a]	301.8 ± 36.6 [b]	p= 0.046
SGR (%.d^{-1})	1.131 ± 0.03 [a]	1.241 ± 0.08 [b]	p= 0.030
TL (mm)	210.2 ± 34.6 [a]	218.5 ± 34.9 [b]	p= 0.004
FCR (g.g^{-1})	0.931 ± 0.03 [a]	0.881 ± 0.02 [b]	p= 0.036
CF	1.161 ± 0.10 [a]	1.091 ± 0.18 [b]	p= 0.001
Survival (%)	92.13 ± 4.79	94.45 ± 3.41	p= 0.518

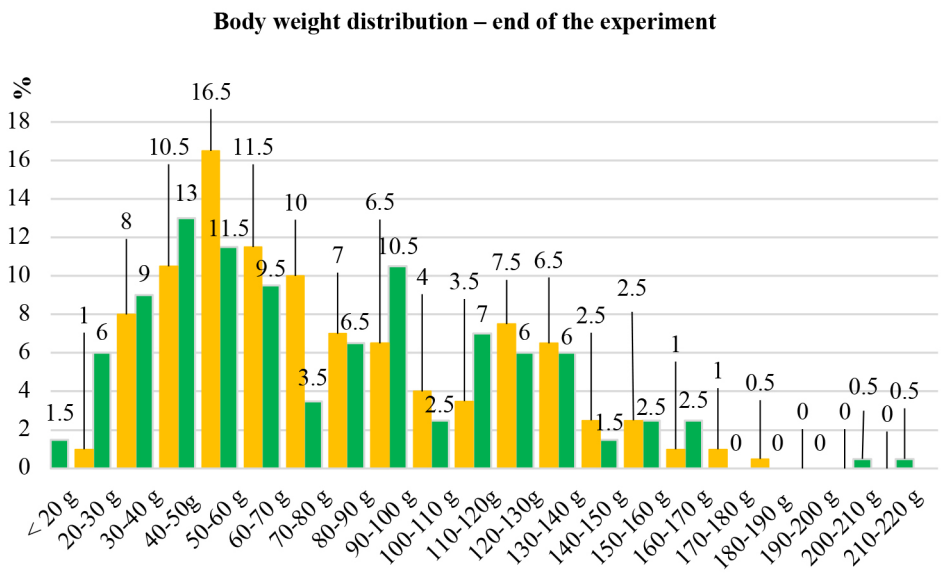

Figure 1- Green – Group fed by sinking feed, Yellow - Group fed by floating feed; Percentual distribution in weight categories a

IMPROVING INTENSIVE LARVAL REARING OF PIKEPERCH (*Sander lucioperca*) - USING DIFFERENT LIGHTING

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Introduction

One of the cornerstones of closed intensive pikeperch farming is the production of habituated high quality juveniles (Ljubobratovic 2015). In the case of pikeperch larvae, lighting is one of the environmental factors that can have a major impact on the success of rearing. The relationship between larval rearing efficiency and light intensity levels has been studied (Tielmann et al. 2017), but the effects of other aspects of lighting on larval rearing are less well understood. In our experiment, the effects of overhead and submerged light sources on survival, growth and larval quality were investigated.

Materials and methods

Fertilized egg originated from broodstock reared in a recirculating aquaculture system were used for the experiment (number of fertilized eggs: 10,000). The hatched larvae were distributed by volume into 6 250L conical bottom upwelling larval rearing tank (1600 larvae per tank). Three tanks were illuminated from above with warm white diffused light, three tanks were illuminated with insulated LED strips emitting warm white light submerged in water (16h light 8h dark). The fish were fed exclusively with artemia (300 nauplius/larvae/day) for two weeks from the beginning of exogenous feeding, and then supplemented with dry feed (10g food/tank/day 0.3-0.5mm Essence Coppens) until the end of the experiment, which lasted 24 days after hatching.

Results and discussion

The fish grew evenly in both treatments. The growth rate was slightly higher in the tanks with submerged light, while the survival rate was higher in the tanks illuminated from above, with a poorer swim bladder inflation in percentage. However, the differences in neither growth nor in survival were not significant ($p>0.05$ t-test).

Experience from the current study suggests that submerged light sources may have a positive effect on larval quality, but further studies are needed to determine the optimal light intensity and duration.

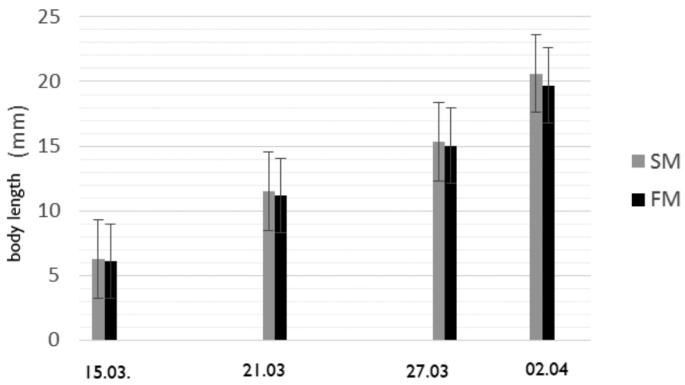

Fig 1 growth of larvae SM: submergend light FM: diffused top light

Table 1 results of the experiment

	number of survived fish	fry without functioning swim bladder (%)	cannibals (%)
submerged light	341±193	19.79±4.46	3.56±3.38
top light	519±115	35.76±18.88	1.12±0.24

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SINGLE-STEP GENOMIC PREDICTION IN GROWTH AND SEXUAL MATURITY TRAITS IN FINNISH RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Genomic selection in aquaculture species has been regarded especially useful for hard-to-record-traits, such as disease resistance, product quality and traits that need to be improved in multiple production environments. In the Finnish national breeding programme for rainbow trout, family tanks are used at the initial phase of growth which allows to maintain a pedigree for a large number of fish, and breeding value evaluation is based on this pedigree (PBLUP) (Kause et al. 2022). Genotyping of a portion of the fish accompanied with a single-step genomic evaluation (ssGBLUP) would maintain high selection intensity and simultaneously make use of possibilities of genomic selection. ssGBLUP has been shown as one of the optimal tools, especially when genotyped population is relatively small (Christensen et al., 2012). The combination of pedigree (A) and genomic (G) relationship matrix improves pedigree recording errors and helps utilize sib information more efficiently. The aim of the current study was 1) to implement ssGBLUP approach for routine evaluation, and 2) to use a validation method to demonstrate the added value of this approach, when breeding for growth and maturity age in two production environments.

Materials and methods

Data from Finnish national breeding programme was obtained from individually tagged fish reared in freshwater (nucleus) and sea stations. Pedigree included 600,409 individuals and 6,234 families made from 3,418 sires and 3,446 dams. Phenotyped fish were born between 1992-2019. Three body weight traits recorded at ages of 2 and 3 years at the nucleus ($Weight_2$, $Weight_3$,) or at age 2 at a sea farm (Sea $Weight_2$) and three binary maturation traits (1=early maturity, 0 = late maturity age) were recorded for males and females at the nucleus ($Maturity_{Male}$, $Maturity_{Female}$) or at sea (Sea $Maturity_{Male}$) (Table 1). Genomic data was available on 4,573 fish born in 2014, 2018, and 2019. Generations 2018 and 2019 were both established from generation 2014, and they had 8,525 and 9,241 tagged fish of which 16% and 28% were genotyped. Samples were genotyped using 57K SNP Axiom™ Trout Genotyping Array. After quality control and imputation 40,374 markers remain for further analysis

Table 1. Number of recorded phenotypes by trait in full and partial data.

Data	Trait					
	$Weight_2$	$Weight_3$	Sea $Weight_2$	$Maturity_{Male}$	$Maturity_{Female}$	Sea $Maturity_{Male}$
Full	96,544	101,509	85,927	41,202	55,404	32,157
Partial	95,038	98,770	83,750	40,014	53,640	31,270

Genetic and genomic prediction was performed using mixed-model equations as shown in Kause et al. (2022). Pedigree BLUP was upgraded to ssGBLUP by replacing A^{-1} matrix with H^{-1} computed as:

$$H^{-1} = A^{-1} + \begin{pmatrix} 0 & 0 \\ 0 & (s_t(1-w)G_{05} + wA_{22})^{-1} - A_{22}^{-1} \end{pmatrix},$$

where G_{05} is a G matrix constructed with assumption that allele frequency of all markers was equal to 0.5, A_{22} is a part of A matrix for genotyped fish, w is residual polygenic effect

equal to 5%, and s_t. is a scaling factor equals to . $\left(\frac{trace(A_{22})}{trace(G_{05})}\right)$.

To test for the predictive ability of PBLUP and ssGBLUP, partial data set was created by removing phenotypes collected in generation 2019 (Table 1). Validation of the model fit was done by linear regression of breeding values computed from full ([G]EBV) and partial ([G]EBV_{par}) data using formulae (Legarra & Reverter, 2018). Model R^2 was assumed as validation reliability.

(Continued on next page)

Results and Discussion

Average validation reliability (R^2, Table 2) in ssGBLUP was on 47% (0.18 reliability units) higher than PBLUP implying more accurate prediction. This resulted even thought our validation design was strict - validation group did not share any full-sibs. Sea weight and sea male maturity trait gave lower R^2 than freshwater traits due to the lower number of records.

[G]EBVs of validation fish were overestimated (b_0>1) in PBLUP and underestimated in ssGBLUP (b_0<1). Dispersion (b_1) results in PBLUP (<1) and ssGBLUP (>1) showed respective under- and overprediction of the [G]EBVs. Low b_0 and high b_1 in ssGBLUP may be explained by a suboptimal validation design. Removing full year 2019 makes training population small. In such a case *n*-fold cross-validation might be a better option to use.

Table 2. Validation bias (b_0), dispersion (b_1), and correlation squared (R^2) in PBLUP and ssGBLUP models.

	PBLUP			ssGBLUP		
Trait	b_0	b_1	R^2	b_0	b_1	R^2
$Weight_2$	46	0.83	0.39	-26	1.18	0.57
$Weight_3$	57	0.87	0.56	-37	1.13	0.66
Sea $Weight_2$	49	0.82	0.33	-48	1.25	0.56
$Maturity_{Male}$	-0.02	0.61	0.69	0.01	0.95	0.80
$Maturity_{Female}$	-0.02	0.93	0.48	0.02	1.12	0.67
Sea $Maturity_{Male}$	-0.01	0.85	0.32	0.01	1.09	0.61

Conclusions

A single-step genomic evaluation was successfully implemented in the Finnish national rainbow trout breeding programme. Genotyping of 16-28% tagged fish in the pedigree increased selection accuracy of body weight and maturity age traits.

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PRODUCTIVITY AND SUSTAINABILITY IN NORWEGIAN SALMON AQUACULTURE: THE ROLE OF PRODUCTION SITE LOCATION

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The Norwegian salmon aquaculture industry is regulated to control sea lice levels and prevent disease outbreaks.

Limited research has explored how the regional location of farms and the mutual positioning of farms within a region affect productivity in the industry. This study aims to estimate the productivity of salmon aquaculture companies while considering factors such as the location of production sites. The hypothesis is that some companies are more productive due to owning well-located or differently-located production sites.

By examining the factors behind differences in productivity and the impact of current regulation on the industry's sustainability, this study contributes to a better understanding of how governance processes can adapt to promote the sustainable transformation of the industry.

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FISH WELFARE MONITORING IN A RAS FACILITY USING MACHINE LEARNING AND STEREO VISION FOR AUTOMATED 3D POSITION TRACKING

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Introduction

Moving fish production from sea to land is gaining popularity due to the ability to control physico-chemical environmental parameters like temperature, salinity, and illumination, as well as to avoid parasitic infestations or harmful algae blooms. To achieve a sustainable production with low water and other resource consumption, recirculating aquaculture systems (RAS) have evolved rapidly in the recent past. RAS facilities does however come with their own challenges of maintaining water quality parameters including different nitrogen compounds-, carbon dioxide- or in a worst-case scenario hydrogen sulphide concentration as real-time sensors for these parameters are typically missing or not even available for industrial scale operations. A change of these water quality parameters can impact the fish welfare and, in worst case, cause a total loss of biomass in a few hours.

Materials and method

We present a system based on stereo vision for 3D tracking of each detected individual fish, utilizing a modified version of Stereo R-CNN (Li et al, 2019) for stereo detection and Norfair (Alori et al, 2023) for tracking of fish in a RAS facility (Figure 1). Using the 3D trajectory data, in x, y, z [mm], three fish behaviour parameters were be calculated, (i) swimming velocity, (ii) swimming pattern and (iii) fish synchronization. These values can be used to describe the general fish welfare and indicate physico-chemical water quality changes, e.g., an increase in carbon dioxide will impact the swimming velocity of the fish.

The method was validated via an experiment conducted in an experimental RAS facility at LetSea (Sandnessjøen, Norway), the experimental setup consisted of an 800 L tank stocked with a density of 10 kg/m^3 (~70 fish) with a submerged stereo camera filming horizontally. The fish was exposed to H_2S to induce a stress reaction and dissolved H_2S, O_2 and CO_2 was monitored using the Aquasense system (SeaRAS, Bergen, Norway). The experiment was approved by the Norwegian Food and Safety Authority (FOTS id. 29143).

Results

During the H_2S exposure an increase in the average swimming velocity was detected (Figure 2, subplot 2), and the fish showed a more erratic swimming pattern (Figure 2, subplot 3) as well as loss off schooling behaviour (Figure 2, subplot 4).

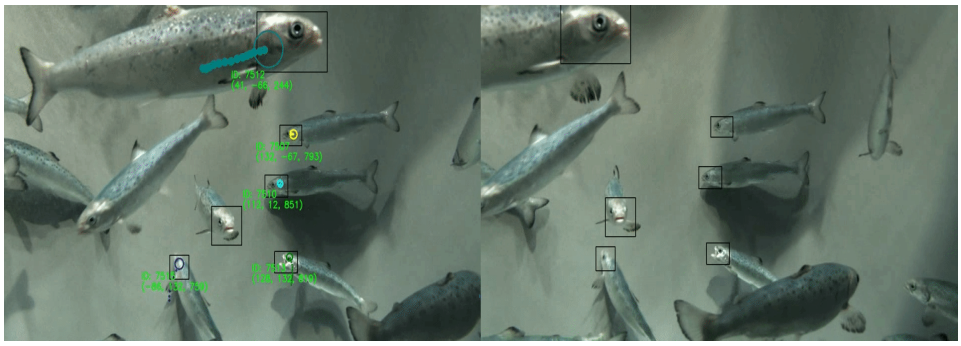

Figure 1. A frame grab from the stereo camera system with image from the left camera (left) and right camera (right), illustrating detections (black bounding boxes) and the 3D swimming trajectory (multicolour) with the detection ID and x-, y- and z coordinates in millimetres (green).

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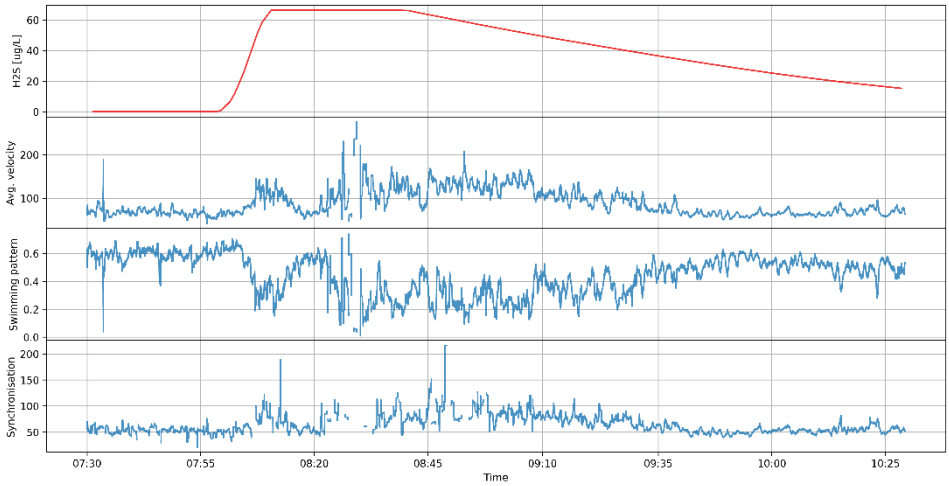

Figure 2. A plot illustrating the stress response of the fish (2nd-, 3rd- and 4th subplot) in relation to the dissolved H_2S concentration (1st subplot).

Conclusions

Using H2S as a stressor with high relevance for fish production in RAS facilities, the here described system showed its potential to serve as a real-time and cost-effective 24/7 surveillance system, that can automatically notify the operator when abnormal fish behaviour is occurring. The operator could then investigate the problem and initiate counteractions before long term damage to the biomass is sustained. Compressing the video streams down to only three essential parameters will ensure that the operator will not get overwhelmed by too much data during normal operation. The here demonstrated proof-of-concept represents a first step for optimizing land-based fish production in the next generation of digital RAS facilities, where fish welfare is in focus.

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REAL-TIME ESTIMATION OF FISH GROWTH IN INDOOR AQUACULTURE FARMS USING VISUAL INFORMATION

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Introduction

Estimating the growth of fish in real-time comes with many benefits for indoor aquaculture farms, such as saving labor time and costs, reducing water pollution during starvation, improving feeding activity, and supporting harvesting.

This study aimed to develop a visual-information-based, real-time method for measuring olive flounder (*Paralichthys olivaceus*) lengths in indoor aquaculture farms in order to efficiently manage fish stocks through accurate growth tracking. This study first compared the actual lengths of olive flounders with the lengths obtained using an image processing algorithm to analyze the morphological characteristics of fish within their rearing environment. Then, estimating the length-weight relationship allowed body weight to be determined based on the estimated length.

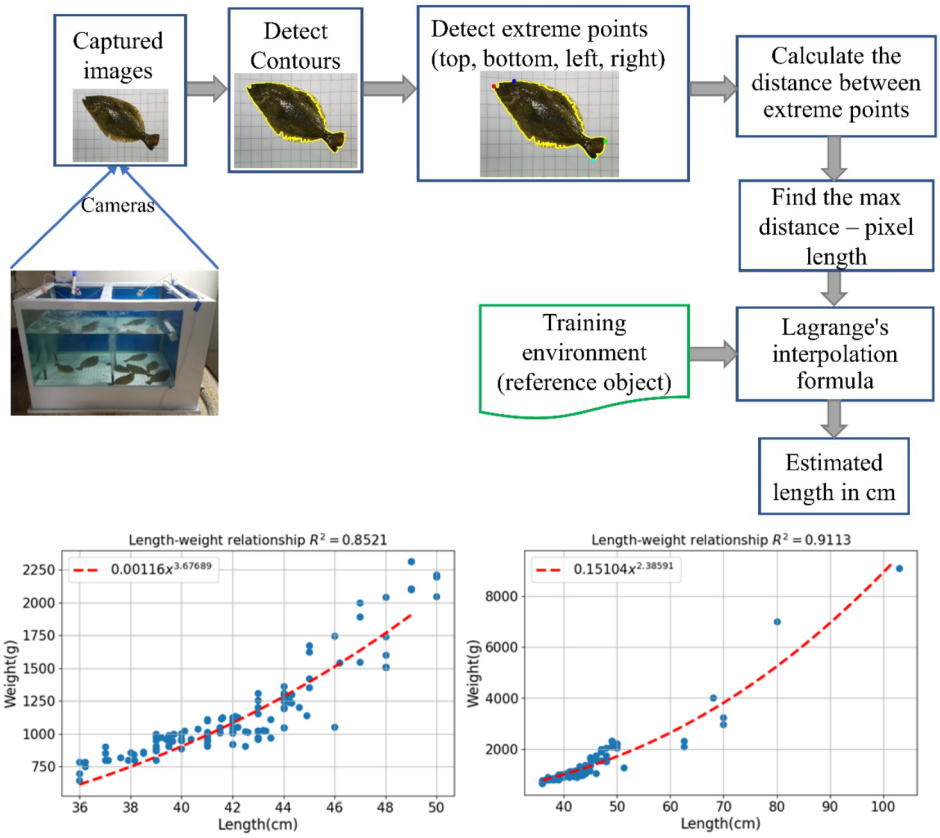

Fig. 1. The process for estimating fish growth. First, the image is captured on the grid. The area of the fish image is calculated by counting the number of pixels, then the area can be converted from pixels to cm^2 based on the relationship between the pixel and the centimeter unit for the grid. The longest length of detected extreme points can be equal to the length of the fish.

(Continued on next page)

Materials and methods

We could estimate the body weight of olive flounders based on the generated length-weight relationship. A 5×5 cm grid paper was placed at the bottom of the water tank for measuring the fish length along with two single RGB cameras. The pixel unit from the fish length in the captured image was converted to millimeters (mm) based on the pixel-mm relationship of the pre-built dataset. A total of 180 lengths were calculated using images captured by the left- and right-side cameras, which have fixed positions above the surface of the water tank. The average length of each fish acquired from both cameras was calculated separately, and Lagrange's interpolating polynomial algorithm was implemented to calculate the overall length of each fish.

Results

Our system estimated the length of olive flounders with a simple, low-cost device comprising two mobile phones as cameras. A 5×5 cm grid paper was used to generate the learning environment as a reference object to convert from pixels to cm. Lagrange's interpolating polynomial algorithm was applied to calculate the length of fish in cm after calculating the pixel length of fish with extreme points. The difference between the actual and computed lengths was minimal, with a high degree of accuracy ($R^2 = 0.994$).

Conclusion

This method was able to reduce the computational complexity, allowing results to be obtained more rapidly and in a user-friendly environment. The power model generated the length-weight relationship, which allowed us to estimate the body weight of each olive flounder based on the length..

COUPLED AEROBIC AND ANAEROBIC GAS FERMENTATION PROCESS FOR CARBON-NEUTRAL SINGLE CELL PROTEIN (SCP)

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The food sector produces ¼ of anthropogenic climate impact. Low-carbon and carbon-neutral feed, particularly for aquacultures, can hence have a very strong impact for climate change mitigation. The methanotrophic strains *Methylomonas methanophilus V1* and *Methylococcus novellus V8* were used to obtain single cell protein (SCP) in continuous fermentation with CH_4 as sole carbon and energy source in an aerobic process. The maximum growth rate was 3.75 g/(l*h of biomass) (dry cell weight). Using CO_2 and H_2, experiments were carried out in a second anaerobic fermenter with *Thermoanaerobacter kivui* to make acetate. Process coupling is achieved via water electrolysis to H_2 and O_2, and fermenting the metabolic CO_2. This work demonstrated at the 30l + 30l scale that by coupling 2 gas fermentation processes, bacterial SCP and value-added side products can be obtained in a CO_2-neutral mode. The SCP has a crude protein content of 51-72%.

RELATIONSHIP BETWEEN BEHAVIOUR TRAITS AND RESISTANCES TO ACUTE HYPERTHERMIA AND HYPOXIA IN RAINBOW TROUT

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Introduction

A significant potential for genetic improvement of resistances to acute hyperthermia and acute hypoxia was demonstrated in rainbow trout (*Oncorhynchus mykiss*) through selection (Perry et al., 2005; Lagarde et al., 2022; Prchal et al., 2023). However, the physiological mechanisms underlying these resistances are still under debate. It is therefore necessary to understand what makes a genotype resistant by identifying the mechanisms underlying these resistances. In previous experiments (Lagarde et al., 2022; Prchal et al., 2023), we observed expression of a large behavioral repertoire during acute hyperthermia and hypoxia stress suggesting that behavior may be linked to resistances.

In the present study, we seek to understand whether resistance to acute hyperthermia and hypoxia could be linked to behavior and discuss the possibility of using some behavior traits as a novel way of phenotyping these resistances. For this purpose, we used the rainbow trout isogenic lines, previously shown to exhibit different levels of resistance to either acute hyperthermia or hypoxia (Lagarde et al., 2023)"ISSN":"00448486","abstract":"Global warming is expected to increase the frequency and intensity of heatwaves, resulting in more common combined acute hyperthermia and hypoxia conditions in fish farms. Such poor thermal and oxygenation conditions induce problems, including growth losses, increased pathogens pressure and mortality. Selective breeding is a promising solution to improve resistance to non-optimal water quality. Indeed, genetic variability to survive in acute hyperthermia or hypoxia conditions has been proved in fish. However, the characterization of these traits is not yet detailed enough to include them in a selection program. Here, we investigated the ranking stability of genotypes for acute hyperthermia or hypoxia resistances over age and between acute hyperthermia and acute hypoxia resistances. To this end, we established rankings of six isogenic lines of rainbow trout (Oncorhynchus mykiss.

Materials and methods

Six heterozygous rainbow trout isogenic lines were produced at the INRAE PEIMA experimental fish farm (doi: 10.15454/1.5572329612068406E12, Sizun, France). At 177 days post fecundation (dpf), 6 fish per isogenic line were randomly chosen and subjected to an individual challenge and then gathered in a group challenge under moderate heat stress. In individual challenge, fish were placed in 12-l aquariums backlighted by an infrared device. The aquarium was divided into two zones: on one side, a gated zone covered by an opaque plate (safe zone) and on the other side, an uncovered zone (risk zone). After a 5-min acclimation in the safe the zone, the gate was open and behavior recorded over 25 minutes. Immediately after the end of the individual challenge, the six fish were transferred in a 75 x 75 cm tank for group challenge. Temperature was gradually increased, from 12°C to 23°C in two hours and kept at 23°C for 0.5 hour. At the end of the challenge, fish were anesthetized, weighed and euthanized. Both tests were repeated three times for each line. Fish activity was video recorded and later analyzed with EthoVision XT15.0 software. A total of seven traits were measured in individual challenge: the maximum acceleration (ACC_MAX, $cm.s^{-2}$), the distance travelled (DIST_TRAV, cm), the zone-change frequency (FRQ_CHAN, #.min-1), the absolute meander (MEANDER, $deg.cm^{-1}$), the moving duration (MOV%, % of the time), the risk-zone duration (RISK%, % of the time) and the maximum velocity (VEL_MAX, $cm.s^{-1}$). In group challenge, 7 traits were measured: ACC_MAX, the average inter-fish distance (DISPERSION, cm), DIST_TRAV, the body contact frequency (FRQ_CONTACT, $\#.min^{-1}$), MEANDER, MOV, and VEL_MAX. A linear mixed model was fitted for each variable, with replica as random effect, time bins and line as fixed effects, body weight as covariate, and the interactions between time bins, line and body weight.

At 182 dpf, isogenic lines were phenotyped for acute hyperthermia and hypoxia resistances with a robust experimental design constituted of 150 fish per line and type of stress. The effect of isogenic line on acute hyperthermia and hypoxia resistances were analyzed by fitting a linear mixed model with replica as a random effect, line as a fixed effect, body weight as a covariate, and the interaction between line and body weight as explained in Lagarde et al. (2023).

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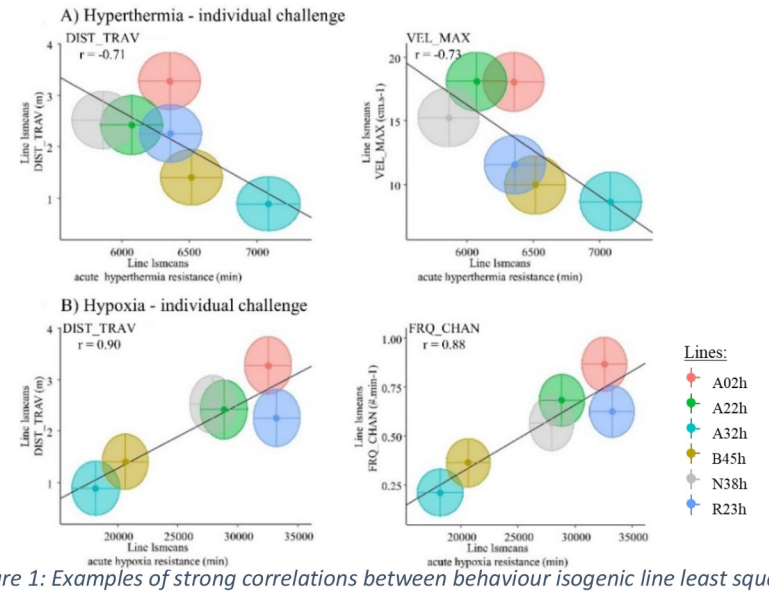

Figure 1: Examples of strong correlations between behaviour isogenic line least squared means and acute hyperthermia or hypoxia resistances isogenic line least squared means in individual challenges

Different individuals were phenotyped for resistance to acute hyperthermia and hypoxia and behavioral traits. Nevertheless, within each isogenic line, fish had the same genotype and could be considered as repetitions of the same genotype. Behavior traits for which least squared means of isogenic lines on behavioral traits were highly correlated with least squared means of isogenic lines on resistance phenotypes were considered as potentially genetically linked with resistance phenotypes and therefore discussed.

Results and discussion

There were significant differences between isogenic lines for most behavior variables. Some behaviors were found to be highly correlated with acute hyperthermia and hypoxia resistances (Figure 1). In general, lines with a higher activity level were found to be more sensitive to acute hyperthermia and more resistant to acute hypoxia than lines with a lower activity level. It suggests that some behavior variables could be used as proxies for acute hyperthermia and hypoxia resistances in fish, leading to more ethical phenotyping methods than classical resistances phenotyping ones.

These results must be confirmed in additional studies as only 6 isogenic lines were used.

Acknowledgements

This study was supported by the European Maritime and Fisheries Fund and FranceAgrimer (Hypotemp project, n° P FEA470019FA1000016).

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HEMATOLOGICAL ADAPTIONS OF *Salmo trutta* TO ELEVATED TEMPERATURE WITH SPECIAL REFERENCE TO ERYTHROCYTE MORPHOMETRY

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Introduction

Hematology is a simple but reliable method to determine the influence of thermal stressors on physiology and performance of fish (Fazio 2019). It is also used to evaluate the effects of temperature stress on fish. A recent study on *Salvelinus fontinalis* and *Oncorhynchus mykiss* demonstrated that erythrocyte size is affected by elevated temperature (Lahnsteiner 2022). Erythrocyte cell volume, nucleus volume, cell surface, and nucleocytoplasmatic ratio were significantly decreased after 32 days exposure to 20 °C in comparison to 9°C. The mechanism and significance of this adaption process are unclear at present.

Therefore, the present study continues the previous investigations. The study is conducted on the brown trout, *Salmo trutta*. Involvement of an additional species allows to detect if the above-described changes in erythrocyte morphometry can be generalized within the Salmonidae. Further, the present study focusses on the chronological sequence of changes in erythrocyte morphometry in response to elevated temperature. It is a goal to find potential regulation and compensation mechanisms. Complementary, growth rate and metabolic rate are investigated, too.

Material and Methods

Brown trout (total length 7.9 ± 0.5, body mass 4.9 ± 0.7 g), acclimated to 10 ± 0.2 °C and kept at a natural photoperiod during their whole life span, were used for this study. Experiments were carried out in accordance with Austrian regulations governing animal welfare and protection and with the EU directive 2010/63/EU for animal experiments.

A number of 250 brown trout, respectively, was stocked in 4 stream channels (190 x 25 x 35 cm, length x width x height) under flow through conditions (0.2 l/sec). Two stream channels were maintained at 10 ± 0.2 °C and served as control. The others were gradually tempered to 15 °C during a 7-d period using a geothermal heat pump and kept at 15°C for 28 d.

The percentage of fish tolerating the experimental conditions, the growth rate, and the metabolic rate were determined in 7-day intervals according to previously published methods (Lahnsteiner 2020b). Also, in 7-day intervals 10 fish per stream channel were haphazardly sampled and euthanized with MS222. Blood was collected via the heart ventricle. 10 µl of blood was fixed in 1 ml 0.1 mol l^{-1} cacodylate buffered 4% glutaraldehyde solution (pH 7.4). A differential blood cell count was made in a light microscope at 1000-fold magnification based on the classification of Fazio (2019). Erythrocyte morphometry was analyzed in twenty erythrocytes per fish as described previously (Lahnsteiner 2020a). Cell dimensions were measured by Image J analysis software and cell volume (μm^3), nucleus volume (μm^3), cytoplasm volume (μm^3), cell surface area (μm^2), volume to surface ratio, and nucleocytoplasmatic ratio, were calculated for the single erythrocytes (Lahnsteiner, 2020a). The mean values per individual were used for further statistical analysis. Data are presented as mean ± standard deviation. Data were analyzed by one-way ANOVA with treatment procedure as independent variables.

Results

Survival rate of brown trout was 98 ± 1% at 10°C and 97 + 2% at 15°C. Growth rate during the course of the experiment was 115 ± 4% at 10°C and 98 ± 5% at 15°C. Erythrocyte volume, nucleus volume, cell surface, surface to volume ratio and metabolic rate revealed specific changes during temperature increase from 10 to 15°C (0 - 7d) and during the exposure period at 15°C (8 - 28 d) (Figure 1). Concentration of erythrocytes, granulocytes, lymphocytes, thrombocytes and aberrant cells did not differ during the course of the experiment.

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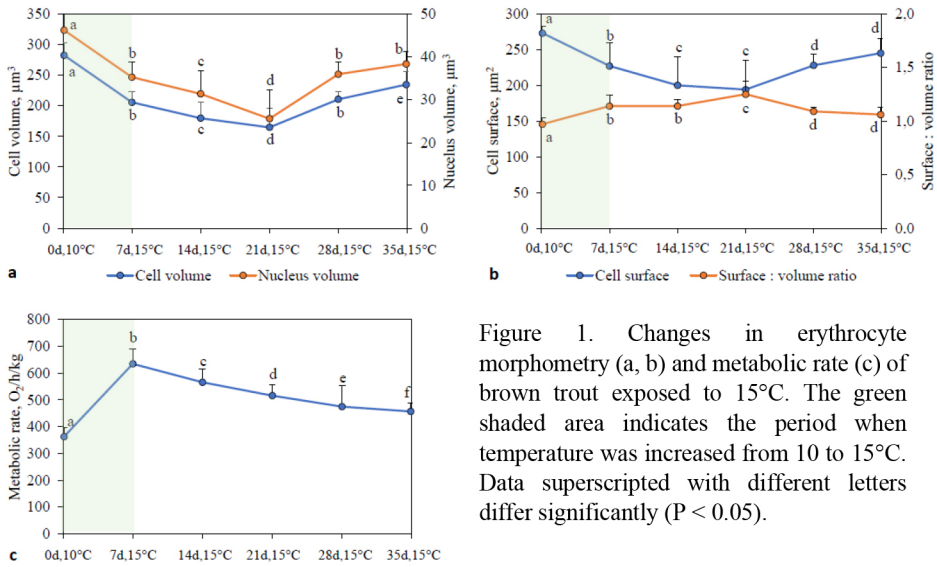

Figure 1. Changes in erythrocyte morphometry (a, b) and metabolic rate (c) of brown trout exposed to 15°C. The green shaded area indicates the period when temperature was increased from 10 to 15°C. Data superscripted with different letters differ significantly ($P < 0.05$).

Discussion

The results demonstrate that metabolic rate and erythrocyte morphometry of *Salmo trutta* change due to a temperature increase from 10 to 15°C. During exposure to 15°C recompensation processes occurred. This is a clear indication that brown trout can adapt to elevated temperature. However, the recompensation was partial as values did not reach the initial ones measured in fish acclimated to 10°C. The reduced erythrocyte size and the increased surface area to volume ratio may improve the oxygen uptake / release capacity of blood which is an advantage due to increased metabolic rate. A reduction of erythrocyte size may also be important in decreasing blood viscosity to optimize blood flow. During the course of the exposure to 15°C erythrocyte size increases again parallelly to the decrease in metabolic rate. This indicates that both processes are coupled with each other.

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SEAWATER GROWTH PERFORMANCE OF ATLANTIC SALMON *Salmo salar* SMOLTS PRODUCED IN A FLOW-THROUGH OR RECIRCULATING AQUACULTURE SYSTEM

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Introduction

Over the last 40 years, Norway has become a world leader in salmonid production, but despite the economic growth, farmers continue to experience high fish mortalities after post-seawater transfer (1). The transfer success of farmed post-smolt Atlantic salmon to sea is largely dependent on the smolt's physiological ability to counteract environmental challenges. Early life experiences, in particular, as a result of different rearing conditions, are known to differentially influence physiological responses and plasticity that lasts later in life (2)"ISSN":"00448486","abstract":"The transfer success of farmed post-smolt Atlantic salmon (Salmo salar. Over the last decade RAS systems have been adopted as a key technology in the production of juvenile salmonids and it is estimated that RAS fish currently make up the majority of the smolts stocked in Norwegian sea cages. In comparison to the more conventional extensive flow-through (FTS) system, RAS provide a more stable and controlled rearing environment, although it is not fully understood how this affect the physiological plasticity and ability to withstand abrupt and seasonal changes in environmental factors once the fish is transferred to the sea. In this study we compared growth performance, physiological traits, and environmental adaptation in Atlantic salmon (*Salmo salar*) transferred into sea cages from fresh water intensive RAS (high and constant temperature) to a similar group produced in fresh water under extensive FTS conditions (natural temperate water).

Materials and methods

Circa 300k fish from RAS and FTS systems, respectively, were released in 6 commercial cages (triplicates cages; 25 x 25 m, 40 m depth.) in one of the commercial breeding facilities of Lingalaks AS. Biometry data and biological samples were collected monthly, from before transfer to seawater and until slaughter (12 months later). Water conditions at different depths (temperature, salinity, current, pO_2, fluorescence) was collected by SAIV-CTD during the entire seawater production.

Results

There were no significant differences in growth and smolt development between FTS and RAS-produced smolts at the end of the freshwater phase. However, after transfer to SW the RAS fish showed several underlying physiological and molecular differences. Remarkably the final slaughter weight was 3646 ± 8.3 g in RAS compared to 4229 ± 70.5 kg in FTS fish; i.e 600 g less. A distinct lower HSI, and plasma levels of lactate and triglyceride were observed in the RAS smolts, before and after seawater transfer. RAS fish showed also lower NKA activity, and plasma levels of Na^+, Ca^{2+}, and P^+ compared to FTS fish once transferred in seawater, suggesting hypo-osmoregulatory maladaptation in the first months in seawater. Both FTS and RAS fish showed physiological adjustments during the seawater production which were mainly linked to the seasonal variation in water temperature, but compared to the FTS, RAS growth showed a stronger negative interaction.

Discussion

The physiological difference observed between the RAS and FTS fish once in sea cages was likely linked to pre-history water temperature in freshwater. The two groups were exposed to different environmental conditions in fresh water, likely allowing the FTS fish to develop a more physiologically plastic response to environmental changes than the RAS fish once in seawater.

In conclusion, the current study emphasises the crucial role that pre-history freshwater rearing conditions play in laying the foundation for the fish successful physiological adaptation to seawater, growth performance, and ultimately the success of farming production.

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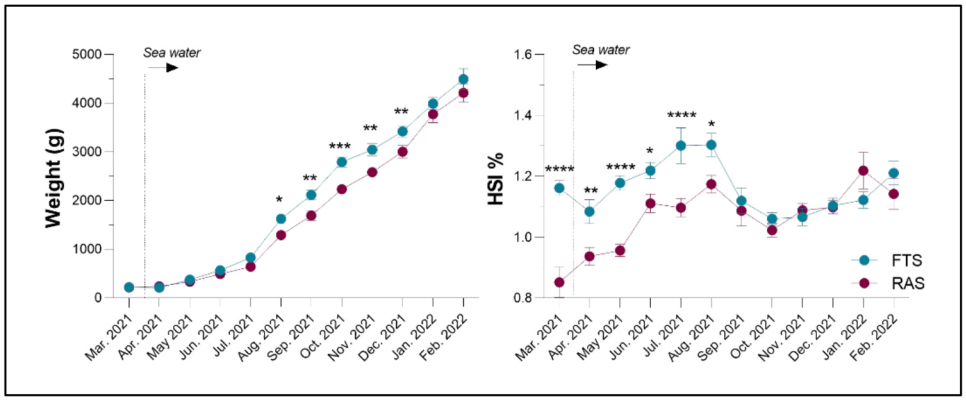

Figure 1 Growth performance of Atlantic salmon reared in the FTS or RAS system prior and after seawater transfer. Data represent mean ± SEM. n= 30/group.

Funding
Supported by Research Council of Norway (grant NoFood2Waste; No. 317770)

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2 Tang, P.A. *et al*. *Aquaculture* 560, 738458 (2022)

FEED INTAKE, GROWTH AND GUT TRANSIT IN ATLANTIC SALMON AT DIFFERENT TEMPERATURES- INVOLVEMENT OF CONTROL PATHWAYS FOR DIGESTION AND APPETITE

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Introduction

During the growth phase in sea cages, Atlantic salmon (*Salmo salar*) is subjected to diurnal and seasonal changes in environmental conditions. As an ectotherm animal, temperature is one of the most important environmental factors influencing and limiting metabolism, growth, energy allocation, and fish welfare. Temperature, in particular, has a strong influence on feed intake, digestion rate, and efficiency, as well as the required supply of nutrients for the fish to grow. However, little is known about the dynamics of gastro-intestinal tract (GIT) transit and how this relates to the signalling factors involved in digestive process control, and the links to appetite-controlling systems in the brain. Thus, we investigated feed intake and fish growth and assessed involvement of hormones and neuropeptides involved in GIT transit and the control of feed intake in Atlantic salmon reared at 8, 12, and 15°C.

Materials and methods

Post-smolt Atlantic salmon of approximately 200 g were reared to either 8°C (low-temperature), 12°C (control) and 15°C (high-temperature) for two months at the Department of Biological Sciences at the University of Bergen. Fish were fed once a day for 2 h using automatic feeders, and feed intake assessed by collecting and quantifying uneaten feed. At the end of the two months, 10 fish from each temperature group were collected 2 h post-feeding, followed by sampling every 4th hour for 24 h. Biometry data for growth and somatic indexes, plasma, GIT compartmental content, stomach tissue and brain samples were collected.

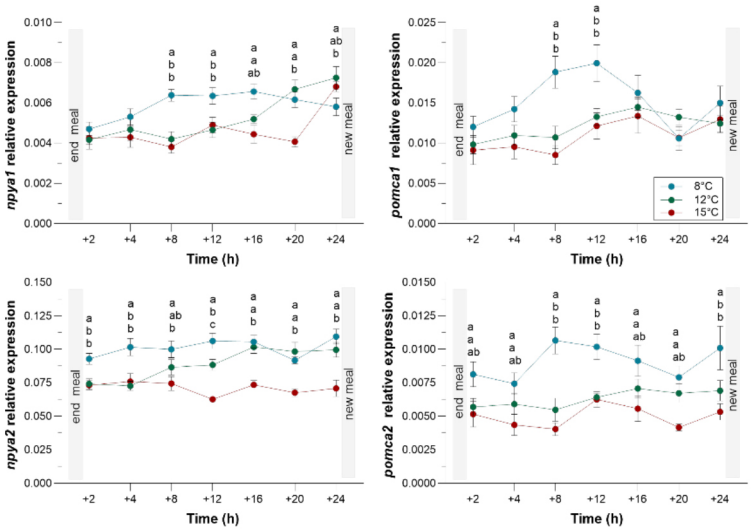

Figure 1. ***mRNA expression levels of npy (orexigenic) and pomca (anorexigenic) paralogs in the hypothalamus of Atlantic salmon at different rearing temperatures.*** *Graph points represent mean ± SEM (n = 10/group) of normalized mRNA copy number to the total ng of RNA for target gene, and the geometric mean copy number of actb and rps20.*

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Results

Temperature had a significant influence on growth rate, feed intake, and appetite-related key neuropeptides in post-smolt Atlantic salmon. The highest temperature groups showed better growth performance, feed intake and gut evacuation. Temperature influenced gene expression of appetite-related genes in the brain (Figure 1), which were found to be higher expressed during the postprandial period in the low temperature group, while the 12°C and 15°C groups had a higher during the pre-prandial period. Plasma levels of ghrelin, on the other hand, was statistically higher at 8°C than at 12°C or 15°C pre-prandial. However, this did not correlate to significant differences in the gene expression of ghrelin nor the enzyme that activates ghrelin (Ghrelin O-acetyltransferase) in the stomach.

Discussion

The different growth regulation and mechanisms underlying feed intake and appetite observed in the three experimental groups highlight the critical role of temperature in establishing the foundation for a physiological adaptation to the environmental condition. Temperature had a significant impact on post-smolt Atlantic salmon growth rate, feed intake, and appetite-related mechanisms. Several differences in the regulation of hormones and neuropeptide appetite key levels between the experimental groups were observed, with a significant difference between the low temperature group at 8°C, and the two groups at 12°C and 15°C.

Funding

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ANTISHARK MEASURES TO AVOID INTERACTION OF SPINY DOGFISH (*Squalus acanthias*) WITH AQUACULTURE INSTALLATIONS

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Introduction

The spiny dogfish is one of the common shark species found in Norwegian coasts and fjords. However, their interaction with aquaculture installation is a financial, welfare and ecological challenge. The fish farmers report that spiny dogfish often bite through the net and get in the fish cages. The holes in the cages cause escapees of farmed fish leading to both financial loses and ecological challenge. The dogfish are usually attracted to dead fish found at the bottom of the cages. But inside the cage, they also eat and harm the live farmed fish causing welfare challenge. To prevent this, farmers continuously remove dead fish and must constantly inspect the cages with the help of divers and underwater cameras for any holes. To date there is no effective method to prevent spiny dogfish incidents in fish farms. Hence, it is crucial to test and develop active and passive methods to prevent spiny dogfish incidents in fish farms.

Materials and Methods

Here, we have tested active measures that acts through sensory system. Spiny dogfish of size 60-85cm were caught and housed in laboratory aquarium (flow through) of size 2m diameter and 85cm water height. It was supplied with sea water at 9 °C; the light intensity was 10 lux at 10cm above the water surface. Experimental tank was equipped with a custom-made low light camera. The ectromagnetic (EM) field change, sound of orca (natural predator) and skin extract from conspecific were used as deterrent stimuli; an extract form mackerel was prepared and used as an attractive stimulus. The animal behavior is recorded before and after application of stimuli and analyzed for change in locomotive behavior.

Results

The spiny dogfish showed change in locomotive behavior (increased/decreased speed) in response to EM, skin extract and food stimuli; however, it showed no change in response to sound of orca. Food stimulus or smell of dead mackerel induced food-seeking behavior- sharks were found probing the odour inlet. Both EM and skin extract induced avoidance response- sharks moved away from the source area.

Discussion

Shark barriers and deterrents have been developed against specific species of sharks; these have been used to keep sharks away from bathing areas, to offer personal protection for swimmers, divers and surfers; some of these have also been tested to keep sharks away from bait and catch in line and net fishing. However, their effectiveness varies depending on the species and geographical area; none of the measures provide a full deterrence. Here, by evaluating the candidate shark-deterrents in a series of laboratory trials, we find that both EM and skin extract could be used as effective shark deterrent. Additional field trials are necessary to evaluate the effectiveness of these methods.

Acknowledgement

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IMPACT OF EARLY COLD TEMPERATURE ON GENOMEWIDE DNA METHYLATION PATTERNS IN JUVENILE RAINBOW TROUT DIVERGENT LINES FOR MUSCLE FAT CONTENT

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Introduction

Recent studies have shown that early exposure to environmental stimuli (such as hypoxia or temperature) could impact fish physiology, growth, metabolism and nutrition at mid-or long term. There are several possible mechanisms underlying this programming phenomenon, among which epigenetic regulations such as DNA methylation (Best et al. 2018). The objective of this study was to understand by which molecular mechanisms early cold exposure (at eyed-eggs stage) can impact fish physiology later in life (at juvenile stage), by analysing DNA methylation patterns in rainbow trout. This study was based on 2 experimental divergent lines for muscle lipid content (Quillet et al. 2005) that have been shown to utilise differently feed and to possess a well differentiated intermediary and energetic metabolism. Our hypothesis is that they will react differently to the cold exposure during incubation.

Materials and methods

At 17 days post fertilization (dpf), eyed-eggs from two experimental lines selected for high or low muscle lipid content, fat line (FL) and lean line (LL), were either incubated at normal temperature (11°C) or incubated at 3°C for 15 days in 12 tanks (2 lines x 2 incubation temperatures x 3 tanks). Hatching and the rest of the rearing was performed at standard temperature (11°C). Eyed eggs were collected at a similar stage of development (220 degree days; 19 and 31 dpf for the control and cold conditions respectively) and analysis of gene expression was performed by qPCR on 5 eggs per line, per incubation temperature and per tank, for 12 genes: *hsp47* (Heat Shock Protein 47), two *cirbp* (cold-inducible RNA-binding protein), *ucp2* (Uncoupling protein 2) and eight *dnmt3* (DNA methyltransferase 3). Genome-wide patterns of DNA methylation was assessed by RRBS (Reduced Representation Bisulfite Sequencing) on liver samples of 48 juvenile trout (2 lines x 2 incubation temperatures x 3 tanks x 4 fish per tank) collected at 189 dpf, snap frozen in liquid nitrogen and kept at -80°C until DNA extraction. Liver was chosen as it is the central organ for intermediary metabolism. RRBS libraries were prepared using MspI and size selection of 40-290 bp fragments and then sequenced on an Illumina NovaSeq6000 sequencer to produce 100 bp paired-end reads (Integragen SA, France). Trimmed reads were aligned to the current Arlee rainbow trout reference genome with the bisulfite mapping tool Bismark. Differential methylation analyses were performed using methylKit (qvalue<0.01, minimal methylation differences between temperature groups>20%). Identified DMCs (Differentially Methylated Cytosines) and DMRs (Differentially Methylated Regions) were finally annotated relative to gene features.

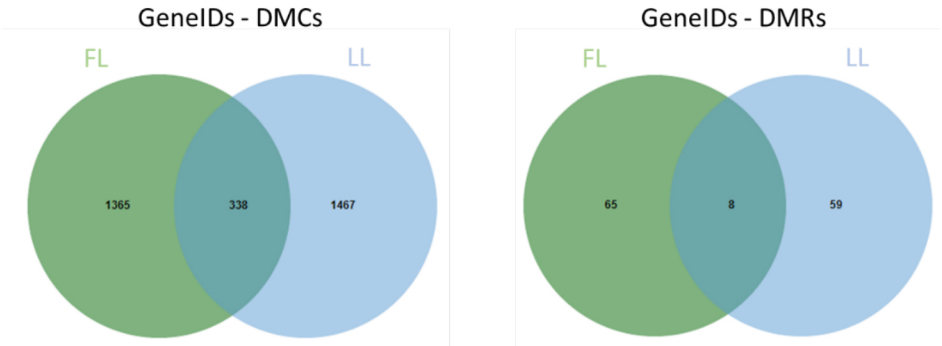

Figure 1. Venn diagrams of geneIDs found in DMCs and DMRs in the two experimental lines, FL (fat line) and LL (lean line).

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Results

Globally, there were significant but low differences in gene expression for 8 of the 12 targeted genes, with a minor impact of incubation temperature or line at eyed-stage. For the RRBS sequencing data, an average of 47 million paired-end reads were obtained per individual (lowest: 28 million; highest: 74 million). Bisulfite conversion rates were very high (>99.7%). Sequences that aligned to unique positions of the genome represented 50% of all reads on average and were used for subsequent analysis. Numbers of CpGs tested by methylKit (i.e. CpGs with a minimal number of 9 samples in each group satisfying the coverage range of 10-500 reads per CpG) were 1,273,685 for FL line and 1,240,712 for LL line. Differential methylation analyses between control and cold exposure groups revealed 3187 DMCs and 89 DMRs for the FL line; 3442 DMCs and 79 DMRs for the LL line. Annotation of DMCs and DMRs revealed that different genes were involved in the two genetic lines, with a certain degree of overlap (Figure 1).

Discussion

Using two divergent lines for muscle fat content allowed testing the impact of the genetic background on the establishment of DNA methylation patterns in response to early cold exposure. Preliminary results suggest that the two lines responded differently, although some genes with DNA methylation varying according to cold exposure were also found in common. Ongoing interpretation of the biological pathways involved will lead to a finer understanding of underlying mechanisms. This study will contribute to use early programming as a lever to improve long term performances of animals.

Acknowledgements

This study was supported by the European Maritime and Fisheries Fund and FranceAgrimer (EpiCOOL project, n° PFEA470018FA1000015). RRBS data analysis used a pipeline previously described by Perrier et al. 2018. We are grateful to the Genotoul bioinformatics platform Toulouse Occitanie (Bioinfo Genotoul, doi: 10.15454/1.5572369328961167E12) for providing computing and storage resources.

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BEFORE-AFTER CONTROL-IMPACT STUDY (BACI) OF BENTHIC EFFECTS OF A FISH FARM IN GREECE

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Introduction

Fish farm cages are commonly used as case studies for the assessment of the effects of organic enrichment, occasionally inducing hypoxic conditions below the cages as a source of disturbance for benthos. As a consequence, this might alter ecosystem services and biogeochemical processes, as well as the structure of benthic communities and result in loss of diversity (Quero et al., 2020; Keeley et al., 2013). However, as the distance from the fish cages increases, the concentration of organic matter and other pollutants decreases and the benthic communities gradually recover from disturbance, as is evident in the successional model of benthic enrichment responses for macrofaunal communities (Karakassis et al., 1999). The commonly used Before-After Control-Impact (BACI) methodology is a useful approach for evaluating fish farming effects through observational studies. By incorporating both time and control sites in the analysis, it minimizes the effects of unmeasured factors on the observed outcomes of the study (Seger et al., 2021, McDonald et al., 2000). The main objective of this study was to employ the BACI methodology to examine the environmental impacts resulting from the relocation of a fish farm's cages, which involved dividing and transferring a portion of the cages to a different, nearby, location.

Materials and Methods

A fish farm located in the northern Evoikos Gulf, operating for more than 20 years planned in 2020 to make a major rearrangement of the fish cages. Until summer 2020, the farm had five large clusters of cages located 50m apart from each over resulting in high precipitation of organic matter in a relatively small area. In 2020, two of the clusters were moved to an adjacent area 500m away, therefore creating two "parks" with the same total fish load as the original. Thus, a BACI study was conducted. Specific sampling stations were designated to assess changes in the environmental impacts of fish cages resulting from the reconstruction process. Benthic samples were collected on three sampling occasions: before the transfer in 2020, immediately after the transfer in 2021, and one year after the transfer in 2022. The sampling stations included the old and new cage locations, as well as control stations without any fish cages. Additionally, a sampling station was positioned near the cages that were transferred, but remained unchanged across time. This station was included to assess the impact of the two cage locations being in close proximity prior to the transfer, and to evaluate the subsequent recovery. Sampling at this location can provide an insight into whether the reduced impact due to the transfer of some of the cages affected the environmental conditions for the remaining cages in the vicinity. From these stations, benthic macrofaunal samples were collected by means of a Van Veen grab ($0.025m^2$), sieved through a 0.5mm mesh size sieve and preserved in 4% formalin. Furthermore, three replicates of every sample were collected from each station. Several environmental variables were also measured. These include temperature, depth, Redox potential (Eh), Organic Matter (OM) with the Loss of ignition (LOI) test, granulometry and Chlorophyll-a.

Results

Prior to the 2020 transfer, sediment redox potential values indicated hypoxic conditions at the impacted site, leading to low benthic diversity and poor ecological status. While there was some improvement in environmental conditions after the transfer, the ecological status at the old site remained moderate throughout the second year (2021), and the relocation of cages did not appear to have a significant effect. However, signs of recovery were observed after 2022 in both the old site and the station with unchanged cages, with a gradual reduction in organic matter, improvement in sediment redox potential values, and an increase in ecological status and benthic diversity. In contrast, pre-transfer, the new establishment area showed higher values of diversity and good ecological status, which was similar to the control station, but the new impact site's environmental conditions deteriorated post-transfer due to increasing impact.

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Conclusions

Based on the results of the study, it is concluded that the translocation of fish cages has a significant impact on the environmental conditions and benthic communities of the affected areas. It is evident that the relocation of cages can have both positive and negative effects on the environmental conditions and benthic communities of impacted areas. While dispersion of the cages can improve environmental conditions locally, relocation can also impact a new, undisturbed area. Further analysis is required to fully assess these effects and assess if the movement of cages improved in total the conditions of the fish farm area.

Overall, this study emphasizes the need for regular monitoring and evaluation of fish cage operations and their impact on the environment to ensure sustainable aquaculture practices as even a cages relocation event can significantly alter the biodiversity and environment of an area.

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EFFECTS OF FISH ASSEMBLAGE AND PRACTICES IN FRESHWATER FISHPOND POLYCULTURE: A TROPHIC WEB MODELING APPROACH

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Introduction

In Europe, freshwater pond aquaculture, which has high potential to meet the growing consumer demand for fish, is facing environmental restrictions, leading to a decrease in the sector's production. Monoculture and polyculture systems are facing many challenges to become more sustainable, that is, more productive, resilient, robust and mature, as well as providing more support for natural and cultural values, human beings and biodiversity (Dong *et al.*, 2022). Current aquacultural research recommends combining multiple species in polyculture, with one reference model being carp polyculture, first established in China. This type of polyculture originally focused more on the fish than on the other trophic compartments of the ecosystem. The SEPURE project aims at improving understanding of complex trophic interactions in pond ecosystems in order to identify innovative assemblages of fish species that use and recycle nutrients better. In this study, trophic web models were built for several polyculture fishponds with different management practices to develop a method to assess the structure and functioning of aquacultural ecosystems.

Materials and methods

Using data for 10 fish ponds (with fish fed or unfed) studied in different regions of France in 2021 and 2022, a model of the trophic web of each pond ecosystem was built using Ecopath software. Each pond was considered an independent case study. The ponds differed in feeding, fertilization, the amount of bird predation and the number of trophic compartments represented. The fish assemblages varied, including the common carp *Cyprinus carpio* and/or grass carp *Ctenopharyngodon idella*, roach *Rutilus rutilus*, rudd *Scardinius eruthrophtalmus*, tench *Tinca tinca*, largemouth bass *Mycropterus salmoides*, pikeperch *Sander lucioperca*, northern pike *Esox Lucius* or white sturgeon *Acipenser transmontanus*. The species selected and their ratios differed among ponds. Using Ecopath software, which models the balance of mass circulating among the compartments, each compartment's contribution to ecosystem productivity was studied. To assess each pond ecosystem, performance indicators such as ecotrophic efficiency (EE), total system throughput (TST), net system production (NSP) (i.e. respiration minus primary production) and system omnivory index (SOI) (i.e. the complexity of feeding interactions between trophic levels) were calculated. Indicators such as Finn's cycling index (FCI) (i.e. the percentage of TST recycled) and the ratio of primary production to respiration (PP/R) were then used to assess the maturity of the system.

Results

Initial model outputs predicted a variety of dynamics among the ponds. EE remained ≤ 1 for all trophic compartments of each pond, which is necessary for a model to be considered a satisfactory representation of a trophic web. The EE were high for macro-invertebrates and zooplankton in most ponds, indicating that they were key factors that limited fish growth in the ponds, even when the fish were fed. TST ranged from 513.8-1095.0 g/m²/cycle, indicating that the size and activity of the ponds differed, with the largest values indicating the highest turnover in the systems. SOI ranged from 0.17-0.21, which indicated many, and thus more complex, feeding interactions among the trophic compartments. NSP ranged from 140.5-264.4 g/m²/cycle, with the larger values indicating more immature systems that are still developing. In addition, PP/R ranged from 4.64-9.40, likewise indicating the immature state and small size of the ecosystems, with no clear differences between systems with fed vs. unfed fish. FCI ranged from 4.4-10.2%, which seemed to indicate high stability and resistance to external disturbances.

Discussion and conclusions

This study is a preliminary approach to build accurate reproducible mass-balanced Ecopath models of European freshwater fishpond systems. SOI, NSP and PP/R indicated that the structures of the food webs during the cycle were incomplete, which means that the primary production could not be fully used. The high variability in performance indicators seems to indicate room for improvement in management practices, fish assemblages and their interactions. Nevertheless, the model results provided guidance on better understanding the functioning of each ecosystem and reflecting on how to improve management of the systems. Analysis of the variety of fish assemblages provided new knowledge to help reconsider polyculture characteristics and potentially develop future experimental designs and recommendations for sustainable management of aquaculture.

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Acknowledgements
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NASAL MUCOSA OF FARMED FISH UNDER THE THREATS OF CLIMATE CHANGE

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Introduction

Climate change poses a significant challenge to aquaculture. In recent years, extreme events such as oceanic heatwaves and unpredictable environmental conditions reveal the need to understand the severity of these impacts and develop evidence-driven mitigation strategies to support the sustainable growth of aquaculture. Climate prediction models have offered insights into these consequences, however, many of these models require experimental verification. Despite significant strides made in recent years, there is still a significant knowledge gap on how climate-related stressors affect the health and welfare of farmed fish.

This study focused on the impacts of several climate-related stressors on the nasal mucosa of two relevant farmed fish in Norway, Atlantic cod and Atlantic salmon. The mucosa of the olfactory organ is considered the first of defence, as it constitutes the interface between the internal and external environments. In terrestrial vertebrates, nasal mucosa protects the host from airborne antigens and hazardous chemicals. Similarly, the olfactory organ of fish exhibits a similar defence function as it is directly and constantly exposed to the biological and chemical threats of the aquatic environment. This paper presents a series of studies on salmon and cod that reveals the consequences of climate-related stressors on nasal health.

Materials and Methods

Atlantic salmon trial: Fish were reared at three thermal conditions – Control group was at 12°C, High temperature group was at 17°C and the Heatwave group was reared at 12°C, then gradually increased to 17°C, then progressively lowered again to 12°C. The experiment was carried out over 2 months. There were 2 rounds of heatwave episodes in the trial. **Atlantic cod trials:** *A*) Interaction of elevated temperature and *Franciscella noatunensis* infection. Fish were reared at two temperatures – either 12°C or 17°C. Thereafter, fish were challenged with the pathogen, and the disease was allowed to develop for 5 weeks. *B*) Interaction of heatwave and ocean acidification. Fish were reared under 4 experimental conditions – 8°C, heatwave (8°C → 17°C → 8°C), 8°C + acidified environment and heatwave + acidified environment. Responses of the olfactory organ in all trials were studied at molecular and histological levels.

Results and Discussion

Elevated temperature altered the transcriptome of the nasal mucosa of both species. Genes related to immunity, extracellular matrix and stress exhibited striking regulation by heat stress. It appeared that in both species, heatwaves and not prolonged exposure to higher temperatures significantly affected the olfactory organ. For instance, in salmon, heatwave affected the genes involved in pathogen recognition, communication and signalling via chemokines and cytokines, humoral and cellular effectors, and development and differentiation of lymphocytes in the olfactory organ. In addition, the migratory potential of epithelial cells of the olfactory organ and the ability of the organ to respond to oxidative stress diminished following a single round of heat wave.

The transcriptomic changes in the olfactory organ of Atlantic cod can be considered local or remote responses to the systemic *F. noatunensis* infection. Five weeks after the challenge, the nasal transcriptome of infected fish, both from 12°C and 17°C, was almost similar to the uninfected control. However, comparing both infected groups identified 754 DEGs, many of which were involved in signalling, defence, transmembrane and enzymatic processes.

Increased temperature, heatwave, acidification and their combinations altered some of the structural features of the nasal mucosa. We found that both heatwave and prolonged elevated temperature resulted in mucus cell hyperplasia in the olfactory organ of salmon. Such a phenotypic response was not evident in Atlantic cod. Nonetheless, elevated temperature and infection showed lower mucus cell counts.

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This paper will also present evidence of how the nasal microbiota is altered by climate-related stressors. In addition, the changes will be compared with the transcriptomic alterations in the olfactory organ. These analyses are currently ongoing.

Conclusion

The results reveal that the olfactory organ of Atlantic salmon and Atlantic cod was sensitive to climate-related stressors. Both species shared a number of common mechanisms in response to these stressors, but some distinctions were also identified. These climate-related stressors affected the immune responses in the olfactory organ, indicating that mucosal barrier functions are likely at risk to future climate scenarios. The results are expected to shed insights into the physiological consequences of climate change in these species, which will be valuable in developing targeted climate mitigation plans that are supported by both predicted and empirical data.

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MARINE BACTERIA AS RELEVANT PROBIOTICS FOR FISH FARMING: GENOMIC CHARACTERIZATION, ANTIMICROBIAL SCREENING AND *in vivo* ASSESSMENT ON EUROPEAN SEABASS

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Introduction

Intensive aquaculture and globalization of trade contribute to the emergence and spread of infections in fish farms. In a context of restriction of antibiotics use, probiotics could represent an interesting bioalternative to control infections and improve the zootechnical performances while minimizing the environmental impact. We investigated the probiotic potential of sporulated bacteria extracted from the marine environment on seabass, *Dicentrarchus labrax*, one of the major marine fish species produced in Mediterranean.

Methodology

Bacillus strains were screened *in vitro* and fully sequenced to identify their potential probiotic properties and assess their safety. The efficacy of probiotics was evaluated by dietary supplementation of juveniles seabass (average initial weight : 5g) with the candidates of interest for several months at 21°C. During the supplementation, their ability to persist in the intestine, their effects on growth (length and size), immune system (blood formulation, phagocytosis activity) were evaluated. After two to five months of supplementation, some fish were experimentally infected with *Vibrio harveyi*, *Vibrio anguillarum* or nervous necrosis virus (NNV) to evaluate their disease resistance.

Results

Genome analyses confirmed the absence of antibiotic resistance and pathogenicity genes and revealed interesting capacities of production of amino acids, vitamins and digestive enzymes. Strains selected showed antibacterial properties against various genus of pathogenic bacteria isolated from farms, associated with the identification of gene clusters encoding secondary metabolites with potential antimicrobial activities.

The survival of probiotics in the intestinal tract of supplemented fish was confirmed with the detection of concentrations up to 1.10^5 CFU per intestine at different time. Probiotic supplementation increased survival rates after infection with *Vibrio harveyi* and *Vibrio anguillarum*. However, survival rates are not impacted for NNV infection. Furthermore, fish supplemented with probiotics showed significantly higher leukocytes counts, with an increased proportion of phagocyte (expression of immunity genes ongoing).

Conclusion

The *In vitro* and bioinformatics analyses performed confirm the probiotic potential and the safety of the marine strains of *Bacillus* selected. These probiotics have beneficial effects on components of the immune system and improve resistance to bacterial pathogens of juveniles sea bass.

NUTRITIONAL-INDUCED AMINO ACID TRANSPORTERS DYSREGULATION IN RAINBOW TROUT IN VITRO: THE BUTTERFLY EFFECT ON GLOBAL AMINO ACID HOMEOSTASIS?

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Introduction

The development of Rainbow Trout (RT) aquaculture industry in combination with limited availability and high prices of fishmeal has prompted feed producers to include more plant proteins (PP) in aquaculture feeds. However, PP do not meet the necessary nutritional requirements of animals, in particular due to imbalances in Amino Acid (AA) profiles compared to fishmeal, the historical ingredient. Although representing building blocks of proteins, AA also have many crucial roles as signalling molecules as well as substrates for cellular metabolism. However, the maintenance of cellular AA homeostasis begins with the absorption and transmembrane traffic of AA within cells, ensured by Amino Acid Transporters (AAT). While characterized in mammals, in which regulation of AAT by nutrients, including AA, has been demonstrated[1], few data exist in fish to date. The aim of this study was to 1) identify for the first time all AAT expressed from RT genome and 2) address their regulations by nutrients, and mainly by AA, to evaluate whether AA imbalance profiles could impact AA homeostasis. To reach these objectives, AAT were first identified in RT genome *in silico* before assessing their expression in different RT tissues, while the study of their regulations by nutrients was conducted by taking advantage of a cellular model: the RTH-149 cell line. Such approach, already validated for the study of AA metabolism in RT[2], allowed us to decipher AAT regulations independently of systemic regulations that could occur *in vivo*. Finally, consequences of nutritional AA-induced AAT dysregulations were investigated through their impacts on intracellular AA contents as well as on the mTOR (mechanistic Target Of Rapamycin) signalling pathway, a major regulator of cell metabolism, also described for being controlled by AA availabilities[3]. Results presented hereafter, not only allowed us to refine AAT classification according to their nutrient-dependent regulations but also highlight several interdependencies regulated by AA availabilities, AAT and signalling pathways and their potential physiological outcomes in fish fed with alternative protein sources.

Materials and Methods

First, potential AATs in the RT genome were identified by *in silico* analysis and primers were designed for each AAT, before being tested and validated on a pool of RT tissues including brain, gut, stomach, liver, ovary, adipose tissue, kidney, spleen and muscle. *In vitro* experiments were performed using the RT hepatocyte cell line called RTH-149 (ATCC® CRL-1710, LGC standards, Molsheim, France). Cells were subjected to 13 experimental conditions using Hank's balanced salt solution (HBSS, #14025-092, Gibco) supplemented in combination or not with total AA, Non-essential AA, Essential AA, Foetal Bovine Serum or using MEM deficient in single AA (c4086, Genaxxon Bioscience: -Arginine, -Lysine, -Methionine, -Leucine) as well as 3 conditions with MEM supplemented with pharmacological activators/inhibitors of key signalling pathways (Halofuginone and Tunicamycine, activators of the Integrated Stress Response pathway, and rapamycin, a mTOR inhibitor). Cells were treated for 24h prior being collected and subjected to the following analyses: RTqPCR analyses of AAT expression, UPLC intracellular AA content analyses and Western Blot analyses of 4EBP1 and S6 phosphorylation, two known targets of mTOR. Data gathered from these 3 sets of analyses were cross-analysed (Pearson correlations, generalised linear models) to define correlations and fitting models that could help to decipher relationship between AA availabilities, AAT dysregulations, AA uptake and mTOR activation.

Results

From the 71 AATs identified in mammals, 212 were found in the RT genome, while at least 116 were expressed in RT tissues. Of the 116 AAT expressed *in vivo* in RT, 74 are identified in the RTH-149 cell line covering 86% of AAT specifically found to be expressed in the liver. All AAT expressed in RTH-149 were shown to be dysregulated by at least one of the experimental conditions tested, the vast majority of which being under the control, positively or negatively, of AA and/or FBS. Interestingly, our study revealed an antagonistic response between two major classes of AAT: Cationic AAT (CAAT)

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being predominantly upregulated by starvation, while Anionic AAT (AAAT) were mainly downregulated, which is mainly explained by AA, and more specifically on essential AA. According to the whole set of dysregulations observed in the 13 conditions tested, a hierarchical clustering of AAT expressed in RTH-149 cells has been generated highlighting the existence of 3 main groups of AAT according to their nutritional regulations. Following the analysis of the outcomes of nutritional-induced AAT dysregulations on intracellular AA contents together with effects on the activation of the mTOR signalling pathway, we potentially identified AAT candidates that appear as the main gatekeepers controlling AA homeostasis, and certainly their cellular functions.

Discussion

For the first time, AATs in RT have been identified in RT genome and characterized for their expression in several RT tissues while *in vitro* assays performed in RTH-149 cells allowed us to refine the current AAT classification by providing new insights in their regulations by nutrients. These results confirm the key role played by AAT in the maintenance of cellular homeostasis, but more significantly illustrate the butterfly effect that a deficiency in a single AA can have. Indeed, we observed that single AA starvations induce dysregulations of large pools of AAT, which consequently strongly influence the intracellular content of many AA and thus the global cellular AA homeostasis. More widely, this questions the actual outcomes of diets with imbalanced AA profiles, when compared to fishmeal-based diets, on the physiology of farmed trout. If such observations are to be soon confirmed (work in progress), results gathered from this study provide a wide understanding of AAT regulations by nutrients in RT, but it also has the potential to supply a new method to assess, in a unique so far, comprehensive way to study AAT functions and activities in cells, and that, disregarding the species considered. Thus, such method could be useful not only for the fish nutrition field but for any biological study related to AAT and AA cellular functions which are still deeply required but overlooked due to notably to the lack of tools and methods[4].

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FATTY ACID ACCUMULATION IN THE FLESH OF JUVENILES *Lipopeneaeus vannamei* SHRIMP FED WITH DIFFERENT OIL SOURCES

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Introduction

Omega-3 (ω-3) are a demonstrated as an essential fatty acid (FA) family for human as their synthesis is either not possible for the a-linolenic acid (ALA, C18:3 w3) precursor or very weak starting from the precursor for the eicosapentaenoic acid (EPA, C20:5 w3) and docosahexaenoic acid (DHA, C22:6 w3) long chain derivatives. They must therefore be supplied by foods. Seafood products are the major source of ω-3 long chain derivatives in human diets and shrimp *L. vannamei* may contribute to this intake. Shrimp flesh fatty acids (SFFA) composition is influenced by feed fatty acids (FFA) composition. Omega-3 sources are rare and expensive, it is therefore essential to optimize their utilisation to produce sustainable and cost-effective feeds.

In this context, the present study aimed to screen the relative part of ALA and EPA among the total SFFA and their kinetics of assimilation regarding two different sources of dietary lipid: linseed oil (LO) and krill oil (KO) respectively. According to IAFFD, a reference database for raw materials composition, LO possesses 53.3%ALA and almost no EPA while KO includes 2.4%ALA and 17.4%EPA.

Materials & Methods

Two batches of experimental feeds were manufactured by coating 3% of the tested oils (LO or KO) on a control feed (37.3% crude protein and 7.8% fat).

135 juveniles of *L. vannamei* (0.1 g) were randomly distributed in 3 tanks (45 individuals/tank) resulting in a total biomass of 4.7±0.1g per tank.

They were acclimatised in the tanks for 7 days with the control feed. A first sampling of 3 individuals per tank was performed at day 0 (9 shrimps). Then, juveniles were fed with the experimental feeds: 1 tank with control+3%KO, one with control+3%LO and one with the control. The juveniles were fed at 5% of their estimated biomass per day. During the next 2 weeks, 4 samplings were made of 5 juveniles per tank for a total of 60 shrimp (20 shrimp per treatment). The SFFA composition was determined by Gas Chromatography with Mass Spectrophotometry (GC-MS) after extraction and saponification of the lipids of the abdomen.

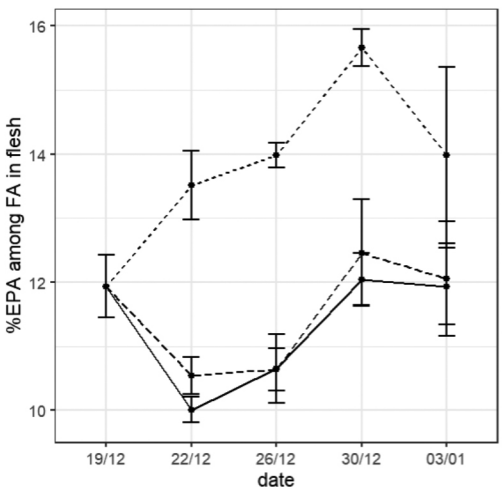

Fig. 1- %EPA among FA in flesh

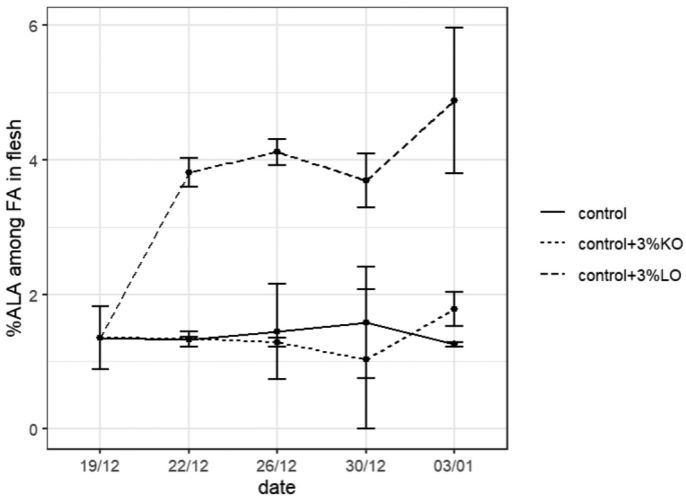

Fig. 2- %ALA among FA in flesh

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Results and Discussion

The results showing the % of ALA and EPA as a function of time are presented in Fig.1 and Fig.2, respectively. The SFFA profile matches the FFA profile obtained with GC-MS and the FA oil's profile described by the IAFFD database. These results suggest that coating is an efficient process to include specific FA in shrimp's flesh.

Moreover, the relative part of ALA and EPA in shrimp flesh fed with control+3%LO and control+3%KO respectively increase by 1,8 % ALA for the juveniles fed with control+3%LO and by 2,5% EPA for the juveniles fed with control+3%KO between the 2 first samplings (T0 and T+3). This result suggests that the kinetics of assimilation of ω-3 in shrimps flesh is quick.

Further experimentations with shorter sampling intervals are needed to precise these results.

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CHARACTERIZATION OF DETECTION AND ATTRACTIVITY BEHAVIOR OF THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Introduction

Litopenaeus vannamei is an omnivorous shrimp species with a scavenger tendency. Its clear preference for feed containing high proportion of marine animal products lead to unsustainable feed formulas. Continues and impressive efforts have been made by feed formulators to spare marine proteins with alternative raw materials. One of the actual limits of this process is the poor attractivity and palatability of those ingredients for the shrimp. There is a consequent need to characterize feed ingredients based on their capacity to attract the shrimp toward sustainable pellets. Ethograms such as the one established by Lee and Meyers (1996) could be improved thanks to new observation methods.

The goal of this study was to observe and analyze individual behaviors of shrimp confronted with pellets or dissolved amino acids (AA). This work was divided into two experiments: the first (Exp I) looked at the behavior of shrimp when confronted with feed pellets and the second one (Exp II) looked at their behavior when confronted with free AA dissolved in water.

Materiel and Methods

Twenty PL20 *L. vannamei* were bought in a commercial shrimp farm and acclimatized in Halieutica research station (Angers, France).

In Exp I, 14 shrimp were tested individually in a random order. Each animal was successively placed in a close tank filled with artificial marine water (salinity 30ppt, temperature 27°C) and was acclimatized for 5 minutes. At this time, experimental pellets were introduced in the tank. Animal behavior was recorded from above for ten minutes (Fig. 1). Videos were analyzed in order to find characteristic behaviors of detection and attractivity.

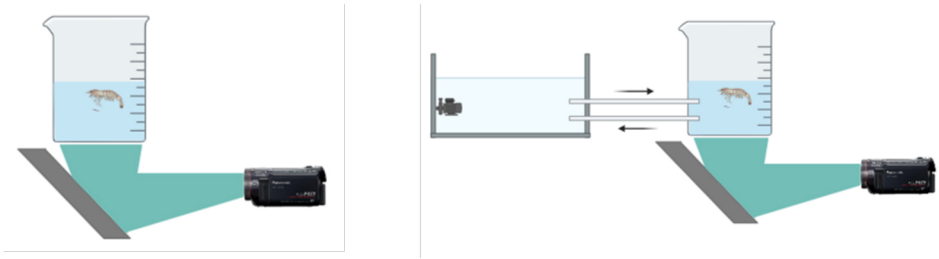

Fig. 1: diagram of devices used for Exp I and Exp II

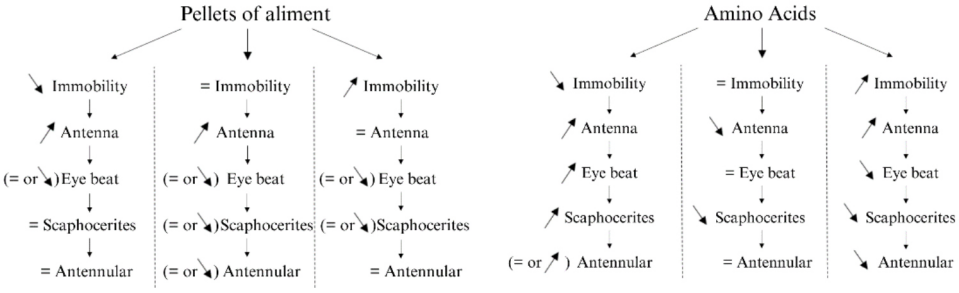

Fig.2: Ethogram of detection and attractivity behavior of L. vannamei *shrimp confronted with pellets (Exp I) or MFAA (Exp II).*

(Continued on next page)

In Exp II 6 individuals were tested individually in a random order. Animal were placed in a small recycling aquaculture system made of two tanks. Animal were kept in a first tank, which was the same tank as in Exp I. The second tank was used as a buffer tank. After 5 minutes of acclimatization, different quantities of a dissolved mix of free amino acids (MFAA, KERASTIM® 50, BCF Life Sciences, France) were placed in the second tank (0ppm; 30ppm; 60ppm and 90ppm). Water movements between the two tanks were allowed by a peristaltic pump (Fig. 1). Animal behavior was recorded for 5 minutes before the adding of amino acids and 5 minutes after. Video recording and analysis were performed the same way as during Exp I.

For the observed behaviors, statistical analysis have been performed in order to show a potential effect of the concentration of MFAA, and an effect of the adding of pellets. Permutation test and Wilcoxon post hoc were made using RStudio (version 4.2.1).

Results

For both experiments, the main behaviors observed were immobility duration, antennal, antennular and scaphocerites flicking and eye beats.

In Exp. I none of those behaviors were significantly impacted by pellet addition. A tendency was however observed for antennal and antennular flicking, eye beat and immobility duration. In Exp. II, behaviors significantly influenced by the concentration of MFAA dissolved in water were immobility duration and scaphocerites flicking. A tendency was shown on antennal and antennular flicking.

Animal behaviors analysis resulted in a classification of the individuals within three different groups for each stimulus (Fig.2).

Discussion/Conclusion

Results obtained through this preliminary study did not allow us to create a clear ethogram. There was a strong individual variability in the studied behaviors. It could be the sign that every shrimp behave differently. Thus, it could be interesting to further this study with a higher number of individuals or to test individuals ingroups.

Acknowledgements

This work is a partial result of the KERASCREEN project, supported by the Région Pays De La Loire.

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ZOOTECHNICAL BENEFITS AND HEALTH FONCTIONALITY OF GRADED INTAKE OF A YEAST-BASED PARAPROBIOTIC IN EUROPEAN SEABASS

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Introduction

Functional health feeds are a pillar of modern aquaculture. Inactivated yeast cell wall (YCW) fractions are widely documented to conditionally confer health and/or performance benefits across a diversity of species. However, only few dose-response studies have been reported and none in marine species limiting the optimal application of this biotechnology. The aim of this study was to document the animal performance and mucosal health response to graded intake of a specific YCW paraprobiotic with the view to inform the strategic use of this ingredient. The specific YCW tested in this study was previously selected based on its specific parietal features and promising functional benefits on the intestinal barrier and immune homeostasis (YCW 2; Rawling et al., In Press).

Materials and Methods

The 9-week trial (Riasearch Lda; Murtosa, Portugal) tested 4 diets in quadruplicate tanks (500 L tanks flow-through indoor, pumped-ashore natural seawater ~22 °C; ~36 ppt) in juvenile European seabass (BWi = 11.3 g; 45 fish/tank) reared under ideal conditions (43 days) then chronically exposed to environmental and handling stress (20 days) (**Fig 1**). The basal diet was formulated to the species requirements and according to current commercial practices (55% plant meal, 20% fish meal; 15% LAPs; 6% fish oil, 6.5% rapeseed oil; 46.3% crude protein; 16,4% crude lipid; gross energy 21.1 MJ/kg). Test diets were prepared by incorporating graded level of a selected, proprietary YCW ingredient (Lallemand Animal Nutrition, Blagnac, France) "on-top" the basal recipe at 0 (Control), 1.5; 3.0 or 4.5 kg/T feed and manufactured by traditional hot extrusion. Test diets were hand-fed to apparent satiation 4 times daily over the trial's duration.

Growth and feed performance were assessed over phase I, phase II and the overall trial's duration based on total tank biomass, individual body-weight (15 fish/tank) and apparent daily feed intake per tank. Tissues were sampled at T1 and T2 as follow: crude skin mucus quantity (4 fish/tank, surgical absorbent sponges), skin mucus, faeces, gill, gut and skin (3 fish/tank). Skin mucus and plasma were assayed for immune biomarkers; faeces for intestinal health markers; mucosae histology focussed on morphometry and goblet cell population and mucosal gene expression was performed using tissue-specific panels of 20 genes (immune, cellular and oxidative stress, epithelium barrier function). Data were analysed by 2-way ANOVA and post hoc analysis with significance accepted at $P < 0.05$.

Results

Growth performance increased with increasing YCW level (**Fig 2a, b**). Mean body-weight was significantly higher in YCW 4.5 compared to the Control at the end of Phase I and II, with YCW 1.5 and 3.0 showing intermediary values. Similar trends were evident for SGR although differences were significant over the full trial period only for YCW 3.0 (+4.5%) and YCW 4.5 (+6.8%) compared to the Control.

There was a significant diet effect on FCR which was increasingly improved with increasing YCW intake over the whole trial's duration (**Fig 2c**). FCR overall improved by up to 8.3 % in YCW 4.5 compared to Control with, interestingly, an apparent mitigation of its degradation over the period of chronic-stress exposure (Phase II) in this group.

Skin mucus excretion was significantly higher in YCW 4.5 compared to the Control at the end of the trial (**Fig 2d**) with numerical increases at lower YCW intakes. Similar trends were measured at the end of the Phase I (ideal conditions).
Further tissues analysis will be presented.

Conclusion

There was a positive dose relationship between dietary intake of YCW and zootechnical performance as well as skin mucus excretion. In particular, the highest level of YCW tested (4.5 kg/T feed) appeared to mitigate the degradation of the zootechnical performances but also to boost skin mucus excretion during chronic-stress exposure.

Taken together, this YCW paraprobiotic as the potential to support the performance and robustness of European seabass juveniles. The feed supplementation strategy can be tailored to the levels of challenge expected and of benefits targeted.

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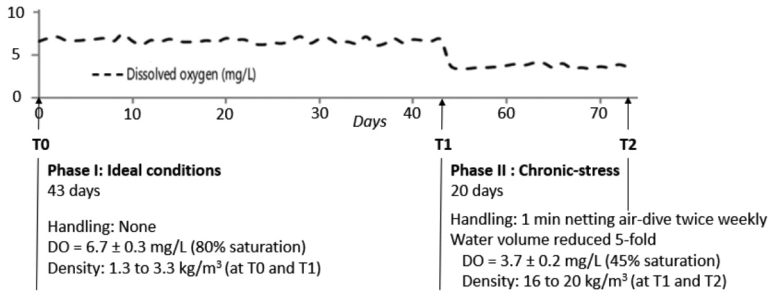

Fig 1. Schematic representation of the experimental periods.

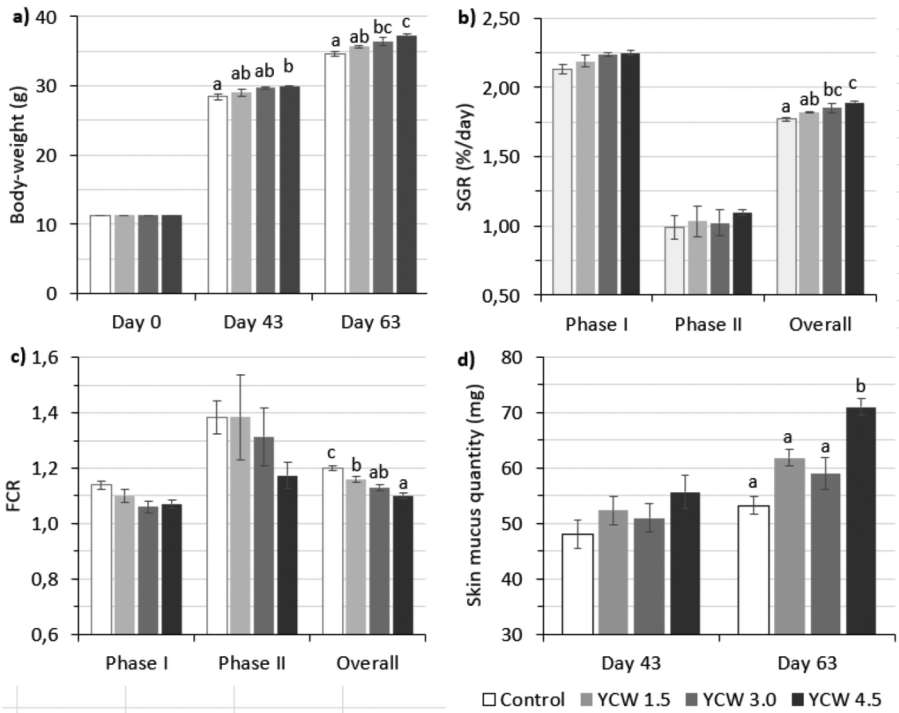

Fig 2 a) Mean body-weight**, b)** Specific Growth Rate**, c)** Feed Conversion Ration and **d)** Skin mucus excretion at the end of or within each experimental period (Day 0 to 43: Phase I, ideal conditions; Day 43 to 63: Phase II, chronic-stress exposure. Data given as mean ± SEM (n = 4). Different letters highlight significant differences between diets.

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CAN WE DISCUSS ABOUT CIRCULAR ECONOMY IN THE ANGLING SECTOR? CASE STUDY THROUGH A HUNGARIAN EXAMPLE

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Introduction

Over the last decade, the need for a circular economy has emerged and is now pervading the day-to-day functioning of the economy. It is attracting increasing attention, will and action from economic and social actors, not only in the EU but also worldwide.

The definition of a circular economy is relatively simple: it is a system or set of systems that does not generate waste material and whose products today (whether they are consumable or usable immediately or even waste material) are the raw materials of the future. The current complex material and energy flow systems are primarily linear units (production-use-disposal), whereas in the circular economy, materials are recycled in various forms back to the production line. The circular economy, even before the term was coined, was instinctively practised by many families and individuals: composting and using kitchen waste as fertiliser at home, or separate collection of waste, are all part of this system, necessary but not sufficient solutions in themselves. Addressing the problem requires more complex, longer-term strategies and planning by all economic actors to make measurable progress (Henriksson et al., 2021).

In the agricultural sector, circular economy and farming is a priority, and there is already a clear move towards conscious thought and action, with the development and deployment of technologies offering different solutions well underway, but the principle of starting with the basics of production technology is the same in almost all sectors.

The aquaculture-angling aspects of the circular economy have recently begun to be examined at a general (global) level. The problem is still being identified and solutions explored primarily at the theoretical level, but pilot systems are being set up to analyse causes and possible solutions in real time and space (Chary et al., 2021).

At EU level, the aquaculture sector operates in an autonomous system, independent of agriculture, given the particular importance of marine fisheries, in particular those with "industrial characteristics". However, this approach cannot be applied to domestic, Central and Eastern European, pond-based fish production units and fishing ponds. Pond fish production and pond fishing cannot be separated from agriculture and the natural and built environment. They must be treated as one and only as such a complex entity can the complexity and simplicity of this system be understood (Muscat et al., 2021).

Materials and methods

The research was based on a 31-question questionnaire. This questionnaire aims to explore the popularity of the circulating system of angling ponds and, in addition to popularity, the attitude and openness of pond owners and pond keepers towards this issue. The research covers general knowledge as well as more specialised areas of knowledge and expertise.

The 56 completed questionnaires aim to develop a comprehensive picture of the attitudes of pond owners and managers towards the issue of pond management, which is representative of the current and potential future situation of the sector in the Hungarian angling sector.

Results and conclusion

A high percentage of those working in angling waters do not know what circular economy/farming is (44.7%). The owners of angling ponds are reluctant to open up to circular systems, and in order to change this and to reach more people with this knowledge, it will be necessary to organise and hold awareness-raising lectures on this topic.

From the answers given to the practical questions in the questionnaire, it was found that 23% of pond managers do not carry out any water quality monitoring, which can have a significant impact on the success and sustainability of their business.

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Focusing on agro-technical interventions, it was found that 13.6% of respondents do not use any interventions on their water bodies. The majority of respondents are not concerned with the quality and quantity of feeds (baits) applied to the water, despite being mostly aware of their harmful effects.

The last and one of the most important observations in the research is the question of education and training. Only a certain percentage (35.8%) of fishing pond managers have some form of education, a lack of knowledge and skills that may be fundamental to the management of problems and the use of technologies. 77% of the respondents would like to participate in further training on angling issues and are open to the learning process.

Historically and based on tradition, aquaculture, and within it pond fish farming, represents and embodies a significant part of the principles of the circular economy. A significant proportion of the fishing ponds in Hungary fall into this category - pond farming - and the findings on fish farming are therefore also applicable to these ponds in general. In addition, this sector is in an excellent position with regard to the various emission standards (emission limit values). Considering that the EU's objective of achieving sustainable agriculture will be a mandatory requirement for agriculture, the angling and pond farming sector is at an advantage. This advantage needs to be reviewed and developed in a targeted way, based on basic information, a thorough mapping of the initial situation and a careful definition of development proposals.

The work is supported by the iFishIENCi project (European Union's Horizon 2020 research and innovation programme under grant agreement No 818036).

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PLANT-BASED DIETS SUPPLEMENTED WITH SUSTAINABLE PROTEIN SOURCES FOR ENVIRONMENTALLY FRIENDLY EUROPEAN SEABASS IN ADRIATIC SEA

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Introduction

Over the last three decades, aquaculture has become the fastest growing sector of the food industry, providing one-third of the seafood consumed worldwide (FAO 2018). An upward trend in the consumption of farmed European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) has led to a significant increase in their production worldwide over the past decade, and these species are now among the most important European aquaculture products (WWF, 2021). However, the farming of carnivorous fish species, such as seabass and seabream, depends on large quantities of fish meal and fish oil, as optimal sources of protein and lipids to ensure normal growth, and consequently on small pelagic fish as the basic ingredient of fish meal and fish oil. This ultimately led to additional fishing pressure on already endangered stocks of small pelagic fish and a significant increased the market prices for fish meal and fish oil (Rana *et al*. 2009). European aquaculture is therefore faced with the challenge of maintaining a balance between the increasing demand for fish production and the need to alleviate pressure on natural fishing grounds. One of the obstacles to the adoption of sustainable aquaculture is the lack of sustainable fish feed ingredients, whose production would be economically and environmentally sustainable while providing optimal nutrients for the farmed fish themselves itself (Aas *et al*. 2016; Luthada-Raswiswi *et al*. 2021). Recently finalized Interreg project Enhancing Innovation and Sustainability in Adriatic Aquaculture (AdriAquaNet) project, funded by the INTERREG V-A 2014-2020 Italy-Croatia program was dedicated to enhancing the innovation and sustainability of Adriatic aquaculture, through development of sustainable feed forumations. Within the project, new feeds were designed and tested at a laboratory scale on sub-adult European sea bass.

Material and methods

Two types of fish feed formulations were developed and tested, one based on vegetable protein and one based on animal protein (fish meal and fish oil): a plant protein-based diet (CV), two plant-based diets in which graded amounts of vegetable protein mixtures were replaced with *Hermetia illucens* meal alone (VH10) or in combination with poultry by-product meal (PBM) (VH10P30), a fishmeal diet (CF) and a fishmeal diet supplemented with *H. illucens* (FH10). The experiment lasted 147 days, after which the effect of the test feed formulations was evaluated by growth, muscle tissue composition, skin color, gut morphophysiology, digestive enzyme activity, and gut bacterial community composition.

Results and discussion

Fish fed VH10 and VH10P30 showed the highest specific growth rates and lowest feed conversion ratios of all groups tested. Fish fed CV diet exhibited significant degenerative changes in the proximal and distal intestine. However, PBM supplementation significantly improved all gut morphometric parameters in the VH10P30 group. Partial substitution of the vegetable mixture with insect meal alone or PBM also induced most BBM genes and stimulated the activation of BBM enzymes, suggesting a beneficial effect of *H. illucens* /PBM on intestinal digestive/absorption functions. Fish fed diets containing *H. illucens* meal also had the highest richness of bacterial communities and increased abundance of beneficial genera such as *Lactobacillus* and *Bacillus*. On the other hand, fish fed CV, although having the highest microbial diversity, lost one of the major components of fish intestinal microbiota, phylum Bacteroidetes. Skin pigmentation most similar to that of wild seabass was also observed in the group of fish fed VH10P30. Our results showed that feed formulations based on vegetable ingredients enriched with *H. illucens* meal, both independently and in combination with a meal derived from poultry by-products, have the potential to be an alternative feed for farmed European seabass, without affecting cellular homeostasis, growth, and overall fitness and even providing some beneficial effects. However, the results of the study also highlighted the importance of animal proteins in the diet of carnivorous species, as the addition of a small amount of insect and poultry meal to plant-based feeds significantly improved all measured parameters compared to the experimental group to which no insect and/or poultry meal was added.

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Acknowledgments
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INFLUENCE OF DIETARY BETA-GLUCANS ON HEALTH STATUS OF FISH AFTER EXPERIMENTAL CHALLENGE

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Bacterial and viral infections in fish including koi herpesvirus disease and furunculosis are among serious problems of modern aquaculture. A number of substances with potentially positive effects on the fish health have been tested in order to reduce the use of chemotherapeutic agents. In recent years, attention has been focused on beta-glucans, too. These are polysaccharide derivatives of bacteria, fungi, yeasts, algae and plants that play an important role in immunomodulation, especially in the non-specific immune response. Their effects have been assessed in various fish species.

In our experiments we assessed the effects of beta-glucans in the feed on haematological, biochemical and immunological parameters of rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*), as well as on the fish ability to resist infection. In common carp, beta-glucan concentration of 0,1 and 0,5 % were tested. After 7 weeks the fish including control group were challenged by *Cyprinid herpesvirus 3*. In rainbow trout beta-glucan concentration of 0,2; 0,5 and 1 % were tested. After 7 weeks the fish including control group were challenged by *Aeromonas salmonicida*. The fish were supplemented with beta-glucans for other 5 weeks. Mortality, fish behavior and feed intake were monitored throughout the whole experiment. Blood samples were taken and used for analyses of haematological, biochemical and immunological parameters. The level of lysozym was determined from skin mucus samples.

If positive effects on fish health are confirmed, the tested food with supplement of beta-glucans could be used for preventive treatment of the fish in intensive aquaculture systems.

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MULTIFUNCTIONAL USE OF FISHPONDS: THE HUNGARIAN EXPERIENCE

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Introduction

Pond fish farming is by by far the most widespread culture method of raising finfish globally. It is also the dominant type of fish production in Hungary just like in several other Eastern European countries. However, pond fish farming - similarly to other food production sectors - faces new challenges such as need for resilience to climate change, increasingly responsible use of water and land resources, adaptation to unexpected external impacts and changing market conditions. Multifunctional use of fishponds can be a possible response to these challenges as some examples demonstrates. However further efforts are needed to explore the opportunities offered by multifunctional pond fish farming.

Structure and function of the Aranyponty Fishfarm as a pioneer in multifunctional pond fish farming

Aranyponty was one of the first aquaculture companies established after the transition of Hungary in 1989. In 1993, the family-owned business acquired the nearly 1000ha large fishpond system called Rétimajor. Besides the challenge of reconstructing an extremely neglected farming infrastructure, the owners also had to adapt to the fact, that the farm is part of the Duna-Ipoly National Park area as well as other international environmentally protected network. The biodiversity attracted by the large fishponds are unique, with special emphasis on aquatic birds, mammals, and amphibians. However, most of these animals are also competing for resources – dominantly fish – with the farmer. Since these ponds are used for production and not for feeding wildlife, a delicate balance had to be found.

The uniquely attractive landscape with its abundant wildlife created another opportunity, which is eco-tourism. In today's extremely fast urban lifestyle, Rétimajor provides an opportunity to relax and reconnect with nature. However, most of the visitors are not looking to completely isolate themselves from civilization, therefore certain hospitality facilities were needed, including a restaurant, accommodations, and other services. These developments in turn brought along another demand by the customers, which was safe, healthy, and locally produced food products, including meats, seasonal fruits and vegetables.

Status and perspectives of multifunctional pond fish farming

Based on extensive survey in Hungary and other countries in Central and Eastern Europe and Asia, where pond aquaculture has a great importance in food fish production, it was found, that managers of pond fish farms and competent experts believe that he role of multifunctionality and diversification will increase. The development of multifunctional pond fish farming is basically driven by the stability and economic growth inherent in the diversification of activities, but it is also important to create harmony between farming and the natural environment. Although fish farmers consider the production function to be the most important, nevertheless they are well aware of the importance of ecosystem services and are ready to strengthen this function if they receive measurable financial recognition. Within the fish production function, fish farmers consider the production of healthy and safe fish products to be the most important but are aware of the importance of animal welfare and ethical functions which indicates the need to meet consumer needs at the highest possible level. Analysing the ranking within the environmental functions, it can be seen that the "conservation, maintenance and protection of natural values / resources / ecosystems, biodiversity and valuable natural habitats" was considered by farmers to be by far the most important in the "regulatory" and "landscape preservation" functions. Analysing the significance of social roles, it can be stated that fish farmers consider the dissemination of knowledge about the aquatic environment and fish to be of paramount importance, preceding even the "recreation and tourism" function, recognizing that raising interest in fish also serves their business interests.

Conclusions and recommendations

Multifunctional pond fish farming is a good development opportunity to maintain sustainable production of species low on the value chain and also the ecosystem services provided by fishponds. Operation of multifunctional pond fish farms also contribute to increasing the awareness of the benefits of pond aquaculture including the valuable contribution to the preservation of natural values and to the enrichment of biodiversity. Aranyponty Fishfarm in Hungary is an excellent example of a well-managed multifunctional pond fish farm. The farm in cooperation with research institutions is ready to collaborate with partners from countries where pond fish production dominates to develop management strategies for the better exploration of opportunities offered by the multifunctional use of fishponds.

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EUROPEAN-ASIAN BUSINESS COLLABORATION IN POND FISH FARMING

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Introduction
Freshwater pond fish farming is a dominant type of aquaculture both in Asia and in Eastern Europe. There have been several collaborations between the two regions in freshwater aquaculture development that included expert consultancies, research and development and training. Hungary has also been active in aquaculture development cooperation with Asian countries, especially with Laos and Vietnam mainly through bilateral institutional collaboration and some specific EU projects (e.g. Aquasem; Eurastip). When Hungary became EU member, new opportunities became available to strengthen economic and business collaboration financed by the Hungarian International Development Cooperation fund and also by tied aid loans provided in the frame of OECD rules. An important objective of the tied aid loan projects was to assist the business development of SMEs both in Hungary and in Laos. Thus, the main contractor of the project was the Hungarian Vitafort Agro Asia Company. The results, experiences and personal contacts gained through traditional institutional collaborations provided a good basis for business partnerships. A good example of such business cooperation is the establishment and operation of an aquaculture joint venture company in Laos, the ADC Aquatic Development Company Ltd. An old fish fingerling farm near the capital city of Vientiane in Nam Houm village was upgraded by projects financed by the Hungarian government, however Hungarian and Lao investors established a joint venture company for the long term operation of the farm.

Establishment and operation of a pond fish farm for the production of high-quality fish fingerling
ADC Aquatic Development Co. Ltd. was established in 2015 as a Lao-Hungarian joint venture company. The Hungarian parent company of ADC is Aranyponty Zrt., one of the largest private aquaculture producers in Hungary, with over 30 years of experience in freshwater fish production.

The primary activity is the production of high-quality tilapia fingerlings to supply the local fish farmers. ADC is also involved in several other activities, including the establishment of common carp breeding with Hungarian genetic background, diversification of farmed species with indigenous fish, training and education of local farmers, research and development of aquaculture production technologies.

Contribution to commercialization of aquaculture in Laos
The development of the high-quality fish seed supply has been integrated into the overall development of the fish value chain in Laos that was the objective of the aquaculture component of the Hungarian tied aid loan project between 2019-2023. The development of the fish value chain also included primary activities such as: development of grow out, feed supply, processing and marketing, and secondary activities such as training, demonstration, R&D and innovation. ADC has also been participating in the implementation of both primary and secondary activities contributing to the fulfilment of the project's goals.

The company has been actively involved with several NGO and donor funded projects in the field of capacity-building in aquaculture. ADC was an important partner of *CRS (Catholic Relief Services*) and *WFP (World Food Programme)* in their "school lunch" programs in the Northern and Southern parts of the country, providing advisory and training services. With the support of the *Hungarian Tied Aid Loan* projects, ADC was assigned to provide training and development services in four provinces in the Northern part of the country, resulting in successful aquaculture businesses.

ADC is also a member of the *SUN (Scaling Up Nutrition) Business Network*, a UN organization aimed at improving nutrition related issues in less developed countries. In 2021 the company was awarded the "UN Best Small Business, Good Food for All" title, a competition where 50 companies were chosen worldwide to become examples of good food production practices. The same year, ADC signed a working agreement with the *National University of Laos* and the *National Fisheries Development Center* to start a public private partnership with the goal of Human Resource development and R&D activities in the field of aquaculture.

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Conclusions and recommendations

It is always challenging to start a business in a foreign environment, let alone one that is different in basically every aspect, culturally, politically or even climatically, just to mention a few. The most important tasks of starting a foreign aquaculture investment in a less developed country is to take the time to assess the opportunities and the business environment in the country as well as finding trustworthy local partners. We were lucky in both regards, due to the long-standing relationship between Hungary and Laos.

Despite the careful evaluation work that has been done before the investment, difficulties still had to be faced. These included the understanding of the works of local government institutions, the mindset of the local employees and the local farmers.

ADC is planning to expand its activities in the field of fish breeding by developing a country-wide network of partner nursery farms. However, this will require extensive HR development work, to ensure the same product quality throughout the country. The company continues to work in close cooperation with active local NGO's, including FAO and WFP.

VALIDATION OF A TEMPERATURE-MATURATION MODEL FOR *M. gigas* IN SWEDISH WATERS: IMPLICATIONS FOR CULTIVATION AND MANAGEMENT

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Introduction

The Pacific oyster, *Magallana gigas,* has been present along the Western Swedish coastline since 2006. However, sea-based cultivation is prohibited to prevent further growth of wild populations. Understanding the reproductive cycle of *M. gigas* in Sweden is the first step towards changing legislation and building management strategies for cultivated oysters. A model based on cumulative heat exposure (Mann, 1979), predicts the progression of gametogenesis in *M. gigas*. It postulates that surpassing a threshold temperature (t_0) of 10.55˚C will initiate gametogenesis and 592-thermal degree days must be accumulated before spawning can occur. This study aims to validate this model for accurate prediction of maturation and spawning in *M. gigas* in Sweden.

Materials & Methods

Over 18 months, 15 wild-collected oysters were harvested every 2 weeks from a submerged culture system. Biometric data, histology, and image analysis were conducted on each oyster to classify the reproductive stage and quantify gonadal material for identification of spawning.

Results

Two consecutive reproductive cycles of *M. gigas* were described for the first time in Swedish waters, with two spawning events occurring in the first cycle. Gametogenesis was initiated before the previous cycle ended and continued throughout the winter. Subsequently, the threshold temperature was adjusted to represent the proliferation period when there was a significant increase in gonad fullness. Evaluation of the temperature maturation model found that using t_0=10.55˚C correctly predicted full maturity after 592-degree days in both years.

Conclusion

This study provides fundamental knowledge for developing an *M. gigas* cultivation industry in Sweden. Predicting oyster spawning will enable strategic management such as pre-spawning removal of cultivated oysters and cultivation in suitable temperature zones. It could also inform timely collection of wild spat for hatcheries. Further in-situ validation studies are needed to refine the threshold temperature in relation to continuous gametogenesis and proliferation.

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A MODELING FRAMEWORK TO INVESTIGATE THE SUB-LEATHAL EFFECTS OF PARASITISM ON FISH: THE CASE OF SEA LAMPREY PARASITISM ON LAKE TROUT

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Introduction

Parasitism is a common occurrence in aquatic ecosystems, where fish often serve as hosts to a variety of parasites. Fish parasites include protozoans, worms, crustaceans, and external parasites like leeches, lice, parasitic fish that influence their hosts through complex and varying mechanisms. These parasites can reside internally or externally, deriving nutrients and protection from their hosts while exerting detrimental effects on host fish, sometimes through reduced growth, impaired reproduction, and altered immune function. Subsequently, fish parasites can have substantial negative implications for fisheries and aquaculture operations.

Understanding the sublethal effects of parasitism on fish performance is crucial. While empirical measurements at the molecular, cellular, or tissue level provide valuable information, they alone are insufficient to grasp the whole impact on individual fish performance. Parasitism is energetically demanding for hosts, and to model the energetic consequences and connect them to individual effects, the dynamic energy budget (DEB) theory is a valuable tool (Kooijman, 2010). To demonstrate the relationship between parasitism and individual processes, we present a case study involving sea lamprey parasitism on lake trout (*Salvelinus namaycush*)), a species of concern in the Laurentian Great Lakes that is cultured for reintroduction and conservation efforts (Firkus et al., 2023).

Material and Methods

To investigate the impact of parasitism on fish growth and reproduction, we first developed a base DEB model (Figure 1) that accounts for energy allocation and dynamics throughout the fish's life cycle, considering the energetic trade-offs influenced by their life history. This model expands upon the standard DEB model (Kooijman, 2010) and incorporates three feeding life stages (larvae, juvenile and adult), as well as metabolic accelerated development during early stages (Lika *et al.,* 2014, Stavrakidis-Zachou, 2019). Additionally, as we focus on the effects of parasitism, a significant stressor impacting maintenance costs, our model also includes rules that account for energy utilization when available energy in the κ fraction is not sufficient to meet somatic maintenance demands.

After parameterizing the base DEB model with available data (Marques et al., 2018) for the target fish species, the next step is to identify the physiological mode of action (pMoA) and specific DEB parameter(s) through which parasitism affects the fish‘s life history (Ashauer and Jager, 2018), based on empirically observed endpoints, such as alterations in length, weight, and ovarian mass. Once the relevant DEB parameter(s) is determined, it is necessary to establish a relationship, referred to as „damage," between the stressor (parasitism) and the model parameter(s). For the sea lamprey parasitism case study on siscowet lake trout growth and reproduction, we used data from studies on the long-term effects of non-lethal sea lamprey attack. The model was parameterized to capture the observed changes in energy allocation towards growth and reproduction in parasitized lake trout. It incorporated an estradiol module to describe the conversion of reproductive reserves to ovarian mass based on estradiol concentration. In our DEB model, parasitism increased maintenance costs, reduced estradiol levels, and lowered the efficiency of converting reproductive reserves to ovarian mass. Additionally, muscle lipid composition influenced energy mobilization and reproductive efficiency.

Results

The model successfully replicated key life history characteristics specific to the siscowet lake trout ecomorph and produced model estimates that aligned well with data collected from field and laboratory studies. The proposed pMoA accurately captured the observed changes in ovarian mass and growth. The model scenarios demonstrated that parasitism led to reduced ovarian mass across muscle lipid concentrations, consistent with findings from laboratory investigations. Importantly, the model also captured how parasitism leads to skipped spawning in host lake trout.

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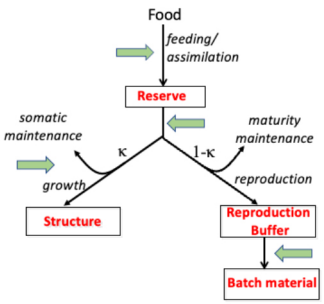

Figure 1. Conceptual representation of the metabolic processes. Energy assimilated from food is added into reserves and subsequently allocated to fuel the metabolic processes: a fixed fraction κ of the mobilized energy flux is allocated to somatic maintenance and growth and the remaining (1−κ) to increase and maintain maturity or to reproduction. The green arrows indicate the potential impact of parasitism on metabolic processes via the DEB parameters.

Conclusions

Parasitism is a complex stressor for host species, affecting multiple physiological processes simultaneously, which complicates the assessment of cumulative impacts on growth and reproduction. The DEB approach presented here offers a means to interrogate these cumulative effects, as well as interactions with other factors such as food consumption and muscle lipid concentrations. By employing this approach, we gain a comprehensive understanding of the overall parasitism-driven changes in growth and reproduction that can be used to better anticipate the effects of parasites on wild fisheries and aquaculture operations.

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GENETIC VARIATION IN GROWTH BETWEEN *Seminavis robusta* STRAINS ASSESSED THROUGH CROSSBREEDING

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Introduction

Micro algae show a large potential to become a future food and feed ingredient. They can be cultured at large scale and hence be a sustainable option to harvest oil rich wild marine species. with stable access and quality of essential fatty acids, not limited by nature (Ganuza et al., 2008). Currently, culturing microalgae is not economically competitive to other fat and protein sources (Ruiz et al., 2016). Optimization of the culturing protocol is thereby necessary, including strain selection and optimization. For micro algae that do sexual reproduction, like the diatom *Seminavis robusta*, new strains can be generated through crossbreeding of existing strains and strain selection hence be made from a broader selection. If successful, this process can be repeated each generation to form a breeding program. The aim of this study was to document whether genetic variation between existing strains is inherited to the next generation after crossbreeding.

Materials and Methods

Eight strains of *Seminavis robusta* were used, four male and four female strains, coming from three different clades, derived from analysis of the rbcL gene. Each male was crossed with each female, to produce as many novel combinations as possible, but not all crosses were successful, producing 64 cross-bred cultures, from 11 full-sib families. The parental and offspring strains were grown in 2 replicates in a 12 days growth experiment where biomass development was measured through CY5 fluorescence data on day 0, 3, 6, 9 and 12, using Cytation™ 3 plate reader and imager from BioTek. Cell-length on day 0 was measured for 30 random cells from each culture. Based on visual inspection of the parental growth curve (Fig. 1), three growth parameters were selected for analyses: growth from day 0 to day 6 (Gr06), growth from day 0 to day 9 (Gr09) and growth during the 3-day interval where this line had the highest growth (MaxGr). The growth traits were analysed with a general linear model in R, fitting replicate, cell-length (as a proxy for culture age) and whether the crossing was performed within or between clades as fixed effects together with the fixed effects of the two parents.

Results

The effects of parents were significant for all three growth parameters ($p<0.01$), demonstrating that genetic variation in growth between the strains exists and is passed on to the next generation when crossing the strains. Replicate and whether the cross was an inter-clade crossing was non-significant ($p>0.05$) for any of the studied traits. Cell length was significant for MaxGr ($p<0.05$), where cultures with longer cells, i.e. younger cultures, grew significantly better than cultures with shorter cells. By comparing r^2 from models with and without parents fitted in the model, the effect of the two parents together could explain 0.25, 0.22 and 0.32 of the total variance in Gr06, Gr09 and MaxGr, respectively, indicating heritability spanning from 0.44 to 0.65.

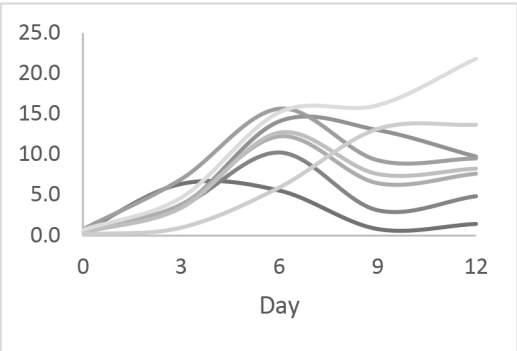

Figure 1 – Growth curves for the parental lines

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Discussion and Conclusion

This study clearly showed a heritable variation in growth between the *S. robusta* strains which was herited from parents to offspring when crossing parents. Hence, selective breeding for growth is expected to be successful. Among the growth traits explored, MaxGr gave the highest heritability, estimated to 0.65. The parental growth curves (Fig. 1) showed that the strains differed in when they reached exponential growth as well as the growth rate in the exponential phase. The fact that MaxGr has a higher heritability than Gr06 and Gr09 indicates that the achieved maximum growth rate is controlled more by genetics and less by other random factors than the time when exponential growth is reached. MaxGr is also the only growth definition significantly affected by average cell-length in the culture. Gr06 and Gr09 seem more affected by other random variables, not controlled in this study, causing larger residual variation. This study is a proof of concept that growth in micro algae may have a significant and considerable genetic component which can be utilized for strain development and optimization. Crossing of existing strains can be used to generate variation to select among when the species of interest do sexual reproduction.

Acknowledgments

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EFFECTS OF POPULATION AND PHOTOPERIOD ON EARLY SPOROPHYTE GROWTH IN SUGAR KELP

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Introduction

Sugar Kelp (*Saccharina latissima*) dominates as a commercially produced seaweed species in Norway, but relatively little is known with regards to how different populations perform in standardized growing conditions. Variation for fundamental traits such as growth are of particular interest to the aquaculture industry as raw material for breeding programs to enhance. In this study, we evaluated the growth performance of young sporophytes from two geographically distant Sugar kelp populations in Bergen and Tromsø.

Materials and Methods

Wild broodstock algae were collected from two areas in Norway; 25 individuals from outside Bergen, located at the west coast, and 25 from outside Tromsø, north in Norway. The sporophytes from Bergen, which under natural conditions would have matured earlier, were collected in November and transferred to flow-through seawater tanks at Havbruksstasjonen in Tromsø for ~2 months to delay sporulation. In late December, wild Tromsø broodstock algae were collected and spores from the two populations were released at the same time, separately, producing offspring populations of the same age. Two months later, ~800 young sporophytes from each population were identified, measured and divided into 10 flow-through seawater tanks with overhead lighting. Two photoperiods were tested: 8 hour light per day and 12 hour light per day. After 7 weeks of growth under this regime, all sporophytes were imaged on a hyperspectral imaging platform from which total area and average spectral absorbance was calculated. Surface area at the end of the experiment was analysed with a linear mixed model in R, fitting population, light-regime, interaction(populationxlight) and length at the start of the growth period as fixed effect and tank as a random effect together with the random residual.

Results

All fixed effects tested in the model were significant ($p<0.01$). The Bergen population grew significantly better than the Tromsø population, under both light regimes (Figure 1). The effect of light regime was larger for Tromsø than for Bergen. Initial length at start of the experiment also differed between populations. The Tromsø population showed reduced growth also in the very early stage and produced fewer sporophytes than the Bergen population. Spectral analyses reveal that the mean absorbance of sporophytes from each population were significantly different from one another at light wavelengths from ~450-675nm, even after accounting for differences in size (Figure 2).

Discussion and Conclusion

These results demonstrate that sugar kelp from different regions of Norway display different phenotypic traits when grown under the same conditions. This may be a consequence of genetic differences between macroalgae collected from different Norwegian regions, although it could also be a result of poor viability of the Tromsø sporophytes, shown by fewer and smaller offspring produced from the same number of parents. However, significant growth of both populations under both light regimes shows both offspring populations were viable with growth potential, supporting the hypothesis that the differences between the populations are genetic.

The growing season for sugar kelp in Norway is during winter and spring but the bulk of growth takes place during the latter half of this period when day length increases. The high latitudes of Northern Norway experience more variation in day length than coastal environments in the West. Even though we tested early sporophyte growth in this study, the results show that population from the northern region were already at this stage more sensitive to photoperiod, which could be a sign of adaptation to different regions in Norway. The distinct patterns of spectral absorbance of the two populations support this theory: suggesting chemical compositions to be affected from origin even when grown in the same environment.

Acknowledgments

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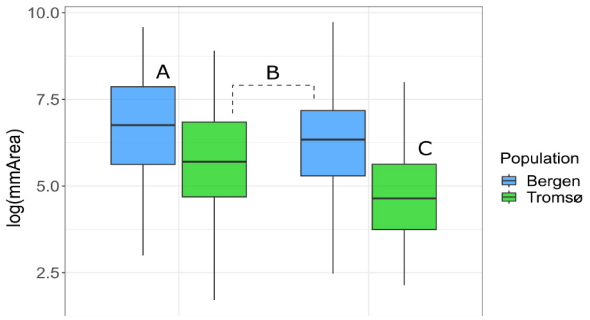

Figure 1 – Box plot of surface area

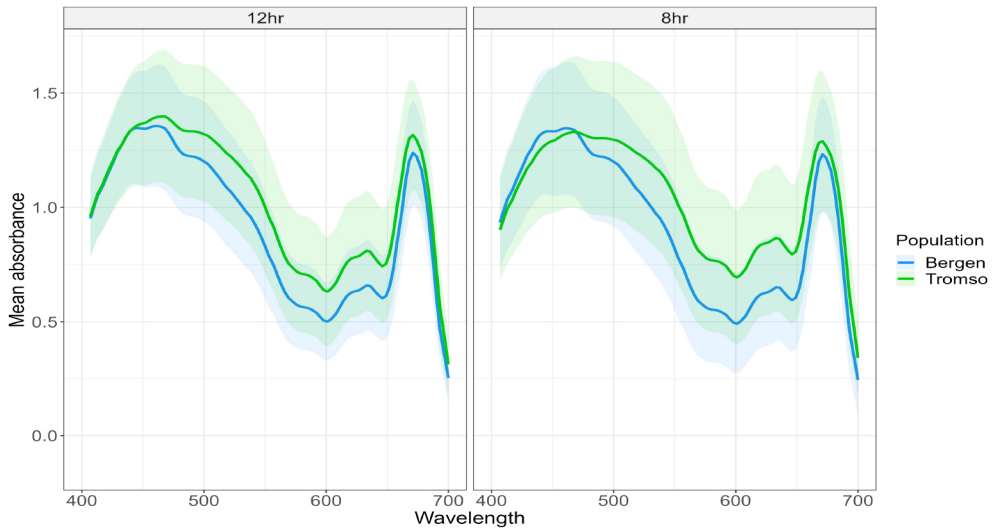

Figure 2 – Spectral absorption of the two populations under each light regime

QUALITY OF RAINBOW TROUT (*Oncorhynchus mykiss*) REARED IN RECIRCULATING AQUACULTURE SYSTEM AND DURING DEPURATION BASED ON CHEMICAL AND SENSORY ANALYSIS

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Introduction

In recirculating aquaculture systems (RAS), off-flavors can accumulate in fish muscle tissue. Dozens of compounds have been identified, including alcohols, aldehydes, and terpenes. They affect the perceived sensory properties in fish and cause earthy, musty or other unwanted flavor to fish flesh. Off-flavors are problematic for consumer acceptance and the reputation of farmed fish products. Accumulated off-flavors are typically removed from the fish flesh by depurating the fish in clean water before sale.

This study aimed to follow the change in sensory quality attributes of rainbow trout during the depuration period and compare the results with the content of off-flavor compounds. We hypothesized that a group of trained sensory panelists could recognize and describe the off-flavors and the change in their intensities during the depuration.

Materials and methods

Rainbow trout (*Oncorhynchus mykiss*) was reared in a pilot-scale RAS as reported by Pulkkinen et al. (2021). Fish were reared for 3 months in RAS until they were on average 1.89 kg (tank density of 46 kg m^{-3}). Selected fish were transferred to the depuration (flow-through in clean water, feed withheld) which continued for 16 days.

Samples were taken directly from the rearing tank in RAS (D0) and during depuration after 4 days (D4), 8 days (D8), 12 days (D12), and after 16 days (D16). Additionally, selected fish individuals were depurated for 26 days and used as a reference (REF). These individuals and were considered to represent fully depurated fish without any off-flavors from the RAS.

The fish were humanely euthanized, instantly gutted, fileted, and carefully washed to ensure high quality for sensory evaluation (ISO-8589:1988). The fish were cooked sous vide at 55°C for 25 min prior to the sensory analysis. The sensory properties of the samples were analyzed using a generic descriptive method. A group of panelists was specifically trained in analyzing rainbow trout samples. The panelists evaluated the fish with a sensory profile of 29 sensory attributes (12 odor, 5 taste, and 12 flavor properties).

For chemical analysis, 14 selected off-flavors compounds were analyzed with a validated method based on automated SPME and GC-MS/MS. The off-flavor compounds were quantified using the method reported in Lindholm-Lehto (2022).

Results and discussion

All the detected concentrations decreased during the depuration. For most of the compounds, the concentrations decreased to below the LODs. Before the depuration (D0), the highest concentrations were found for GSM (950 ng kg^{-1}), MIB (1 600 ng kg^{-1}), and octanal (600 ng kg^{-1}), while the concentrations of other off-flavor compounds remained below 250 ng kg^{-1}. D0 differed significantly from the other samples in fish-like, mud-like, and in total intensity of odor and flavor.

During depuration, the concentrations of some of the compounds decreased to below the level of detection (LOD), while the others (acetoin, hexanal, octanal, octanoic acid, phenyl acetaldehyde, and vanillin) remained at a certain low level. Even after 26 days (REF), no further decrease in concentrations was observed.

The intensities of mud-like, earthy, and musty odor and flavor already began to decrease in the early stages of depuration but decreased to low intensities between days 8 and 12 of depuration at the latest (Figure 1). However, the certain flavors seemed to slightly increase between D16 and REF. Many off-flavor compounds are lipophilic and accumulate in the lipid tissue of fish. The lipid content of fish affects the perception of these off-flavor compounds by increasing the sensory threshold values. As the lipid content of fish decreased during the depuration due to withheld feeding, the intensities of the compounds can increase. However, no statistically significant differences were detected.

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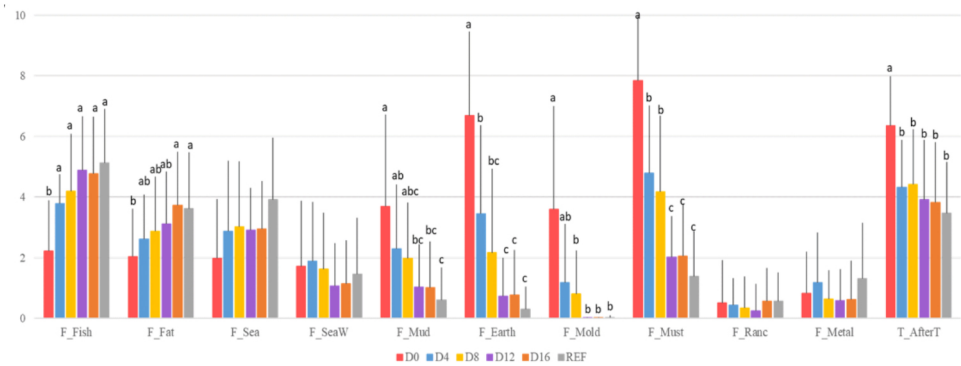

Fig. 1. The means and standard deviations of the flavor properties in the rainbow trout samples from different stages of depuration (n = 24). REF: reference, 26 days of depuration; D0-16, 0-16 days of depuration; F, flavor; Fish, fish-like; Fat, fatty; Sea, sea-like; SeaW, seaweed-like; Mud, mud-like; Earth, earthy; Mold, moldy; Must, musty; Ranc, rancid; Metal, metallic; AfterT, total intensity of aftertaste. a,b,c The different letters within each sensory property indicate a statistically significant difference between the samples at a 95% confidence level ($p < 0.05$).

This study showed the consistency between sensory observations and chemical analyses. Intense earthy, mud-like, and musty odors and flavors were observed. However, not all the odors and flavors induced by the studied compounds were observed by the panelists, possibly due to the masking of other compounds or higher sensory threshold limits than the concentrations in the samples.

This study gives important information about the effect of the depuration period in RAS on the chemical and sensorial quality of rainbow trout. Fish producers could benefit from using chemical and sensory analyses more extensively for the evaluation of off-flavors and sufficient depuration time.

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TOWARDS PRECISION FARMING OF MEAGRE: DEVELOPING MODELS FOR MANAGING FEED INTAKE

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Introduction

The efficient management of feed intake plays a vital role in the successful and sustainable aquaculture production. Feed is the main input and constitutes the primary fraction of production costs, which can exceed 50% of the total production cost (Buentello et al., 1999; Iversen et al., 2020). This fact highlights the importance of developing tools to support the definition of better feeding management practices, aiming to contribute to improved economic performance and precision farming. In particular for species, like meagre, for which not much information on feeding and nutrition is yet available.

Feed intake is a complex behavioral process, regulated at different levels, and affected by both physiological and environmental factors. With the use of feed intake models to support feeding operations at the farm it is possible to optimize feeding strategies and avoid overfeeding or underfeeding. In order to evaluate and quantify the effects of nutritional and environmental factors (such as oxygen levels) on feed intake, it is important to characterize the effect of central factors (such as fish size and water temperature) through the development of feed intake reference models.

In this work, we develop reference feed intake models for meagre to support the characterization of the effects of some key environmental and nutritional factors on feed intake and the development of more advanced models of meagre feed intake.

Material and methods

To develop the reference models, data from commercial feeding tables and growth trials from scientific publications was collected and processed. In total, 54 commercial feeding tables and 55 scientific publications were collected and the information therein extracted (name, recommended feeding rates according to fish body weight, feeding rate or FCR values, and water temperature, feed nutritional composition,) for downstream analysis.

Using the data collected, mathematical models were developed using the general model (equation 1):

$$FI(BW, temperature) \approx \boldsymbol{a} \times f(BW) \times g(temperature) \quad (1)$$

Assuming that the effect of body weight on the feed intake of fish follows a specific power law, while the effect of temperature follows an exponentiated quadratic relationship, the following specific form is obtained (equation 2):

$$FI(BW, temperature) \approx \boldsymbol{a} \times BW^{2/3} \times \exp(b \times temperature + c \times temperature^2) \quad (2)$$

Taking the logarithm on both sides and applying a mixed-effects modelling function (to account for possible different values of "a" in each data source), the unknown coefficients are estimated to obtain a reference model.

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Results

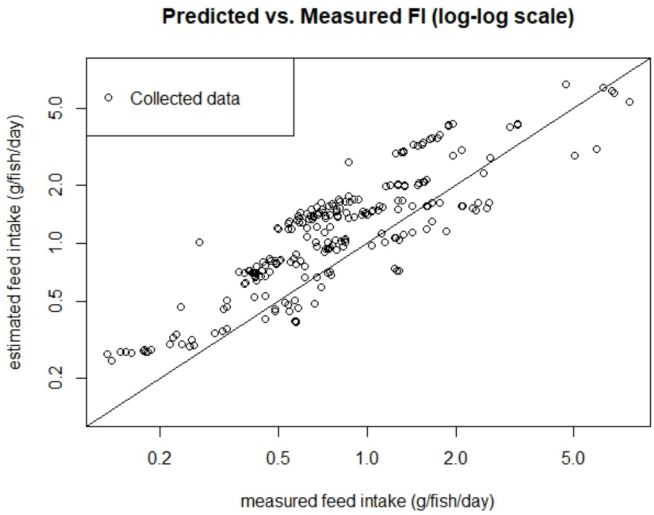

Figure 1 – Scatter plot showing estimated feed intake (g/fish/day) values as a function of measured feed intake (g/fish/day). Black points represent independent feed intake information from the growth trials reported in the scientific publications collected.

Testing of the developed reference models against independent data (see example in Figure 1) suggests a good prediction of measured feed intake data ($R^2 = 0.77$). Preliminary analyses using feed intake data normalized using the reference model illustrated in Figure 1 indicate that the levels of gross energy and fiber in meagre diets have an impact on feed intake.

Discussion and Conclusion

Temperature and body weight play crucial role in determining the feed intake of meagre, since a simple reference model using these factors explains most of observed variance. The validation of the reference model for feed intake represents a significant achievement, since it can be used to support the investigation of different variables that affect the feed intake (e.g., nutritional factors, oxygen levels).

Acknowledgements

This work is part of the NoviFEED project, financed by Iceland, Liechtenstein and Norway, through EEA grants, in the scope of the program Blue Growth, operated by Directorate-General for Maritime Policy (DGPM), Portugal, under reference PT-INNOVATION-0099

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ENHANCING ANTIOXIDANT PROPERTIES OF LETTUCE THROUGH NUTRITIONAL DEFICIENCY IN AQUAPONIC SYSTEMS WITH AEROPONIC CULTIVATION

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Introduction

In recent times, sustainable farming has evolved with the rise of soilless plant cultivation techniques, particularly aeroponics, where plants grow suspended in the air with nutrient-enriched aerosol sprays, ensuring consistent growth conditions. Notably, aeroponics offers faster growth and superior nutrient uptake than traditional hydroponic systems while minimizing water, fertilizer, and pesticide usage (4). The nutrient composition, mainly ionic forms like NH4+ and NO3-, critically affects plant growth and quality (6-8). Though nutrient deficiencies are known to hinder yield and bioactive compound content, comprehensive research on the effect of deprivation of essential ions in soilless systems is still wanting. While aeroponics often employs hydroponic nutrient solutions, the potential of aquaponic solutions, where fish, bacteria, and plants symbiotically interact, has seen limited exploration. Although balancing aquaponic systems presents challenges, especially with specific mineral deficiencies, they can still match hydroponic system yields, possibly due to beneficial microbial interactions (22). The study delves into comparing aquaponic solutions with hydroponics in aeroponic settings, focusing on growth and nutritional content, aiming to enrich discussions on nutrient management in sustainable soilless farming.

Materials and methods

Lettuce (Lactuca sativa L.) seeds were germinated and transplanted into an aeroponic growing system. Two different nutrient solutions were used: a conventional hydroponic solution (HS) and an aquaponic solution (AS) derived from external fish farming systems. Both systems were maintained under the same controlled conditions (e.g., light period, humidity, and temperature), enabling the comparison of growth parameters, leaf area, antioxidant activity, mineral content, and sensory characteristics of the lettuce.

Results

Lactuca sativa L. grew differently in hydroponic (HS) and aquaponic (AS) systems. HS exhibited significantly higher fresh weight of lettuce heads, fresh weight of roots, and number of leaves compared to AS. However, the AS system outperformed HS regarding the dry matter content of lettuce heads and root-to-shoot ratio. There was a marked increase in leaf area in HS compared to AS, with the difference peaking at later stages of cultivation. Water quality assessment showed elevated NO3- levels in HS, almost 15 times higher than in AS, while AS dominated in Cl and Na concentrations. Elemental analysis showed that HS generally contained higher concentrations of several elements, such as P and K, with a few exceptions, such as Mg, Na, Cl, and S. AS outperformed HS regarding total flavonoids and phenolics and showed significantly higher antioxidant activity with an ORAC value of 2. 6 times higher and DPPH radical scavenging activity 2.4 times higher than HS, both in terms of dry weight. While both systems showed comparable vitamin C content, HS showed significantly higher vitamin B2 concentration. Analysis of the mineral content of lettuce leaves revealed significant differences between the systems, with lettuce grown in HS containing significantly more P and K, whereas lettuce grown in AS contained significantly more Na, Mg, and nearly three times the amount of Si. Organic acid and anion evaluations showed a different profile, with AS being higher in tartrate and citrate and elements such as Cl-, SO42-, while HS showed elevated NO3- and PO43- content. Sensory analysis showed that, except overall acceptability, the two systems had no significant differences in flavor characteristics. The majority favored the HS lettuce mainly for its aesthetic appearance and size.

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Conclusion

This study underscores the aquaponic system‘s potential in sustainable agriculture. While the aquaponic system (AS) demonstrated a lower nutrient composition than the hydroponic system (HS), it exhibited resilience with sustainable yields. Despite its lower nitrogen content, AS effectively produced satisfactory lettuce yields without traditional fertilizers, emphasizing resource efficiency. Notably, lettuce grown in AS showed heightened flavonoids, phenolic compounds, and antioxidant activity, possibly due to increased salinity and nutrient scarcity. Elevated silicon content in AS lettuce indicates a potential role in boosting antioxidant activity, warranting further exploration. Despite yield constraints, AS showcases resource efficiency and potential nutritional enhancements, underscoring the need for continued aquaponic research.

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Acknowledgment

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ESTABLISHMENT OF A PISCINE MYOCARDITIS VIRUS (PMCV) CHALLENGE MODEL AND TESTING OF A PLANT-PRODUCED SUBUNIT VACCINE CANDIDATE AGAINST CARDIOMYOPATHY SYNDROME (CMS) IN ATLANTIC SALMON *Salmo salar*

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Cardiomyopathy syndrome (CMS) is a severe cardiac disease occurring in the grow-out sea phase of farmed Atlantic salmon with approximately 100 outbreaks annually in Norway. Piscine myocarditis virus (PMCV) is believed to be the causative agent of CMS. There is no vaccine available to control CMS, partially because PMCV withstands propagation in known cell cultures. In the present study, we selected the putative capsid protein of PMCV as the candidate antigen for immunization experiments and produced it in the plant *Nicotiana benthamiana* by transient expression. The recombinant PMCV antigen formed virus-like particles (VLPs). To evaluate the efficacy of the plant made VLP vaccine, a PMCV infection model was established. In an experimental salmon vaccination trial, the VLP vaccine triggered innate immunity, and indicative but not significant inhibition of viral replication in heart, spleen and kidney tissues was observed. Similarly, a reduction of inflammatory lesions in cardiomyocytes and subendocardial infiltration by mononuclear leukocytes were observed. Therefore, there was no difference in efficacy or immune response observed post the plant made PMCV VLP antigen vaccination. Taken together, this study has demonstrated that plant made VLP antigens should be investigated further as a possible platform for the development of PMCV antigens for a CMS vaccine.

CHARACTERISATION, DEVELOPMENT AND ECONOMIC BENEFIT OF VARICON AQUA'S CELL-HI NUTRIENT PRODUCT LINE

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The Cell-Hi All-In-One product range is the only complete, free-flowing, dry and fully soluble algae nutrient blend available on the market. The products incorporate a balance of all essential, macro and micro nutrients coupled with vitamins. Each of the Cell-Hi products is based on industry-standard formulations from literature and culture collections. The product is widely used in academic settings, as well as aquaculture and production facilities worldwide. There are over 24 peer-reviewed academic journal articles in which the Cell-Hi product range is referenced [1].

We estimate between 2-5% [2] of OPEX costs associated with microalgal production at typical hatcheries as being attributable to the costs associated with making media for algae cultivation. To prove the efficacy of the Cell-Hi range, we benchmarked the biological and economic performance of the product line against commercially available alternatives. Experiments were undertaken in a Varicon Aqua (CTC) controlled temperature chamber using 250 ml conical flasks aerated with 1% CO_2, and surface illumination of 150 µmol m^{-2} s^{-1} achieved with red/blue/white (400-760 nm) LEDs. The temperature conditions were fixed at specific temperatures depending on the trial strain 25°C ± 1°C for *Nannochloropsis* spp. and 20°C ± 1°C for *Tetraselmis suecica*.

Our results showed that *Nannochloropsis* spp. grown on Cell-Hi F2P had identical performance to laboratory-made or commercially supplied nutrient (no significant statistical difference, average productivity of 0.15 g L^{-1} d^{-1} and final yield of 1.1 g L^{-1} over a 7-day batch). Similarly, no significant differences were observed in growth rates between *T. suecica* grown on Cell-Hi-WP and laboratory-made equivalents (average productivity 0.25 g L^{-1} d^{-1} and a final yield of 1.7 g L^{-1} over a 7-day batch).

Furthermore, techno-economic evaluation demonstrated 60% cost savings per kg of nutrient used within a hatchery when substituting conventional media protocols with Cell-Hi products. We have shown that our Cell-Hi range is comparable in biological performance and superior in economic performance to many conventional alternatives (both pre-formulated and formulated from analytical grade chemicals). These findings provide a compelling argument for making the switch to Cell-Hi All-In-One within commercial hatchery operations.

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PREDICTABILITY OF LATENCY TIME USING THE IN VITRO OOCYTE MATURATION IN PIKEPERCH (*Sander lucioperca)*

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Introduction

The main problem of artificial reproduction of pikeperch presents the unpredictability of the latency time (LT) (Żarski, et al., 2019). Therefore, a special final oocyte maturation (FOM) classification was developed in order to tackle this (Żarski et al., 2012). Although useful, this procedure still requires frequent handling especially in cases of out-of-season reproduction when the fish are commonly hormonally stimulated in the first stage of FOM. A recent paper showed high agreements in the post-stimulation maturation dynamics in pikeperch between *in vivo* and *in vitro* oocyte maturation (Ljubobratović et al., 2023). Therefore, using the in vitro maturation (IVM) technique, we are aiming at developing a method that predicts the individual latency time and thus minimizing the number of handling procedures that require the biopsy of oocytes.

Materials and methods

In early November 2022, during out-of-season pikeperch reproduction, in total 13 females were hormonally stimulated with salmon gonadoliberin analog using the warming thermal regime (Ljubobratović et al., 2021). At the time of ovulation, a sample of oocytes was taken from each female using a catheter (CH06 infant feeding tube). Immediately upon biopsy, the oocytes of each female were stocked in separate wells of 6-wells cell culture plates. Each well was filled with 90% L15 medium and 5 IU/mL of human chorionic gonadotropin. Wells were further placed onto an orbital shaker and incubated at 13°C for in total 96h. Starting from 48 h post-stimulation, IVM of oocytes were monitored each 4 h, while the same procedure in females was started 120 h post-stimulation and was performed each 24 h. On this way, FOM dynamics and both *in vivo* and *in vitro* latency times were assessed and correlated.

Results

All injected females ovulated, while all the IVM oocytes samples reached germinal vesicle breakdown (GVBD). The LT in females ranged from 6 to 9 days, while *in vitro* oocytes took 56 to 76 h to GVBD. The strongest correlation between the LT and IVM was found in 52 and 56 h post-stimulation, showing higher correlation compared to the *in vitro* LT.

Discussion

The IVM techniques appear to be a promising tool for estimation of optimal female state for hormonal induction as well as the prediction of the latency time in out-of-season pikeperch reproduction. Rather than using the time until GVBD during the IVM, a given FOM stage at a certain time post-stimulation might be more useful to predict the state of female readiness for ovulation induction as well as the prediction of its LT.

Acknowledgements

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SIMULTANEOUS BIOMETHANE PRODUCTION AND NUTRIENT REMINERALIZATION FROM AQUACULTURE SOLIDS

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Introduction

The rapid expansion of the aquaculture industry has brought about a heightened focus on the waste produced by high intensity fish farming. In closed-containment, recirculating aquaculture systems (RAS), fish solids are mechanically separated and/or coagulated before being disposed as waste. Subsequent revalorization is typically limited to the direct dispersal of aquaculture solids onto agricultural fields.

Here, we developed a novel, continuous flow, low-cost solids waste treatment system for freshwater and saline RAS. Rotating drum filter backwash was collected as the primary feedstock for anaerobic digestion. A laboratory scale set up was used to monitor the conversion of the solids into a methane-rich (60-80% purity) biogas stream. Iron supplementation (ferric iron at 100 mg/L and 1000 mg/L) improved salt tolerance of the methanogenic community, leading to higher methane yields in a supplemented ($FeCl_3$ at 1000 mg/L) saline treatment than the saline control. The application of iron additionally improves pH stability and volatile fatty acid utilization. The methane yield ranged from 0.1-0.4 NL CH_4/ g VS across the three freshwater treatments and the iron-supplemented saline treatment, however, it was significantly lower for the saltwater control: ranging between 0.08-0.25 NL CH_4/ g VS. These values correspond to a percentage yield of 57% - 86% of the total biomethane potential.

Overall, implementing anaerobic digestion for RAS waste valorization may generate significant amounts of biomethane to be used in electricity and heating for large-scale aquaculture facilities, while even for smaller facilities it may off-set costs and mitigate environmental impacts of the waste streams.

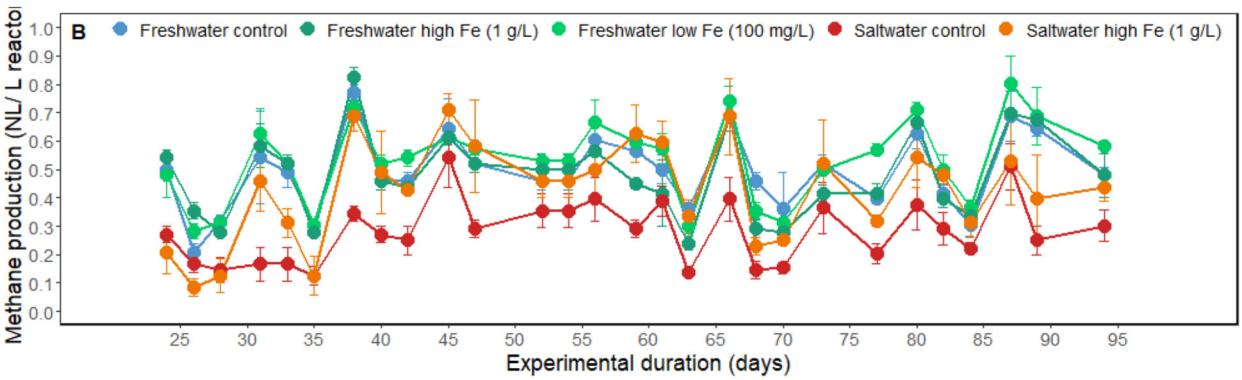

Figure 1. Methane production across treatments over the experimental period.

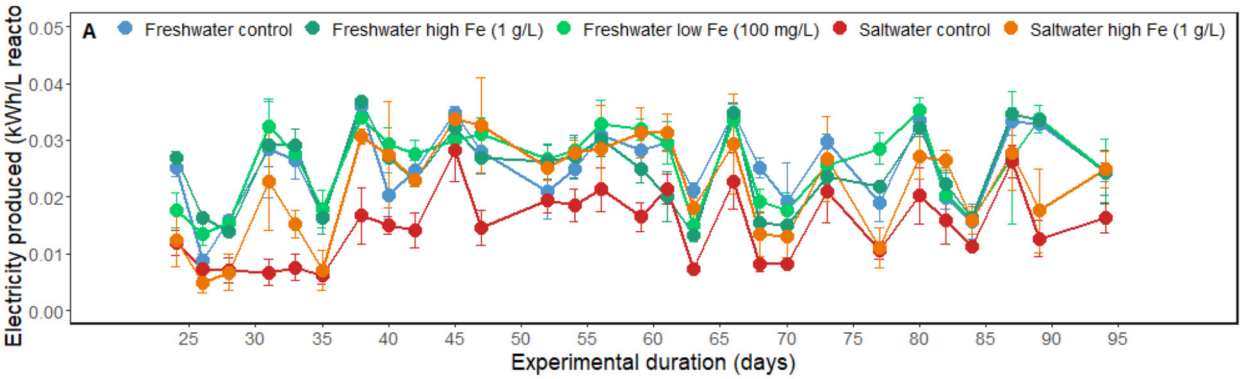

Figure 2. Electricity production across treatments over the experimental period.

SINGLE CELL PROTEIN AS FEED INGREDIENT FOR RAINBOW TROUT (*Oncorhynchus mykiss*) COMPARED TO FEEDS BASED ON FISH MEAL, SOYBEAN MEAL AND FEATHER MEAL

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Introduction

Recently, several products have emerged on the market as new sustainable fish feed ingredients with presumable health promoting effects. One such product is Coryne Pro (Agrifeed), a single cell protein (SCP) of bacterial nature as by-product from fermentation of *Corynebacterium glutamicum* for industrial production of threonine. Coryne Pro has a high protein level (~70%) and is described to constitute a good protein source for growth, as well as having beneficial health effects. While *in vivo* trials are the best way to evaluate new ingredients, it would be helpful to develop complementary *in vitro* methods to reduce the number of animals involved according to 3R principles. The H2020-FETOPEN project Fish-AI, aims to develop such a platform but it requires an experimental set-up that facilitates the *in vivo* vs. *in vitro* correlation. To this purpose, as part of this project, we tested Coryne Pro at two different inclusion levels in the feed for rainbow trout and compared it to three reference feeds: one presumably healthy fish meal-based feed, one challenging feed with high soybean meal content and one feed with high inclusion of feather meal with presumable low protein digestibility value. Rainbow trout were fed the five different feeds for nine weeks, and growth performance, organ indices, histology, gene expression, blood values as well as digestive enzyme levels were investigated.

Material and methods

Rainbow trout (~160 g) were fed five different diets; fish meal diet (FM), soybean meal diet (SBM), feather meal diet (FTHM) or diets containing single cell protein at 10% and 20% inclusion levels (SCP-10 and SCP-20, respectively) (Table 1). Feeds were produced by extrusion with pellet size 2mm, and yttrium was added to calculate nutrient digestibility. Macro and micronutrient content were adjusted to meet the NRC standards when formulating the feeds and kept constant in protein and lipid level.

The feeding trial was run at Skretting Aquaculture Innovation's research facility in Mozzecane, Italy. Rainbow trout were randomly distributed in 0.6 m^3 tanks, 35 fish per tank and three replicate tanks per diet. The feeding regime was two meals per day with overfeeding. Fish were held at 15°C water temperature and 24-hours light regime. After 9 weeks of feeding, 6 fish per tank were randomly sampled and euthanized with an overdose of MS-222. Weight, length and organ indices were recorded from individual fish. Blood samples were collected in heparinized syringes from the caudal vein and analyzed for plasma indicators of nutrient metabolism. The intestinal tract (posterior to the stomach) was dissected from the same fish, and divided into pyloric intestine, mid intestine and distal intestine. The intestinal sections were opened longitudinally, and intestinal content collected for analyses of bile salt and trypsin activity. Tissue samples were fixed in formalin for histology, RNA later for gene expression and snap frozen in liquid nitrogen for enzyme analyses. Fecal samples were pooled by tank.

Results and discussion

The SCP diets performed equal to the fish meal diet concerning fish growth, feed intake, and feed conversion ratio, indicating that the SCP may partially replace fish meal as a protein source in diets for rainbow trout. Results indicated however some immune stimulation from the SCP diets by slightly higher numbers of mucosal immune cells in the distal intestine and elevated levels of certain immune genes. Increased levels of liver enzymes in the blood from SCP were moreover observed, although large variation within the groups requires further analyses to conclude. As expected, the SBM diet induced intestinal inflammation in the distal portion of the rainbow trout gut evidenced by infiltration of inflammatory cells in the gut mucosa, reduced intestinal fold height, loss of supranuclear vacuoles, as well as reduced expression of functional brush border genes and reduced organ indices. Further, results indicated that the FTHM diet had a lower protein digestibility compared to fish meal, as feed intake and final body weight were significantly higher in this diet group, although there was no difference in feed conversion ratio. Also, increased organ indices of the pyloric intestine was observed in this diet group possibly from lipid accumulation as known from choline deficiency in salmon.

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Conclusions

The SCP diets performed equal to the fish meal diet concerning growth and organ indices. Gene expression results indicated however slight immune stimulation from the SCP. Results confirmed intestinal inflammation in the distal intestinal region from the SBM diet and indications of sub-optimal digestibility from the FTHM diet. The experimental set-up used in the current study with three contrasting reference diets with known characteristics, was thus successful for testing of new feed ingredients and will provide a clear reference for testing the predictive ability of the *in vitro* platform that is under development by the Fish-AI consortium.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 828835.

Table 1. Feed composition and nutrient content for experimental feeds (%)

	FM	SBM	FTHM	SCP-10	SCP-20
Fish meal	35.00	5.00	5.00	25.00	15.00
Feather meal	0.00	0.00	20.00	0.00	0.00
Soybean meal	0.00	30.00	0.00	0.00	0.00
Single cell protein	0.00	0.00	0.00	10.00	20.00
Wheat gluten	10.85	21.86	9.16	7.76	8.18
Fish oil	9.29	10.25	10.35	9.45	9.55
Rapeseed oil	9.44	10.41	10.52	9.60	9.71
Faba bean	5.00	5.00	5.00	5.00	5.00
SPC	10.00	10.00	18.00	15.00	15.00
Phosphate	0.00	1.26	1.44	0.42	1.00
Fat %	23.90	23.40	24.20	23.30	23.00
Protein %	42.70	43.40	44.40	43.20	44.50
Moisture %	6.20	7.30	6.80	7.20	7.30
Ash %	6.30	4.50	3.80	5.90	5.00

*Constant between the diets: Vit and Min premix (0.75), Yttrium premix (0.1) and Astaxanthin (0.05)

A COMPARISON OF GENOMIC INBREEDING IN WILD AND FARMED EUROPEAN SEABASS AND GILTHEAD SEABREAM POPULATIONS

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Introduction

The most important marine fish species farmed in the Mediterranean are European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) and for both species, selective breeding programmes have been initiated in recent years. In these programmes, the control of inbreeding is crucial to achieve sustainable production. Monitoring inbreeding is also important when dealing with wild populations.

Recently, a combined ~60K SNP array for both species has been developed (Peñaloza *et al*. 2021). The use of this tool (the MedFish SNP array) allows to obtain more accurate estimates of inbreeding coefficients than those obtained using pedigree information and also enables the evaluation of inbreeding patterns across the genome. Villanueva *et al*. (2022) compared inbreeding of wild and farmed populations for both species using the aforementioned array but their analysis was limited to average genome values. This study aims to compare patterns of inbreeding throughout the genome of wild (*W*) and farmed (*F*) populations for European seabass and gilthead seabream.

Material and Methods

Samples of both species were the same as those used for the SNP array development and were collected from *W* and *F* populations located from East to West Mediterranean. Three Atlantic populations of seabream were also sampled. For seabream, SNP genotypes were available for 462 fish from 14 *W* and 12 *F* populations. For seabass, SNP genotypes were available for 516 fish from 9 *W* and 15 *F* populations. After quality control, the total number of SNPs was 24,548 and 21,797 for seabream and seabass, respectively.

The proportion of genome-wide homozygosity and the molecular inbreeding coefficient were obtained using the software PLINK (Purcell *et al*., 2007) and an R script. The measure of molecular inbreeding (F_{mol}) used was that based on deviations from Hardy-Weinberg proportions (Li and Horvitz, 1953). Patterns of genomic homozygosity and inbreeding were calculated using a sliding window approach. For both species, the length of the windows was ~ 1 Mb and they were moved one SNP at a time. For each window, homozygosity and inbreeding were estimated by averaging the values for all SNPs lying in that window. Afterwards, values were averaged across individuals.

Results

For seabream, patterns of observed homozygosity were very similar across the genome in both *W* and *F* populations (Fig. 1). Also, differences between *W* and *F* populations were negligible. Values for homozygosity ranged from 0.485 to 0.725. In general, seabass populations showed higher variation among them than seabream populations. In fact, some distinct patterns were observed in seabass populations (Fig. 1). For instance, the Moroccan wild population (purple line in Fig. 1C) showed high peaks of inbreeding across the genome while one of the Greek farmed populations, exhibited lower levels of homozygosity (green line in Fig. 1D). Values for homozygosity ranged from 0.415 to 0.880. Values for F_{mol} ranged from -0.386 to 0.193 in seabream and from -0.463 to 0.366 in seabass. Negative values appear because current population frequencies were used when computing F_{mol} and then its values are centered around zero.

Discussion

This study shows that differences between *W* and *F* populations in terms of inbreeding are small in both species. Results are in agreement with previous population structure results where *W* seabream populations showed lower levels of differentiation than *W* seabass populations and where the Moroccan seabass population presented a higher genetic differentiation when compared to other populations (Villanueva *et al*. 2022). Here, the measures used to evaluate inbreeding have been the observed homozygosity and F_{mol}. Many other measures have been proposed, but they can lead to inconsistent results in terms of loss or gain of genetic variability (Villanueva *et al*. 2021). In conclusion, in most cases the selection pressure exerted on farmed populations for a number of generations (7-8 for the older breeding programmes) did not drastically increase homozygosity.

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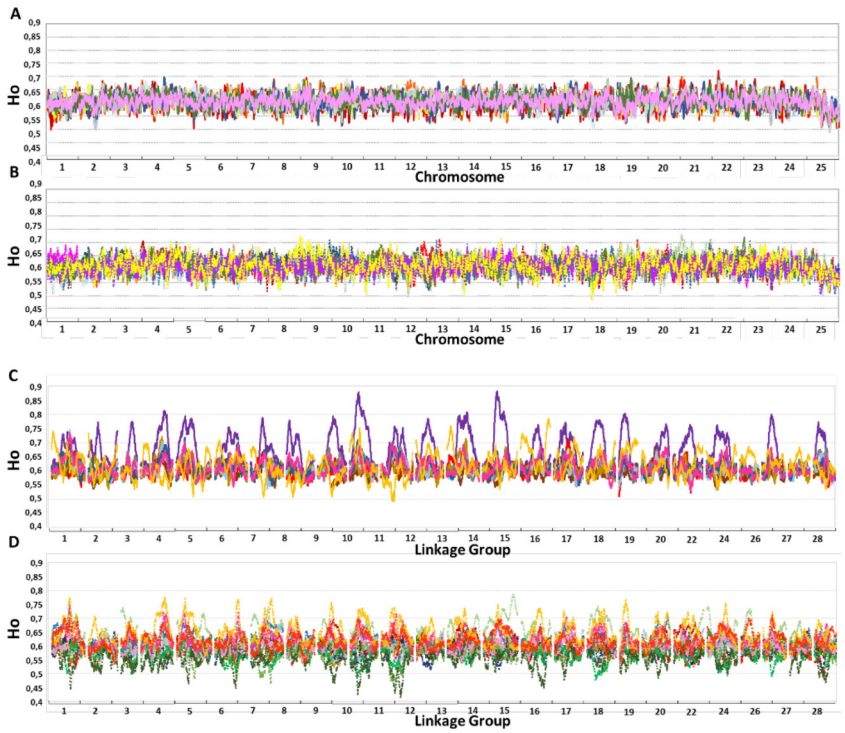

Fig 1. Patterns of observed homozygosity (*Ho*) for seabream (A: 14 Wild; B: 12 Farmed populations) and seabass (C: 9 Wild; D: 15 Farmed populations) across the genome. Each population is represented with a different colour.

Acknowledgements

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THE FRENCH ASSOCIATION OF BREEDING COMPANIES (SYSAAF): AN ORIGINAL AND UNIQUE MODEL FOR BUSINESS ORGANIZING AND DEVELOPING GENETIC SELECTION

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Introduction
Genetic selection has played a crucial role in the success of agriculture and animal husbandry, driving the systematic adaptation of the evolution of domesticated plants and animals to the societal demands. In recent decades, industrialization of the agri-food industry has opened up new possibilities for the creation of completely new products. In this paper, we describe the non-profit organization (cluster) of 35 French breeding companies selecting 31 poultry, aquaculture or insect species. and compare it to other models of industrial organization of business groups and companies in which genetic selection has been integrated.

Methods
The data were acquired from SYSAAF's historical records since 1952, and the model was described owing to collaborative effort with the aquaculture department's management and surveys conducted with the leaders of the enterprises and departments.

Results
Our results show that the non-profit and collective nature of SYSAAF is adapted to invest in R&D projects in sharing results within or between species and companies. It's interactions with INRAE and Ifremer or ANSES and the French ministry of agriculture is insuring management the national genetic resources and their improvement in stimulating multiple innovative processes and transfer and implementations of results.

One of the main advantages of this style of organization is that it allows small companies to access high-value technologies (as genomic selection or GWAS assisted selection), shared computational capacities and expertise (23 geneticists) while reducing their individual costs Teece, D. J. (2010).

Conclusions
Overall, this paper sheds light on the feasibility to develop collective and shared knowhow in genetic selection, a unique economical Model adapted for the domestication of new species and the support of SME's.We believe that the SYSAAF organization serves as an excellent example of how a public and collective model can benefit both large and small companies in the industry to ease implementation of innovations.

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THE EFFECT OF FEEDING WITH THE COPEPOD *Acartia tonsa* DURING THE FIST DAYS OF LARVAL REARING ON THE ONTOGENY OF THE INTESTINE AND LIVER IN GREATER AMBERJACK (*Seriola dumerili)* AND GILTHEAD SEA BREAM *(Sparus aurata)*

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Introduction

Ontogenesis is a process of growth, differentiation and maturation of cells, tissues, organs, and systems (Chambers and Leggett, 1987), where fish larvae undergo dramatic changes in body shape, swimming ability, metabolism and behavior. Nutrition is one of the most important factors influencing the ontogeny of the organs in the digestive tract (Chambers and Leggett 1987). Copepods have the advantage that the fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are found in the phospholipids of cell membranes. Copepods do not need enrichment like rotifers and *Artemia* metanauplii and so their use does not increase the problem with the oily surface of the rearing tanks (Conceição et al. 2010). In addition, copepods are very rich in free amino acids, which stimulate the sense of smell of fish larvae and enhance feeding behavior and food search in the tank (Rønnestad et al. 2013).

Materials & Methods

In a marine hatchery (Galaxidi Marine Farms A/S), four cylindroconical tanks of 2,700 were stocked with 150,000 greater amberjack larvae (*Seriola dumerili*) in each, which were reared until 45 days post-hatching (dph). The larvae were initially fed from 3-17 dph in two tanks with copepod nauplii and rotifers (*Brachionus* sp.) (Copepods group), while in the other two tanks, the larvae were fed only with rotifers (Control group). All the tanks were fed with rotifers (3-27 dph), Artemia nauplii (12-22 dph), enriched Artemia metanauplii (20-30 dph), and formulated diet (25-45 dph).

A second experiment with gilthead seabream was carried out at the facilities of the Department of Biology at the University of Patras. Six cylindroconical tanks (three tanks – Control and three tanks – Copepods group) were stocked with 10,000 seabream eggs (*Sparus aurata)* in 100 L tanks and these were reared until 25 dph. The feeding protocol was similar to the greater amberjack experiment with the only difference being that the Copepods group fed with *Acartia tonsa* nauplii days 3 to 14 after hatching. In both experiments, samples were taken every three days anaesthetized and transferred to a fixative (4% formaldehyde solution). Fixed samples were rinsed in distilled water, dehydrated in graded ethanol 70, 80 and 100% and then embedded in methacrylate resin (Technovit 7100 Heraeus Kulzer, Germany). Serial sections of 5 mm were obtained with a microtome (Leica SM200R, Germany), stained according to Bennett et al. (1976) and observed through a light microscope. The factors that we choose to study to determine the effect of the copepods in the ontogeny of the intestine and the liver were the length of the villi, the abundance of goblet cells (intestine), and the percentage of area covered with lipid vacuoles - ACLV (liver).

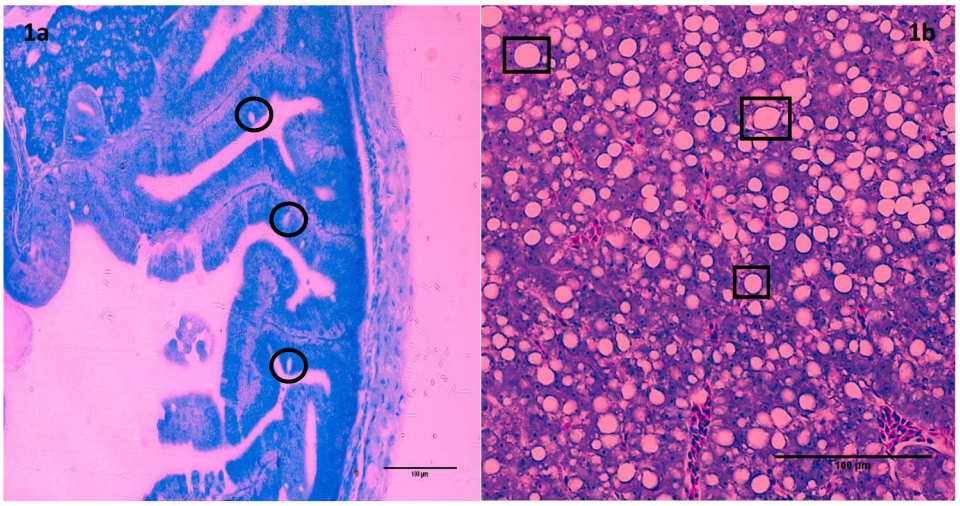

Fig. 1 1a: Intestine of *S. aurata* 25 dph. Black squares indicate the goblet cells. 1b: Liver of *S. dumerili* 36 dph Black squares indicate the lipid vacuoles (Scale 100 μm).

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Results

Villi and goblet cells in the intestine of *Sparus aurata* 25 dph are shown in Figure 1a and in ACLV in the liver of *Seriola dumerili* juveniles 36 dph are shown in Figure 1b.

The use of copepods during the first days of rearing affected positively the intestine and the liver in both the species, *S. dumerili* and *S. aurata*. More specifically, the length of the villi and the abundance of the goblet cells in the intestine were greater in the Copepods group throughout the whole rearing of both species with statistically significant differences in several days after hatching ($p<0.05$). Moreover, it seems that the percentage of ACLV in the liver was also greater in the Copepods group throughout both experiments, with statistically significant differences during the copepod feeding period ($p<0.05$).

Conclusions

In conclusion, the use of copepods in the rearing of both species, *Seriola dumerili* and *Sparus aurata* affected positively the ontogeny and development of the intestine (length of the villi and abundance of goblet cells) as well as the lipid accumulation in the liver.

Acknowledgements

This study was supported through the research project "Improvement of broodstock management and fingerling production methods for greater amberjack (*Seriola dumerili*)" with grant number MIS 5045873, funded by the General Secretariat for Research and Innovation (GSRI) in Greece.

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HEALTH ASSESSMENT OF GILTHEAD SEABREAM (*Sparus aurata)* AND SEABASS (*Dicentrarchus labrax* L.) REARED IN RAS BY APPLYING HISTOLOGICAL ANALYSES AND 18S rRNA BASED MONITORING OF PROTOZOANS

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Introduction

The aim of this study was to assess the health status of the farmed fish gilthead (*S. aurata*) and seabass (*D. labrax*) at ANDROMEDA AS facilities in Vonitsa, Greece by histological examination and evaluation of tissues from fish sampled from RAS. In addition, we made surveillance of protozoans encountered in various parts of the rearing system as well as in the intestine of the fish.

Materials and Methods

Five fish for each species (seabream 102 dph and seabass 142 dph) were sampled, anaesthetized, dissected, and pieces of isolated tissues (gills, intestine, liver, and spleen) were transferred to fixative (4% formaldehyde solution). Fixed tissue samples were rinsed in distilled water, dehydrated in graded ethanol 70, 80 and 100% and thereafter embedded in methacrylate resin (Technovit 7100, Heraeus Kulzer, Germany). Serial sections of 5 mm were obtained with a microtome (Leica SM200R, Germany), stained according to Bennett et al. (1976) and observed through a light microscope. To evaluate the pathology of the tissues we applied a scoring system (Multiparametric Semi-quantitative Scoring System – MSSS, scoring range 1-5) for some descriptors (Table 1). The scoring system MSSS was a modification of Pacorig et al. (2022).

For the surveillance of protozoans, eight samples were taken for both species seabream and seabass which consisted of Tank water (TW), Tank biofilm (TB), Biofilter biofilm (BB) and Intestine (GUT) samples. For these samples DNA isolation was done using the ZymoBIOMICS DNA Miniprep Kit, followed by 18S rRNA gene amplicon library preparation and Illumina MiSeq sequencing.

Results

The histological evaluation showed that gilthead seabream was in a better health state compared with seabass indicated by a lower incidence of oedema in the gills (Fig. 1a), a higher abundance of goblet cells in the intestine (Fig. 1b), and a lower incidence of steatosis (Fig. 1c) and necrosis (Fig. 1d) in the liver. On the other hand, melanomacrophage centers, multifocal exudates (Fig. 1e), and necrotic areas (Fig. 1f) in the spleen were more abundant in gilthead seabream.

Five genera of protozoans were identified based on the 18S rRNA amplicon sequences: *Pseudovorticella*, *Zoothamnopsis*, *Zoothamnium*, *Dysteria* and *Acineta*. The highest relative abundance of protozoans was recorded in the tank biofilm dominated by members of the *Zoothamnopsis* in seabream tanks and *Zoothamnium* in seabass tanks (Fig. 2).

Conclusions

1. Based on the histological analysis, seabream showed a better health state compared with seabass.
2. Abundances of protozoa in the rearing systems of the two fish species showed a different pattern.

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Table 1. Descriptors used in each tissue of both species (*S. aurata* and *D. labrax*).

Gills	Intestine	Liver	Spleen
Chloride cell hypertrophy	Goblet cells	Hyperemia	Multifocal exudates
Clubbing of secondary lamella	Desquamation	Vacuolar degeneration	Melanomacrophage
Hyperplasia of secondary lamella	Inflammatory infiltrate	Multifocal glycogen accumulation	Necrosis
Basal fusion of secondary lamella		Steatosis	
Oedema		Necrosis	
Goblet cells			
Inflammatory infiltrate			

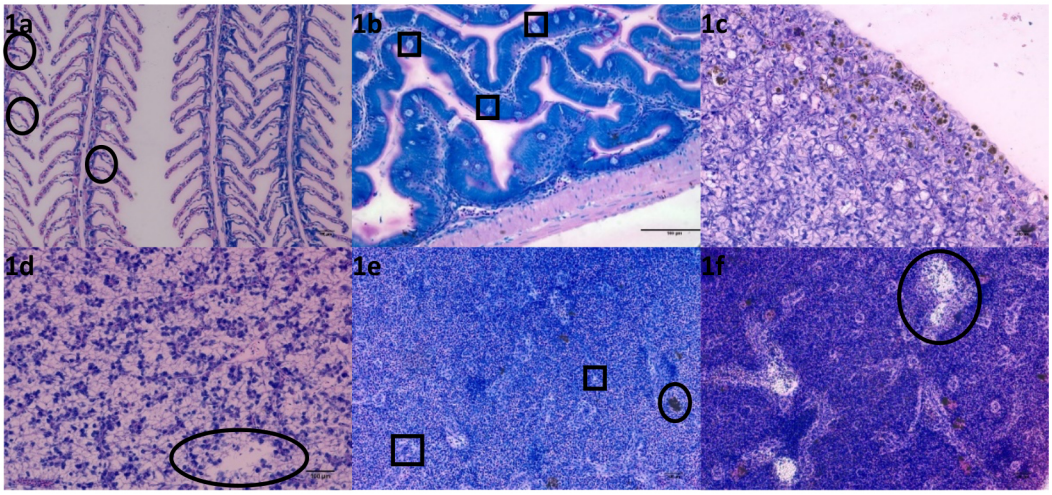

Figure 1: Histological sections of gilthead seabream and seabass. 1a: oedema in the gills of *D. labrax*. 1b: goblet cells in seabream anterior intestine. 1c: steatosis in seabass liver. 1d: necrosis in seabass liver. 1e: multifocal exudates (squares) and melanomacrophage centers (circles) in gilthead seabream spleen. 1f: necrosis in gilthead seabream spleen.

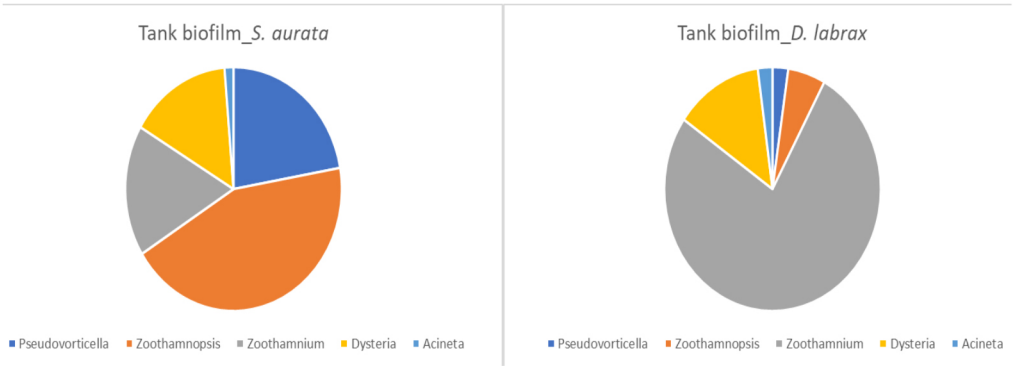

Figure 2: Relative abundance of protozoans in tank biofilm in seabream and seabass tanks.

EFFECT OF DIFFERENT BROODSTOCK DIETS ON LARVAL DEVELOPMENT OF THE SEA URCHIN *Paracentrotus lividus* (LAMARCK, 1816)

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Introduction

Maternal provisioning and nutritional level can have a significant impact on the reproductive performance and offspring quality of marine invertebrates. In sea urchins, the energy for embryo and larval development up to the onset of feeding is primarily driven by nutrients acquired from the egg (Carboni et al., 2013). The high mortality rates occurring during larval development remain a major bottleneck to the full-cycle production of sea urchin aquaculture. The provision of broodstock feeds with key ingredients could help to improve offspring development. Carotenoids, especially b-carotene, are known to improve gonad growth, fecundity, and larval survival. While lipids and fatty acids, particularly the absolute levels of polyunsaturated fatty acids (LC-PUFAs) such as docosahexaenoic (DHA, C22:6n-3), eicosapentaenoic (EPA, C20:5n-3) and arachidonic (ARA, C20:4n-6) have been proven to be critical factors in regulating the reproductive performance, egg, and larval performance. The present study aimed to evaluate the growth and offspring performance of the sea urchin *Paracentrotus lividus* (Lamarck, 1816), after conditioning broodstock on different feeding diets.

Materials and Methods

P. lividus broodstock was acclimated and maintained in recirculating aquaculture systems (RAS) and starved for one month before conditioning to the experimental diets. The individuals were conditioned for two months on five different jellified diets consisting of different proportions of carotenoids (High and low content - HC and LC) and lipids (Fish oil – FO, and Colza oil - CO) as the main ingredients, and seaweed-based diet (*Saccorhiza polyschides*). By the end of the feeding experiment, the maturity level of both males and females was assessed and compared with a wild group. Spawning was chemically induced in both sexes and eggs from each broodstock diet were collected for measurements. The larval rearing experiment was carried out in 600 mL glass flasks (in triplicate) and maintained in a climate chamber. Larvae were stocked at a density of 4 ind.mL^{-1}, kept at a salinity of 35, an average temperature of 18 °C, continuous fluorescent light (12.62 µE.m^{-2}.s^{-1}), and reared in aerated static seawater. Larvae were fed with a mixed microalgal diet (*Chaetoceros calcitrans* and *Tysochrysis lutea*) during their exponential growth phase. Additionally, a group of larvae were left unfed to assess the effects of, exclusively, egg maternal reserves. Larval body length (BL), body width (BW), post-oral arm length (POAL), and stomach length (SL) were measured to characterize the larval growth and morphology. Larval survival rate (%) was assessed volumetrically every two days. Larval competence was defined as the number of days post-fertilization (DPF) required for at least 75% of the larvae to reach competence for settlement.

Results

After two months of feeding, all individuals fed with seaweed-based and LC diets were at stage II (growing), whereas when fed with HC-FO and HC-CO were equally distributed between stages III (premature), IV (mature), and V (partly spawned). Across females, the broodstock fed with HC-FO (103.84 ± 3.17 µm) and HC-CO (110.30 ± 8.83 µm) produced larger eggs in relation to the other diets. Only the individuals fed with HC-FO, HC-CO, and LC-FO and the individuals from the wild produced viable gametes. The competence for settlement was reached earlier for fed larvae from the wild broodstock at 20 DPF, while larvae from experimental fed treatments attained at 22 DPF. Significant differences in survival rate at competence were found between feeding treatments (H_{KW} = 16.37, df=2, *p-value* < 0.001), with a higher survival for larvae from HC-FO (38.93 ± 6.21%). At 20 DPF, larvae from the wild broodstock showed a larger BL (529.13 ± 67.35 µm), BW (477.97 ± 57.92 µm), and SL (202.51 ± 27.35 µm) while larvae from the HC-CO diet showed a longer POAL (326.96 ± 54.94 µm) when compared with the other treatments. Across the non-fed larvae, the larvae from broodstock maintained on HC-FO, HC-CO, and from the wild survived for the longest amount of time (13 DPF), when compared with larvae from LC-FO treatment (11 DPF).

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Discussion

The broodstock diets tested supported the gonadal development and maturation, except for the seaweed-based diet. Fed larvae from HC-FO broodstock showed the best larval survival and optimal growth throughout larval development. Across the non-fed larvae, the larvae from broodstock maintained with a HC-FO diet survived for the longest amount of time, when compared with a low-carotenoid diet. This indicates that this specific diet is an effective broodstock conditioning feed that can be assimilated for reproductive purposes and likely had a greater extent of maternal provisioning. In contrast, non-fed larvae from wild broodstock had the longest post-oral arms, which indicates that their maternal reserves were depleting, and these were being used to extend their arms to capture food. Future research should focus on the development of formulated feeds to improve reproductive performance, rather than gonad enhancement, to improve sea urchin reproductive success in aquaculture.

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IMPACT OF DISSOLVED OXYGEN LEVELS ON GROWTH, FEED INTAKE AND BLOOD PARAMETERS OF MEAGRE *(Argyrosomus regius)*

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Introduction

The success of a commercial aquaculture depends on providing the optimum environment for rapid growth at the minimum cost and resource use. Oxygen is an important and critical parameter, requiring continuous monitoring and is one of the most significant costs at non-cage intensive aquaculture production. Several studies carried out with seabream and seabass, indicate that low levels of oxygen affect feed intake and, consequently, growth performance (Araújo-Luna et al., 2018; Pichavant et al., 2001; Samaras et al., 2023). A concentration of at least 5 mg L^{-1} is usually considered the minimum required for a normoxia condition and less than 4 mg L^{-1} is considered hypoxia, and is reported to cause fish stress. The mechanisms involved in hypoxic stress have not yet been fully investigate on meagre rearing, a relevant species for Mediterranean aquaculture. The aim of this study was to investigate two levels of hypoxia in comparison with a normoxia condition on the feed intake, blood parameters and meagre growth performance.

Materials and methods

The study was carried at the Aquaculture Research Station of the Portuguese Institute for Sea and Atmosphere (EPPO/IPMA). A total of 576 meagre (*Argyrosomus regius*) with an initial weight of 256.0 ± 1.8 g were distributed in 9 fibre-glass tanks (density of 10.9 ± 0.1 kg m^{-3} per tank). Three different levels of hypoxia were tested: moderate hypoxia (DO-1: 2-3 mg L^{-1}), mild hypoxia (DO-2: 4-5 mg L^{-1}) and normoxia (DO-3: 6-7 mg L^{-1}), in triplicates. The 1.5 m^3 tanks were set up in a flow through system with a water renewal of 60% per hour, salinity kept at 37 ppt and temperature at 20.7 ± 1.0°C. Oxygen and temperature were registered automatically every hour through Synergia® equipment, and when oxygen values dropped, pure oxygen was injected in the water column until oxygen levels were re-established, according to the levels defined for each treatment. The experiment lasted 31 days. Fish were fed with an experimental diet, produced by Sparos, Lda. (Olhão, Portugal), four times a day, until apparent satiety. The uneaten pellets were counted and registered. For the final sampling, all fish were individually weighed and six fish per tank were anesthetized for blood collection. Haematological parameters were analysed: haematocrit (Hct) and haemoglobin (Hgb), glucose (Glu) and potassium (K+) using an EPOC® card reader.

Table 1. Effect of dissolved oxygen on meagre (*Argyrosomus regius*) rearing performance

	Treatments		
Parameters	**DO-1**	**DO-2**	**DO-3**
Feed intake (g day^{-1})	227.7 ± 58.3[a]	257.1 ± 63.3[b]	286.0 ± 72.2[c]
Initial body weight (g)	245.5 ± 31.7	261.8 ± 28.3	249.5 ± 26.4
Final body weight (g)	391.2 ± 62.0[a]	404.0 ± 60.9[b]	411.5 ± 56.3[b]
SGR (g day^{-1})	0.0137 ± 0.0004[a]	0.0148 ± 0.0004[b]	0.0152 ± 0.0003[b]
FCR	0.90 ± 0.12	0.85 ± 0.02	0.84 ± 0.07

Values are shown as means and standard deviation. Means within the same row and different letters are significantly different ($p < 0.05$).

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Results

Survival rate was considered 100%, as only 2 fish died during experiment, because they jump out of the tank, a normal behaviour for this species. Meagre from treatment DO-3 had a higher feed intake than DO-2, which also had a higher feed intake than DO-1 (Table 1). Final fish weight on DO-1 treatment was significant ($p < 0.05$) lower than DO-2 and DO-3 and the same trend was found for Specific Growth Rate (SGR; Table 1). Despite the increase in feed intake and SGR with the increase of oxygen concentration in the water, there were no significant differences in the FCR, although there was a decrease tendency with the increase of oxygen concentration. In all blood parameters analysed, no significant differences were found between treatments ($p > 0.05$).

Discussion

In this experiment, fish survival was not affected by oxygen levels, thus confirming the high tolerance of meagre to low oxygen concentration. The low feed intake and low growth observed in fish reared at low DO conditions can be explained through the restricted metabolic capacity caused by low DO, and the allocation of energy needs to be prioritized for other body functions, resulting in lower energy available for growth (Araújo-Luna et al., 2018). No differences were observed in the blood parameters analysed, as verified for other species (Araújo-Luna et al., 2018; Samaras et al., 2023), indicating that meagre may have adapted to the different rearing conditions (for instance by the increase on the opercular movements and lowering feed intake). Additionally, the duration of the experiment may have not been enough to observe significant differences on those parameters and future experiments should be longer. In conclusion, the concentration of 4-5 mg L^{-1} of oxygen can be used for meagre aquaculture production, reducing the costs and the need of pure oxygen addition in non-cage farms. Still, the decision should take in consideration the eventual negative impact on growth.

Acknowledgment

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WHITE-WASHING AND ILLEGAL TRADE OF DANUBE STURGEON

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Introduction

The Danube River is the most important refugium for sturgeon populations in the EU inhabited by four species (Huso huso [beluga], Acipenser gueldenstaedtii [Russian sturgeon], Acipenser stellatus [stellate sturgeon], and Acipenser ruthenus [sterlet]). Whereas the first three species are on the brink of extinction, the fourth species (sterlet) shows a declining population trend since decades. In particular, the illegal hunt for caviar is the major risk for populations. Probably, caviar is the most expensive animal products in international trade with prices exceeding 5000 € per kilo of beluga caviar even from aquaculture. Today, sturgeon fishing is no longer permitted in the Lower Danube and the Black Sea. A study was conducted to quantify the amount of illegal sturgeon caviar and meat in trade. Therefore, 149 samples of caviar and meat from the the four Lower Danube countries (Serbia, Romania, Bulgaria, and Ukraine) were investigated in a combined genetic-isotope approach.

Materials & methods

For sturgeon meat and caviar, species or hybrid identification is rarely possible by visual inspection alone. Only DNA analysis can determine the species or hybrid in question, while isotope patterns are essential for detecting wild (poached) fish and identifying the geographic origin. Sampling was managed by authorities. Caviar and tissue samples were stored frozen or in ethanol. DNA was extracted using standard procedure. A combined approach using several genetic markers (mitochondrial DNA; Single Nucleotide Polymorphisms; Microsatellites) was used for secure species/hybrid identification.

The stable isotope method is the leading standard analytical tool to verify the authenticity of biological materials. The method relies on the principle that stable non-radioactive isotopes occur in nature in different relative proportions, because biological processes (such as the water cycle) influence their abundance variations. Stable isotope patterns deliver various information on the geographical origin and the source (e.g. wild versus farmed). The stable isotopes of the elements carbon, nitrogen and sulphur measured in sturgeon tissue reflect the available feed and therefore indirectly the source of an animal or in that study if it was wild-caught or captive-bred.

Results and discussion

Isotope analysis uncovered that wild sturgeon products were on sale in all four countries. Alarmingly high numbers were observed of wild-caught sturgeon (31 samples/21%); additional 17 samples (11.4%) were in violation with CITES and EU regulations, and 25 cases (17%) were found of consumer deception. Of the 31 samples originating from wild-caught sturgeons, 28 yielded results with confidence intervals ≥95%. Within these samples, DNA analysis identified all Danube sturgeon species in varying proportions, but with the sterlet being the dominating species. In addition, 25 samples were sold as wild products but originated from aquaculture, thus indicating an existing consumer demand for wild sturgeon products, which fuels a niche market of wild products that guarantees greater profit. Three were fake caviar and three samples were from non-sturgeon fishes. Our results indicate the urgency of establishing an effective monitoring and enforcement network against poaching and illegal trade, which must be coordinated between all Lower Danube countries including producers (e.g. aquaculture), transit and consumer countries.

ARTIFICIAL INTELLIGENCE AND HIGH-PERFORMANCE COMPUTING FOR DECISION MAKING IN AQUACULTURE

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Introduction

Aquaculture has become one of the fastest-growing industries in the world due to the constant increase in demand, limitations in fishery supply, and innovations in the production process. However, decision-making and planning processes remain particularly complex as they are affected by many technical, biological, environmental, and socioeconomic factors. Therefore, the aquaculture industry is still considered a high-risk and complex industry, in which decisions made throughout the production process are not based entirely on planning and control systems.

In this context, industries would benefit from technological innovation in management and decision-making support systems aiming to maximize efficiency and minimize the environmental impact and risks associated with this activity. In this regard, the main objective of this work is the development and evaluation of new optimization methodologies based on the use of Artificial Intelligence techniques and high-performance computing, to support aquaculture producers in decision-making processes. Thus, the capacity and effectiveness of these techniques to optimize the results of marine farms, from both economic and environmental points of view, will be evaluated.

Materials and Methods

This study follows a two-phase process to achieve the aforementioned objective:

- First, a methodology that integrates a multicriteria model and a Particle Swarm Optimization (PSO) technique, proposed by Luna et al. (2020), is applied to find the optimal production strategy in a farm with multiple cages, batches, feeding alternatives, and products. In this case, this method is focused on improving the results of aquaculture companies from a multicriteria point of view (Figure 1), with low computational cost, addressing decisions regarding stocking and harvesting and the synchronous selection of feed and fry for a large number of cages.
- The optimization process described above would entail a very high computing time due to not only the fact that companies tend to have an increasing number of production units (farms) but also because of the large number of decision criteria included. For that reason, parallelization techniques in distributed systems have been used, based on the techniques published by (Ibáñez et al., 2023), to improve the model computational cost, in terms of performance and energy consumption.

The integration of these techniques is especially important since one of the keys to these methods, and what would allow them to be scaled to different production systems, is their ability to find good solutions in a reduced time, even under complex conditions (Blum and Roli, 2003). To this end, the efficiency of the previous design will be compared to new parallel developments executed with 60 and 80 cores, respectively.

Results

That methodology has been tested for different scenarios of sea bream marine farming with respect to location, sea temperature, and scheduling dates. The information used has been collected from primary sources, such as oceanographic buoys or Spanish market prices, and secondary sources of information, as described by Luna et al. (2020).

On the one hand, the optimization phase has proven to be useful for aquaculture farms as it could achieve an improvement in the aggregated results for the criteria of Figure 1 between 20% and 50%. On the other hand, it proves the importance of high-performance computing in these scenarios as they face exponential growth in the computational cost with increasing complexity, either due to the increase in the number of production units or the inclusion of constraints in the process.

As can be seen in Figure 2, parallelization techniques in distributed memory systems (with 60 and 80 cores) have shown great ability to significantly reduce execution time. The use of these techniques has allowed the optimization of 80 cages in a time of 12 minutes or even 200 in just over 20 minutes. Additionally, the problem of searching for feasible solutions has also been solved due to the possibility of improving optimization parameters without experiencing a significant impact on computing time.

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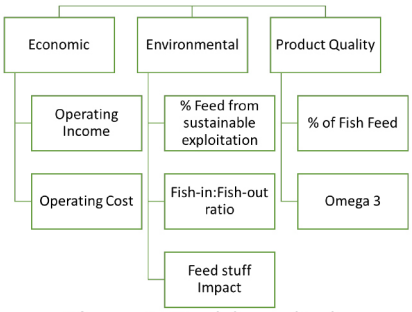

Figure 1. Decision criteria

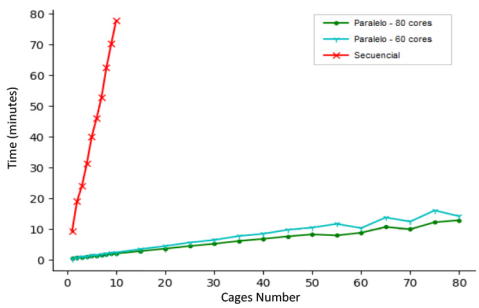

Figura 2. Computational Cost

Conclusions

Decision-making processes are particularly complex in the aquaculture industry. But, as the results of the present study show, decision-support techniques and systems could be the perfect solution to optimize the results of aquaculture companies, from both economic and environmental side.

This is in line with previously published studies regarding the application in the aquaculture industry of decision support systems (Cobo et al., 2019) or multicriteria decision-making techniques (Vergara-Solana et al., 2019). However, this study shows that parallelization techniques in distributed memory systems allow decision-makers to achieve that in sufficiently low computing times, enabling its application to large companies or conglomerates of companies. Furthermore, these results open the door to the application of these techniques in aquaculture companies with different production processes, some with hundreds of production units, and to decisions and scenarios of greater technical complexity.

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GLOBAL OVERVIEW OF NATIONAL REGULATIONS FOR ANTIBIOTIC USE IN AQUACULUTRE PRODUCTION

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Introduction

Aquaculture is the fastest growing food producing industry in the world. It has been subjected to an intensification by an increasing proportion of global farmers (Lulijwa et al., 2020). Especially fed aquaculture has seen a rapid increase and is only expected to grow further (FAO, 2020, IFFO, 2021). This intensification has led to a higher exposure for the animals to disease outbreaks and subsequently to antibiotic treatments (Schar et al., 2020). In turn, this has led to an increasing problem with antimicrobial resistant bacteria (AMR), which quickly has become one of the most pressing public health issues of our time (Léger et al., 2021, Choi et al., 2020). The amount of antibiotics used for aquaculture is expected to increase with 33 percent by 2030 (Schar et al., 2020). With the open nature of most aquaculture production, residues of antibiotic treatments spread into the surrounding waterbody and are very difficult to control (Choi et al., 2020, Bondad-Reantaso et al., 2023). As a result, it affects wildlife, plants, drinking water and subsequently people, making AMR in aquaculture a truly multi-faceted issue. The interconnectedness between public health and animal welfare is at the core of the One Health Approach which aims to design programs, policies, research and legislations to achieve better public health outcomes (WHO, 2017). In this paper we look at the role aquaculture has had in AMR strategies on an international scale up until this point. Furthermore, we analyze whether regulations for antibiotic use in 17 of the largest aquaculture-producing countries match the recommendations set up by the One Health approach by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO). Finally, we compare how two leading aquaculture certification schemes, namely Aquaculture Stewardship Council (ASC) & Best Aquaculture Practice (BAP) work towards limiting the use of antibiotics in aquaculture operations. To retrieve this information, this paper answered the following research questions:

1. How has the presence of AMR been translated into international steering documents and how does it relate to aquaculture?
2. How do the antibiotic regulations in 17 of the largest aquaculture-producing countries/regions compare to these recommendations as defined by the WHO and two leading aquaculture certification programs?

Materials and Method

The findings of the study are based on national regulations, gray literature and international standards and recommendations as well as the standards for best practice set up by the certification schemes. We limited the amount of countries studied to 17 due to document availability as well as production volumes in the observed countries.

For the first research question, we used policy mapping, which is a type of content analysis that is used to make objective and replicable inferences from texts or other forms of communications in this particular context (Krippendorff, 2013, Bengtsson, 2016). More precisely, looking at how the presence of AMR has developed in international commitments over time and how aquaculture has been included. For the second research question we used elements of a comparative case study, where we analyzed and synthesized similarities and differences across multiple cases (Goodrick, 2014).

Results & Discussion

Our results indicate that aquaculture was not included in international steering documents to combat the spreading of AMR until the One Health approach was adopted in 2015 and even now it is not explicitly mentioned but is incorporated in a general animal husbandry consideration. Furthermore, we found that all but three countries allow antibiotics listed as "highest priority" by the WHO in their aquaculture operations. The certification programs have even more stringent standards for the use of antibiotics, banning all antibiotics listed as critical for human health and limiting the number of treatments to maximum three (depending on the species). Other important findings were that nine out of the 17 countries allowed prophylactic use of antibiotics in aquaculture practices and that neither state had a limit to the number of treatments per production cycle. As a result, states still have a long way to go in order to perform according to international recommendations and should start to prioritize this issue in their policies, regulations and strategies for aquaculture production. We argue that a higher emphasis should be put on animal welfare as a means to limiting the use of antibiotics.

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URCHIN-ULVA IMTA IN SOUTH AFRICA: FROM RESEARCH TO PILOT COMMERCIAL SCALE

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Introduction

Sea urchin gonad (uni) is regarded as a premium seafood product. High international demand for these products has, however, led to extensive exploitation and overfishing, and the consequent decline of natural stocks has stimulated research and commercial interest in echinoculture. *Tripneustes gratilla* is a tropical fast-growing shallow water echinoid that occurs along the east coast of South Africa and is one of the most commercially important sea urchin species in Asia, especially Japan. Our group has been investigating *Tripneustes* echinoculture since 2008 and we have successfully addressed all key areas of the life cycle to facilitate commercial-scale production. However, technology for upscaling from the research phase to commercial-scale production needs to be developed further. As part of the EU funded ASTRAL project, we are taking echinoculture from the research phase to the commercial phase and aim to develop the first commercial echinoculture system, integrated with seaweed, in Southern Africa. This contribution will discuss research that is underway to enable commercial scale cultivation of *Tripneustes* under local conditions.

Materials and Methods

Effect of basket depth and feed type on spine loss and consumption was investigated using a 2×3 factorial experimental design with two-treatment levels, basket depth and food type. Three feed types tested (in triplicate) included: formulated feed (pellets) containing 20% dried *Ulva lacinulata* (Cyrus et al., 2015); fresh *U. lacinulata*; and fresh kelp (*Ecklonia maxima*). Both deep (40×15×35cm, L×W×D) and shallow (40×40×10cm, L×W×D) baskets had an internal surface area of $0.32m^2$ and stocking density in both treatments was ~$6kg.m^2$. Trials were conducted over 3 days, with baskets cleaned and animals fed ad libitum daily. The effect of basket removal on spine loss was also conducted. Effects of stocking density on growth and gonad development of *Tripneustes* fed fresh *Ulva* ad libitum were assessed over 3 months. A separate gonad enhancement trial was conducted for 2 months, where animals were fed formulated feed containing 20% dried *Ulva* (1.5% $BW.day^{-1}$). Initial stocking density of baskets (40×40×15cm, L×W×D) for both trials was 4, 6, and 8 $kg.m^2$. Urchin growth, gonad development and gonad quality were assessed before and after treatment. Effects of varying stocking densities on urchin parameters (consumption, faecal production, and spine loss) were also assessed. The first pilot commercial scale urchin-*Ulva* production and hatchery systems were designed and constructed at Buffeljags Abalone farm in South Africa. Several important functionality aspects were tested in the IMTA, including cycling of nutrients (N, P, NH_3), nutritional content and growth of *Ulva* and optimal harvesting rates of *Ulva*. Data from these studies was used to develop a farm-scale model to inform biotechnical feasibility of *T. gratilla*-*Ulva* IMTA systems and assist prospective farmers.

Results & Discussion

Literature suggests production of various urchin species is reduced when cultivated in deeper baskets. The present study confirmed these findings, with deeper baskets resulting in significantly lower consumption of various feed types ($p<0.026$). This is likely the consequence of lower feed accessibility, which in turn causes the observed reduced yield. Baskets of ~10cm depth are therefore recommended to enhance production of *Tripneustes*. Trials provided important management recommendations (e.g., removal of urchins from baskets for extended periods (>5s) or feeding rigid feeds (*E. maxima*) increases spine loss ($p<0.0001$)). While higher stocking densities did significantly reduce mass SGR ($p<0.044$), mortality, cannibalism and gonad size/ quality were not influenced by stocking density. Difference in SGR are attributed to spine loss from negative behavioural interactions. From our data, the optimal stocking density for both grow-out and gonad enhancement of *Tripneustes* is ~20% coverage (surface area of urchins' tests by surface area of basket). The farm-scale model suggests *Ulva* can remove 100% Total Ammonia Nitrogen (TAN) from urchin effluent in the IMTA, but TAN emissions from urchins is insufficient to sustain *Ulva* production. The implications of this study for *Tripneustes* echinoculture development will be discussed, and additional key findings from on-going commercial-scale hatchery and grow-out trials at Buffeljags Abalone will be shared.

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INFLUENCE OF TEMPERATURE AND IRRADIANCE ON THE FIXATION CAPACITY OF THE GREEN ALGA *Codium taylorii* IN DIFFERENT TYPES OF ROPE FOR ITS SUBSEQUENT CULTURE

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Introduction

The culture of macroalgae can constitute an important and sustainable source of food and feed, which is why its promotion is an important part of the new agricultural and food policy of the European Commission, formulated in the communication to Parliament "Towards a Strong and Sustainable EU Algae Sector" (COM (2022) 592). These crops usually require significant initial labor, which has an impact on their production profitability. In order to provide a solution to this problem, this experiment has been carried out for developing a more profitable cultivation techniques. The objective of the same has been to evaluate the effect of two environmental variables (temperature and irradiance) and the substrate for the fixation capacity of utricles and filaments of the green algae species *Codium taylorii*. The species of the genus *Codium* are expected to have great potential in application both in food for humans and animals (Figueroa *et al*., 2022).

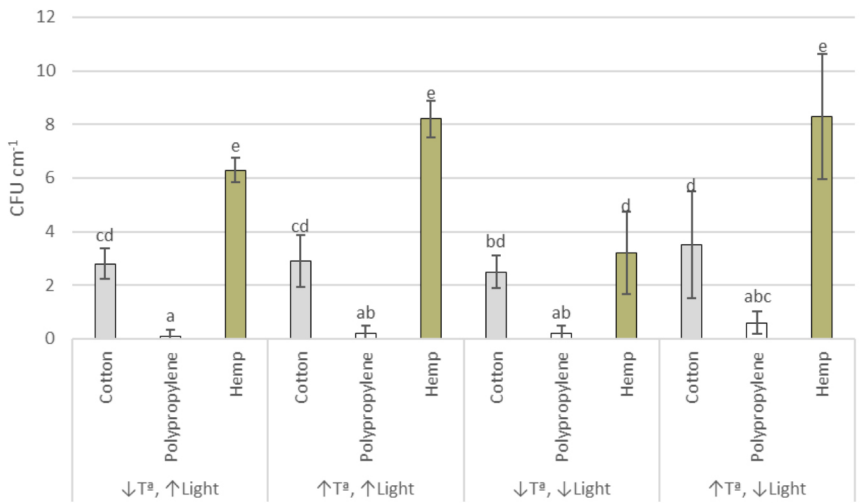
Material and methods

Specimens of *Codium taylorii* were collected in the inner bay of Cadiz (approx. 36° 28' 17" N, 6° 14' 39" W) in southern Spain and kept in culture for two weeks before the start of the trial in February 2023. Parts of the algae thallus were crushed with a blender in order to obtain the isolated utricles and medullary filaments, following the protocol of Hwang *et al.* (2005). To determine the optimal conditions for the fixation of utricles and filaments, a factorial experiment was carried out considering two light intensities (60 and 100 µmol of photons m-2 s-1) and two temperatures (15 and 20 °C). For each condition, fixation on three types of substrate was studied: cotton, hemp and polypropylene ropes.

Results and discussion

A significant effect was observed for the temperature factor and for the substrate on the appearance of CFUs. In addition, the ANOVA indicated the existence of three second-order significant interactions: between temperature and substrate, between substrate and light, and between light and temperature. The highest number of CFUs (11 units/cm) occurred in the high-temperature, low-irradiance treatment for hemp yarn (Graph 1).

In accordance with the results, previous studies agree that the greatest growth occurs at high temperatures, while for irradiance the results vary widely between differents authors for species of the genus *Codium* ((Park & Sohn, 1992; Yang *et al.* 1997; Hwang *et al.* 2005).



Graph 1. *Codium* forming units per centimeter of seeding yarn (CFU cm^{-1}) for each of the four treatments and for each type of substrate. Error bars represent the standard deviation. The letters above the bars show the significant differences for $P<0.05$, according to Tukey's test.

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OPTIMISING CULTIVATION DEPTH TO ENHANCED GROWTH PERFORMANCE FOR COMMERCIAL KELP AQUACULTURE

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In order for seaweed aquaculture to be economically sustainable, the operational costs must be balanced by the production generated in each cycle. The depth at which seaweed is cultivated plays a crucial role in determining the amount of light energy that the cultivated biomass receives, which is essential for achieving optimal growth. If the cultivated biomass receives Photosynthetically Active Radiation (PAR) that exceeds the capacity of photosynthetic tissues, a phenomenon known as photoinhibition can occur, resulting in reduced growth rates. Optimizing PAR throughout the cultivation cycle is unlikely to be achieved by selecting a single cultivation depth, due to the seasonal variations in PAR with depth. Therefore, a careful evaluation of the appropriate cultivation depth for each stage of the cycle is necessary to optimise PAR exposure and achieve optimal growth of the cultivated biomass.

This study investigates the affect of cultivation depth adjustments for twine seeded *Saccharina latissima* and *Alaria esculenta* as a strategy to optimise growth performance and farm efficiency. For each species, two replicate 50m double-catenary lines were connected together using 9 x 4m seeded lines maintaining growing lines under equal tension at 9 depth intervals (0.1m, 0.5m – 4m in 0.5m intervals). In January 2022, lines seeded in October 2021 were removed and yield and morphology established. These growing lines were replaced with growing lines seeded at the same time but maintained on 50m long lines at a depth of 1.5m. This process was repeated in April and the trial concluded in June. Based on the results from this trial only one depth adjustment (spring equinox -21/03/23) was chosen for the following cultivation cycle (October 2022- May 2023). In addition to the double-catenary lines, the affect of depth adjustments was measured across replicate (n=2) 50m longlines maintained at depths of 0.5m (shallow), 1.5m (deep) and 0.5m then 1.5m (shallow-deep) throughout the cultivation cycle. PAR and temperature were recorded at 15 minute intervals at each depth interval using a newly developed sensor chain. Salinity and nutrients were recorded at monthly intervals throughout the trials.

Results show that cultivation depth had a profound impact on growth (yield), morphology and development and this could be correlated with average PAR at each depth selected. *Saccharina latissima* and *A. esculenta* lines maintained at 0.5m depth achieved almost twice the yield by January (2020 and March (2023) when compared to lines maintained a 1.5m optimum previously measured at this site across the entire season. Adjusting cultivation depth from shallow (0.5m) to deep (1.5m) improved yield and overall biomass quality at the point of harvest compared to lines maintained shallow (0.5m) and deep (1.5). Whilst cultivation depth choice is likely to be site specific these results validate the utility of making at least one depth adjustment to the supporting buoys of growing systems or across the entire farming infrastructure to improve productivity and biomass quality.

FUNCTIONAL ANNOTATION AND COMPARATIVE ANALYSIS OF THE DUPLICATED GENOMES OF SALMONID FISHES

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Introduction

High-quality genome functional annotation crucially underpins fundamental and applied genomics studies in all species. The genome sequence of aquatic species, however, typically have limited functional annotation datasets publicly available. Several projects within the international 'Functional Annotation of Animals Genomes' (FAANG) initiative (Giuffra et al. 2019) seek to enhance the quality of functional annotations in diverse farmed animal taxa. AQUA-FAANG (https://www.aqua-faang.eu/) has generated extensive functional annotation data for six commercially important finfish species, including Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). The comparative nature of AQUA-FAANG datasets provides scope to explore the evolutionary conservation of fish-specific genomic features controlling gene expression phenotypes. The aim of this study was to comprehensively map expressed genes and regulatory elements across the genomes of both salmonid species, including their evolutionary conservation following the salmonid-specific whole genome duplication (ssWGD) event, which occurred 100 MYA (Lien et al. 2016; Gundappa et al. 2022).

Methods

For Atlantic salmon and rainbow trout, we generated matched functional genomics data for samples spanning ontogeny, including 14 stages of embryogenesis and a common panel of tissues (liver, brain, gill, intestine, muscle, head kidney, ovary and testis) from males and females at sexually immature and mature stages. The data was generated using standardized methods, and represents approximately 1,000 sequencing libraries, including 130 mRNA-Seq, 70 small RNA-Seq, 80 ATAC-Seq and 270 ChIP-Seq (H3K27ac, H3K4me1, H3K4me3, H3K27me3 histone marks) datasets. Primary analysis for mRNA-Seq, ATAC-Seq and ChIP-Seq was done using nf-core pipelines (Ewels et al 2020). Small RNA data was annotated by the fish microRNA database (Desvignes et al. 2022). All data was submitted to the FAANG data portal (Harrison et al. 2021), and is being used to up-date gene annotations and produce the first regulatory annotations for any teleost species in the Ensembl Genome Browser (ATAC-Seq data for both species publically available as of release 109). We generated novel comparative tools to navigate this new dataset, including a homology prediction integrating phylogenetic and synteny criteria to capture >10,000 high-confidence duplicate gene pairs per species, in addition to a multispecies genome alignment including duplicated regions from ssWGD. ChromHMM (Ernst and Kellis, 2012) was used to model chromatin states in embryonic stages and adult tissues combining ATAC-Seq and ChIP-Seq data, separately for each species. We clustered and visualized gene expression and open chromatin regions across samples using self-organizing maps (SOM) and Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction. Enrichment in transcription factor (TF) binding within regulatory elements was done using GimmeMotifs (van Heeringen & Veenstra, 2011).

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Results & Discussion

SOM clustering and UMAP identified modules of genes showing specific expression at different stages of embryogenesis in both species, for example genes upregulated during the maternal-to-zygotic transition. In adult tissues, we identified tissue-specific, stage-specific and sex-specific clusters of gene expression. ChromHMM identified several distinct chromatin states, including active and bivalent promoters, active, primed and poised enhancers, polycomb repressed regions, and unmarked open chromatin. We observed the expected enrichment of predicted TF binding in promoter and enhancer elements according to sample types, e.g. Sox2/Oct in early embryo stages, Hox in mid-embryogenesis, DMRT3 in testis, HNF in liver, NeuroD in brain, among many others.

In Atlantic salmon, we identified 313,000 high-confidence open chromatin regions across sample types. SOM clustering and UMAP captured the dynamics of regulatory element activity across ontogeny, and revealed regulatory elements specific to tissues, stages and sexes. In embryos, most stage-specific open chromatin regions were present at the end of segmentation, a stage where conserved non-coding elements were strongly overrepresented. We quantified the average divergence in expression of duplicated genes retained from ssWGD across sample types. We also quantified the conservation of chromatin accessibility and regulatory elements in duplicated and singleton (i.e. where one duplicated copy was lost) regions within the Atlantic salmon genome. These analyses revealed extensive variation in evolutionary constraint acting on duplicated gene expression and regulation across ontogeny, broadly matching with expectations of the hourglass model of development (Uesaka et al. 2022).

As a next step, we will overlay our comprehensive annotations of regulatory elements with genetic variation, including single nucleotide polymorphisms and structural variations defined in the Atlantic salmon genome. This will enable the prioritization of candidate causal genetic variants responsible for gene expression phenotypes, with diverse future applications in support of sustainable and profitable aquaculture.

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A COMPARATIVE STUDY OF THE PHYSIOLOGICAL RESPONSES OF ACUTE AND CHRONICALLY STRESSED DIPLOID AND TRIPLOID ATLANTIC SALMON (*Salmo salar*)

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Introduction

One of the main concerns for the environment due to salmon commercial aquaculture are salmon escapees (Keyser et al., 2018; Besnier et al., 2022). Escapees, breeding with wild conspecifics (Diserud et al., 2022), have the potential to endanger local wild populations by introducing genetic changes that may be maladaptive in nature (Besnier et al., 2022). Induced triploidization has been adopted as a strategy to provoke sterility in commercialy farmed Atlantic salmon. However, triploid salmon have shown reduced performance and welfare in commercial settings (Madaro et al., 2021, Stien et al., 2023). This has been suggested to be due to farming suboptimal conditions and impaired stress coping capacity. The aim of this study was to compare physiological responses of diploid and triploid salmon siblings subjected to unpredictable chronic stress.

Materials and methods

Atlantic salmon were reared at the Institute of Marine Research (IMR, Matre, Norway). Diploid and triploid Atlantic salmon were subjected to a 21- days unpredictable chronic stress (UCS) regime and compared with fish that were not stressed. The UCS regime involved exposure of fish to seven unpredictable stressors two times a day. At the end of this period all groups were tested with an acute stressor (netting and transfer in a new tank). Blood chemistry and selected pituitary genes expression were used to assess the stress response. Plasma parameters were sampled before (0) and 15, 30, 45, 60, 90, 120, 240, 300 min post-stress and included measurements of plasma ACTH, cortisol, ions, and metabolites. Gene expression analyses were performed at 0, 30, 60, 120, 240 min post-stress.

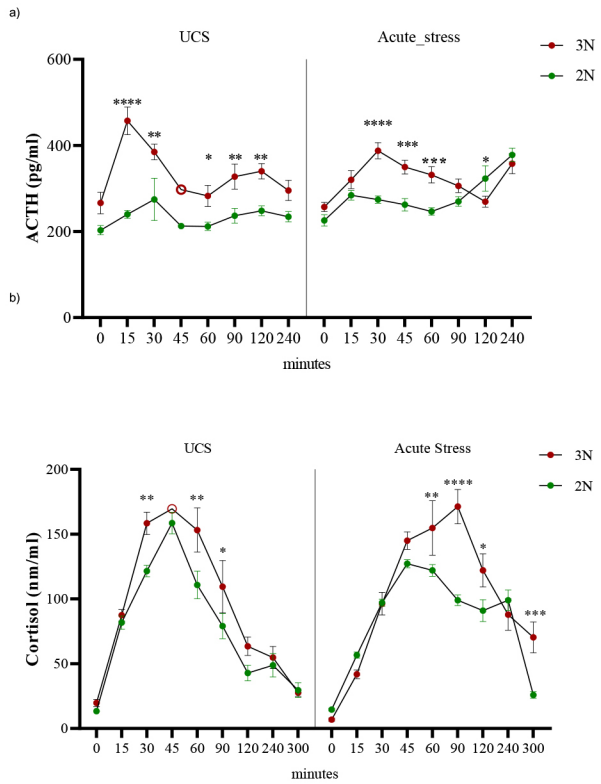

Figure 1 Plasma ACTH (a) and cortisol (b) levels of diploid (2N) and triploid (3N) salmon parr exposed to unpredictable chronic stress (UCS) or naïve fish (Acute Stress) following a stress test.

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Results

Circulating level of ACTH (Fig 1a) and cortisol (Figure 1b) were significantly affected by fish ploidy (diploid vs triploid), type of treatment and time. After the stress test, plasma ACTH levels were mostly higher for triploid than for diploid in both UCS and naïve fish. While pre-stress levels of cortisol were similar between ploidy in both treatments, plasma cortisol levels increased predominantly in both UCS and control triploid groups. More results will be presented at the EAS conference in Vienna (Austria, 2023).

Discussion

The two salmon ploidy groups showed noteworthy differences in the stress response. Post-stress ACTH and cortisol release in plasma were generally higher in both triploid groups. Also, triploid fish showed wider post-stress fluctuations in most of the other plasma parameters. In addition, there were noteworthy ploidy differences in the expression of genes regulating the stress response at the pituitary levels. The study will be discussed in more detail at the EAS conference of Vienna (Austria, 2023).

Funding

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WHITE GRAPE MARC EXTRACTS AS POTENTIAL ANTIMICROBIAL PRODUCTS AND MODULATORS OF EUROPEAN SEABASS GROWTH AND IMMUNE STATUS

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Introduction

Aquaculture has an indubitable contribution to global food production, reducing the gap between supply and demand for aquatic food. The Food and Agriculture Organization of the United Nations estimates that total aquatic food production will increase by 15 percent by 2030, and this growth is mainly attributed to aquaculture.

Intensification of aquaculture rearing conditions contributes to increased stress and susceptibility to disease of farmed fish. Antibiotics use in aquaculture has a positive effect on controlling fish diseases but negatively affects the environment and consumers' opinions about aquaculture fish. Furthermore, the indiscriminate use of antibiotics increases the potential for antimicrobial resistance (AMR) development among pathogens, leading to strong regulations and a drastic reduction of their use in many countries. Moreover, antibiotics are not allowed as prophylactic agents in many countries, namely in Europe. Consequently, the development of efficient and sustainable alternatives to antibiotics is an urgent need to reduce losses due to disease and ensure the economic viability of the aquaculture sector. White grape marc extracts are rich in polyphenols, with antimicrobial and antioxidant capacity, and are an interesting alternative to replace conventional antibiotics and reduce AMR.

In this work, we assessed the potential of three white grape marc extracts to inhibit important bacterial fish pathogens and evaluated their *in* vitro potential against several bacteria pathogens, and the effect of their dietary inclusion at three concentrations on European seabass growth, feed utilization, and immune status.

Material and methods

The *in vitro* antimicrobial activity of three extracts: grape marc extract obtained with ethanol/water 50/50 (ET), with propylene glycol/water 50/50 (PG), and with 100% volatilized acetone (AC) was assessed against fish bacterial pathogens by the well-diffusion assay method and each solvent was used as a negative control. The growth trial was performed with groups of 15 European seabass (*Dicentrarchus labrax*) fingerlings (initial body weight = 23.3 g) distributed in 30 tanks and receiving ten isoproteic (45%) and isolipidic (18%) diets: a control diet similar to a commercial aquafeed and nine diets with the different extracts included at three polyphenols concentrations: 45, 200 and 450 ppm. The growth trial lasted for 42 days and then the plasma of 9 fish per diet was sampled for evaluation of lysozyme and peroxidase activities. Statistical analysis of data was done by two-way analysis of variance (ANOVA) with the extract and concentration as fixed factors and non-orthogonal contrasts between the control and each test diet.

Results

The grape marc extracts obtained from ET and PG produced clear inhibitory halos against *Vibrio vulnificus*, *Tenacibaculum maritimum,* and *Photobacterium damselae* subps. *piscicida*. Additionally, the same extracts interfered with the bacterial growth of *Aeromonas bivalvium*, *V. harveyi*, *V. parahaemolyticus*, *Pseudomonas aeruginosa*, *P. damselae* subps. *damselae* and the gram-positive *Streptococcus agalactiae*. Grape marc extract AC produced clear inhibitory halos against the growth of *P. damselae* subsp. *piscicida*, *V. harveyi*, *T. maritimum*, *S. agalactiae*, and small inhibitory halos (< 2 mm) against *P. damselae* subsp. *damselae* and *V. vulnificus*. Regarding the growth trial, fish accepted well the experimental diets and no mortality was registered (Table 1). No differences were found between groups in the final body weight, weight gain, daily growth index, feed intake, feed efficiency, and protein efficiency ratio. Also, no significative differences were found in the plasmatic lysozyme and peroxidase activities of European seabass fed with the experimental diets, but in absolute values, lysozyme activity was higher in fish fed with all the extracts than in the control group, and peroxidase activity was also higher in fish fed with the PG450 diet than the control (Table 1).

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Table 1. Growth performance, feed utilization, survival, and plasmatic immune parameters of European seabass fed the experimental diets.

Diets	*AC45*	*AC200*	*AC450*	*ET45*	*ET200*	*ET450*	*PG45*	*PG200*	*PG450*	*CTRL*	*SEM*
Initial body weight (g)	23.3	23.3	23.3	23.3	23.3	23.3	23.3	23.3	23.3	23.3	0.01
Final body weight (g)	43.9	42.1	44.6	43.7	45.5	46.1	42.5	43.5	45.8	42.8	0.49
Weight gain (g kg ABW^{-1} day^{-1})	14.6	13.6	14.9	14.4	15.3	15.6	13.8	14.3	15.4	14.0	0.49
Daily growth index[a]	1.60	1.48	1.64	1.58	1.69	1.73	1.50	1.57	1.71	1.52	0.03
Feed intake (g kg ABW^{-1} day^{-1})	19.6	19.8	20.4	20.6	21.1	21.5	20.5	19.7	21.4	20.7	0.25
Feed efficiency[b]	0.74	0.69	0.73	0.70	0.72	0.73	0.67	0.73	0.72	0.68	0.01
Protein efficiency ratio[c]	1.63	1.53	1.60	1.53	1.57	1.60	1.48	1.58	1.57	1.52	0.02
Survival (%)	100	100	100	100	100	100	100	100	100	100	0.00
Lysozyme (µg ml^{-1})	9.66	9.23	9.50	9.70	8.69	9.54	9.59	9.74	9.43	8.32	0.17
Peroxidase activity (U mL^{-1})	85.0	76.5	85.9	84.3	81.3	98.5	103.4	83.4	137.1	91.5	4.41

Values are presented as means (n = 3) and standard error of the mean (SEM). No statistical differences were found in the two-way ANOVA or the non-orthogonal contrast analysis.
ABW: average body weight = ((initial body weight + final body weight)/2).
[a]DGI: daily growth index = ((final body weight1/3 – initial body weight1/3)/time in days) × 100.
[b]FE: feed efficiency = wet weight gain/dry feed intake.
[c]PER: protein efficiency ratio = wet weight gain/crude protein intake.

Conclusions

All grape marc extracts showed antimicrobial activity against *V. harveyi*, *V. vulnificus*, *P. damselae* subps. *piscicida*, *T. maritimum*, and *S. agalactiae*.

Although no statistically significant differences were observed in the growth, feed utilization, and plasma immunity parameters evaluated, there was a trend for a positive effect of the extracts on the immunological response in European seabass fingerlings fed the extracts-supplemented diets. Once demonstrated the safety of the administration of these products on diet, further studies are required to confirm the potential of the grape extracts as modulators of the immune response and resistance against pathogens in European sea bass.

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Gelidium sp. BY-PRODUCTS AS A VALUABLE INGREDIENT FOR EUROPEAN SEABASS AQUAFEEDS

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Introduction

The human population will surpass 10 billion and aquaculture presents itself as a solution to cope with the food demand issue, being a fast-growing food sector. Fish meal (FM) is still an important ingredient as a protein source for aquafeeds, especially for carnivore diets, as it has no anti-nutritional factors, high protein content and balanced amino acid profile, and high palatability and digestibility. Although, its often massive utilization leads to the overexploitation of wild fish stocks and its price is continuously increasing. It´s also important to substitute traditional aquafeed ingredients that are used as human food such as soybean (SBM) wheat meal (WM) and gluten (WG). Thus, this study intents to use low FM diets and look for more eco-friendly and less expensive alternatives with a reasonable protein content such as macroalgae by-products to substitute the traditional plant ingredients for aquafeeds. Therefore, it aims to use *Gelidium* industrial residues from agar extraction, promoting environmental sustainability by reusing nutrients that would be neglectfully discarded. However, the biggest challenge is to satisfy marine carnivore fish nutritional requirements, as macroalgae ingredients have pourer protein and amino acid contents, along with anti-nutritional factors. Thus, this study aims to evaluate how different inclusion levels of *Gelidium* by-products (up to 15%) in diets for European seabass juveniles will influence fish growth, feed utilization, and whole-body composition.

Material and methods

Five diets were formulated with low FM content (15%) and to be isoproteic (44% crude protein) and isolipidic (16% crude lipid) including graded levels (0, 5, 10, and 15%) of *Gelidium* residue after the agar extraction in substitution of SBM,WM and WG. The fifth diet contained 15% of treated *Gelidium* with 1N NaOH (solid: liquid ratio of 4:3) and autoclaved for 30 min at 120ºC. The growth trial was performed at CIIMAR, Matosinhos, Portugal in a recirculating water system (RAS) at 24 °C. A total of 300 European seabass (*Dicentrarchus labrax*) were evenly distributed in the system in triplicate groups of 20, with an initial body weight of 38g. At the beginning of the trial, 10 fish from the initial stock were randomly collected and stored at −20 °C until whole-body composition. Fish were fed 2 times a day, 6 times a week, for 49 days. After that period, fish from each tank were lightly anesthetized with 0.3 mL L^{-1} of 2-phenoxyethanol, and 3 of them were euthanized by decapitation and collected for whole-body composition. Statistical evaluation of the data was done by one-way ANOVA. When p-values were significant ($p < 0.05$), means were compared with Tukey's multiple range test. All statistical analyses were performed using SPSS 27.0 software package for Windows (IBM® SPSS® Statistics, New York, USA).

Results

Results showed no differences in final body weight, weight gain, daily growth index, feed efficiency, and protein efficiency ratio between the diets, with similar results (Table 1). Nevertheless, a slight increase in feed intake was found in fish fed diets with 5 % untreated and 15% treated *Gelidium* by-products compared to fish fed a diet with 15 % untreated Gelidium by-products (Table 1). Whole-body composition, nitrogen, and energy retention (% intake) were not affected by diet composition (Table 2).

Conclusion

In conclusion, European seabass juveniles accepted very well the experimental diets, with no negative effects on growth performance and feed utilization with inclusion levels of *Gelidium* by-products up to 15 %. Therefore, this study showed that diets with low FM (15%) and 15 % of SMB+WM+WG substitution by *Gelidium* by-products can be utilized without negative effects on fish growth, feed efficiency, whole body composition, and nitrogen and energy retention.

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Table 1: Growth performance and feed utilization of European seabass fed the experimental diets.

Diets	Control	G5	G10	G15	G15+T	SEM
Initial body weight (g)	38.0	38.0	38.0	38.0	38.0	0.0
Final body weight (g)	95.0	94.5	88.0	82.2	87.3	2.4
Weight gain (g kg ABW^{-1} day^{-1})	17.5	17.4	16.1	15.0	16.0	0.5
Daily growth index[1]	2.4	2.4	2.2	2.0	2.2	0.1
Feed intake (g kg ABW^{-1} day^{-1})	20.9^{ab}	21.9^{b}	21.6^{ab}	20.5^{a}	22.1^{b}	1.7
Feed efficiency[2]	0.8	0.8	0.7	0.7	0.7	0.0
PER[3]	1.8	1.7	1.7	1.6	1.7	0.0
Survival (%)	100.0	100.0	95.0	100.0	100.0	1.0

Values are presented as means (n = 3) and pooled standard error of the mean (SEM). Means in the same row with different superscript letters differ significantly ($P < 0.05$).
[1]DGI: daily growth index= ((final body weight $^{1/3}$ – initial body weight$^{1/3}$) / time in days) × 100.
[2]FE: feed efficiency = wet weight gain/dry feed intake.
[3]PER: protein efficiency ratio = wet weight gain/crude protein intake.

Table 2. Whole-body composition (% wet weight), and nitrogen and energy retention of European seabass juveniles fed the experimental diets.

Diets	Initial	Control	G5	G10	G15	G15+T	SEM
Dry Matter (%)	26.3	32.0	31.1	31.6	29.9	32.1	0.4
Protein	14.3	15.8	16.3	16.4	15.7	16.5	0.2
Lipids	5.6	10.8	9.3	10.3	9.4	10.4	0.3
Ash	6.4	5.0	4.9	5.1	5.0	5.0	0.0
Gross energy (kJ g^{-1})	5.2	7.5	7.4	7.5	6.9	7.4	0.1
Protein nitrogen retention (% NI^{a})[b]	-	30.8	31.0	29.9	27.1	30.0	0.7
Energy nitrogen retention (% NI)[c]	-	35.7	33.0	31.8	29.2	31.7	1.0

Values are presented as means (n = 3) and pooled standard error of the mean (SEM).
[a] NI: Nitrogen intake
[b]Nitrogen retention = ((FBWxFBN -IBW x IBN)/NI) x 100.
[c]Energy retention = ((FBWxFBE -IBW x IBE)/EI) x 100.

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A NOVEL APPROACH FOR RECIRCULATING AQUACULTURE SYSTEM (RAS) BASED ON PHISICOCHEMICAL WATER TREATMENT PROCESS

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Aquaculture is the fastest-growing sector in animal protein production projected at 100 million tons by 2030. But with the limited availability of land and water, the only viable solution is intensification – to produce more fish per unit of area and water. This shifted the focus towards longevity and sustainability of aquaculture, now driven by innovative, highly sustainable and cost-effective solutions. One such solution, Recirculating Aquaculture Systems (RAS), are based on the treatment and reuse of water via the application of mechanical filtration, followed by biofiltration, disinfection, and oxygenation. Available RAS technologies suffer from several limiting factors restricting their wide application: (1) Difficulty in meeting desired environmental standards, namely related to inefficient removal of nitrogen and phosphorus compounds; (2) bio-filter limitations, such as long start-up time, temperature dependency, possible amplification of pathogens within the biofilter (3) generation of fish off flavor agents such as MIB & Geosmin generating a muddy taste of the fish causing tremendous lost for the farmer. These factors result in increased production costs due to environmental-related expenses, fish health issues affecting both growth performance and survival rates, and high capital costs particularly apparent in RAS focusing on cold-water fish, which require large bio-filter surface areas.

A new operational approach for RAS, is based on physicochemical water treatment techniques. Within this concept the fish are grown at high TAN concentration and around neutral pH that is calculated to maintain the toxic NH_3 concentration lower than a predetermined threshold. The inherently high Cl^- concentration in seawater enables efficient electro-generation of $Cl_{2(aq)}$ which consequently oxidizes ammonia directly into innocuous $N_{2(g)}$. The system's water passes in a semi-batch mode through the water treatment unit and then returns to the fish tanks, supplying disinfected water with zero TAN and off-flavor agents, and most of the acidity required for maintaining the alkalinity mass balance in the RAS. A powered controller with a highly-intuitive graphic UI monitors and controls the water's pH, temperature, O_2, ORP, Cl_2 and NH_4^+ levels for maintaining optimal water quality for the fish. The BioFishency ELX enables real-time data collection and management via an intuitive dashboard through a cloud-based solution. Remote monitoring and operation are facilitated by an easy-to-use mobile app, accessible from any location, at any time, via any mobile device or tablet. Intelligent process adaptation using Machine Learning technologies, are planned for future versions.

The presentation will include a technical description of the Biofishency ELX technology and describe it value proposition to both investors and fish farmers in three major market segments: Off-flavor purging under regular feeding regime, Full grow-out period in RAS, and Zero discharge transportations of live fish in welboats.

Tetradesmus obliquus AND *Raphidonema monicae* BIOMASS PRODUCED THROUGH A CIRCULAR ECONOMY APPROACH FOR ATLANTIC SALMON (*Salmo salar*) FEEDS

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Introduction

Biomass from microalgae has the potential to replace fish- and soybean-based ingredients in aquafeeds, improving the sustainability of the aquaculture sector. Nevertheless, the incorporation of microalgae can have an impact on the end cost of feeds. Furthermore, the rigid cell wall of microalgae can lower the nutrient bioavailability and digestibility of farmed fish. Through the present work, we show that by adopting a circular economy approach that relies on re-using nutrients and water from hydroponic drain water for microalgae growth, we can upgrade the produced biomass using a biorefinery process, thus developing innovative bioproducts for agriculture and aquaculture markets.

Material and methods

Tetradesmus obliquus and *Raphidonema monicae* were produced in an industrial 19-m^3 tubular photobioreactor at Necton S.A. facilities, using hydroponic drain water as culture medium, reaching a dry weight of 1.8 and 1.6 $g.L^{-1}$, respectively. After harvesting the biomass, a simple biorefinery approach (high-pressure homogenization and centrifugation) was applied to generate two bioproducts: aqueous extracts for the agriculture sector and residual biomass for the aquaculture sector. The present study focused on the residual biomass of *T. obliquus* and *R. monicae* that was incorporated at 10% into experimental aquafeeds as a substitute for fishmeal. Two experimental diets containing the respective microalgal biomass and a commercial-like diet (control diet with fishmeal) were offered to Atlantic salmon (*Salmo salar*) post-smolts with an average start weight of 150.70±0.06 g. The experimental fish were reared in flow-through tanks in an indoor seawater research facility, and fed to satiation, twice a day, for 9 weeks. The feeding trial aimed to examine fish growth, feed performance, fillet quality as well as the intestinal health of the fish.

Results

At the end of the experiment, the final average weight of the fish was 368.70±1.20 g. We did not detect significant differences in specific growth rate (average 1.38±0.02 $\%.day^{-1}$) and feed conversion ratio (average 0.75±0.02) among the dietary treatments. The proximate composition of the whole body did not reveal treatment-linked differences: protein, fat, and ash content ranged between 52.69-54.55% dry matter (DM), 13.83-14.22% DM, and 1.28-1.45% DM, respectively. The fatty acid profile of the salmon fillet was determined as well, and the total polyunsaturated fatty acid content was in the range of 30.42-30.56%, with the sum of eicosapentaenoic and docosahexaenoic acids ranging between 10.74-11.09% of total fatty acids. With regards to digestibility, the diet containing *R. monicae* demonstrated a significantly higher protein digestibility when compared with that of *T. obliquus* and the control diet, while displaying a significantly higher lipid digestibility compared to that of *T. obliquus*. Histological analysis revealed that the fish fed *T. obliquus* had shorter villi and the fish fed *R. monicae* had significantly higher number of goblet cells per villi and significantly higher number of neutrophils. Nevertheless, analysis of the expression of genes connected to inflammation and tight junction proteins did not point to the adverse effects of the microalgal biomass.

Conclusion

The processed microalgal biomass of *Tetradesmus obliquus* and *Raphidonema monicae* can be effective substitutes of fish meal in diets for Atlantic salmon smolts. This study points to the potential value of the tested circular approach in developing new ingredients for aquafeeds.

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EVALUATION OF SAFETY AFTER INTRAPERITONEAL VACCINATION OF SEA BREAM (*Sparus aurata)* WITH THE EMULSION VACCINE AGAINST PASTEURELLOSIS AND VIBRIOSIS, ALPHA JECT MICRO 2000

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Photobacteriosis (*Photobacterium damselae* subsp. *Piscicida*) and classical vibriosis (*Vibrio anguillarum* O1) are two bacterial diseases that have the potential to cause problems for farming of sea bream. Safe and efficacious vaccines, including Alpha Ject micro 2000, for prevention of photobacteriosis and vibriosis in sea bass (*Dicentrarchus labrax*) have been commercially available many years but no vaccine for sea bream (*Sparus aurata*) has been licensed. The aim of this study was to investigate under controlled laboratory conditions, the safety of Alpha Ject micro 2000 after intraperitoneal (IP) administration to sea bream of the minimum recommended weight at vaccination. Fish groups of 10 - 12 g and >12 - 15 g were IP vaccinated with the recommended dose of the vaccine. The study groups were observed daily for abnormal behaviour and mortality throughout the study period of 12 weeks post vaccination (wpv). Local reactions (adhesions and melanisation) and amount of vaccine residues were evaluated according to Pharmaq scale for local reactions in sea bass and sea bream after IP vaccination, 3 -6- and 12-weeks post vaccination. Individual weights in the study groups were measured at vaccination and at 3, 6 and 12 wpv for assessment of growth.

No mortality post vaccination was observed in any vaccinated group. The severity of adhesions was quite similar between vaccine groups at the different evaluation time points, with 1.4 mean adhesions score for both groups at 12 wpv. The observed severity of adhesions was mild. No melanin was observed on the abdominal organs or the abdominal wall for any group at termination. Moderate amounts of vaccine residues were detected at termination (score of 1-2).

The growth of both vaccinated groups was considered normal and as expected under laboratories conditions, showing that vaccination with oil-adjuvanted vaccines containing inactivated antigens of *P. damselae* and *V. anguillarum* O1 did not result in any growth reduction in sea bream in the 12 weeks observation period of this study.

Safety was satisfactorily demonstrated until 12 wpv for the two investigated fish sizes of sea bream IP vaccinated with Alpha Ject micro 2000.

EFFECTS OF FREEZE-DRIED *Rhodotorula mucilaginosa* IN FISH FEED OF *Sparus aurata* ON THE FISH GUT MICROBIOME

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Introduction

As the aquaculture sector continues to grow at a rapid pace, there is a need to discover effective feed supplements that enhance the growth and welfare of fish bred in intensive environments. The capacity of yeasts to transform low-value materials into valuable resources has garnered interest as a promising novel ingredient in aquaculture. Among these resources, polysaccharides (e.g. b-glucans, mannans) and pigments (carotenoids) are important ingredients and have attracted significant research efforts. Studies have shown that the dietary inclusion of yeasts could reduce the adverse effects of soybean meal inclusion in Atlantic salmon (Agboola et al., 2021). Dietary hydrolyzed *Rhodotorula mucilaginosa* has improved the growth performance and antioxidant capacity of Nile Tilapia (*Oreochromis niloticus*) (Chen 2019). In the current study, the yeast *R. mucilaginosa* was used as a fish feed additive to investigate its effects on juvenile seabream *Sparus aurata* individuals' microbiome.

Materials and methods

Rhodotorula mucilaginosa strain ACA-DC 5340 were grown on Potato Dextrose Agar petri-dishes and freeze-dried into a dry powder. Four experimental isolipidic, isoenergetic and isoproteic diets containing lyophilized yeast at at 0%, 1%, 2%, and 3% inclusion levels, were formulated by the Aquaculture Laboratory of the University of Thessaly. One hundred twenty (120) fish were acclimatized to laboratory conditions. The experiment consisted of four experimental groups (control, R1, R2, and R3), and each group encompassed three replicate tanks. Fish were hand-fed twice daily at a rate of 1,2% BW. At the end of the experiment, three (3) fish from each tank (36 fish in total) were sacrificed and the distal intestine was dissected and stored at -20°C until downstream applications. Bacterial DNA was extracted with DNA Nucleospin Tissue (Macherey – Nagel, Duren, Germany) according to manufacturer instructions. V3-V4 variable region of 16S rRNA was amplified with 341F/785R primers. The corresponding libraries were normalized, pooled, and sequenced (paired-end, 2×250, v2 chemistry) using Illumina MiSeq (Microbiome Core Facility, Technical University of Munich). Normalization of OTU tables and downstream analysis was carried out with Rhea 1.1.6 software.

Results

The results showed that a total of 1.1 million high-quality reads for the 36 samples accounted for 572 operational taxonomic units (OTUs). Corresponding OTUs were mainly classified into five (5) groups with Firmicutes being the most abundant followed by Bacteroidota, Proteobacteria, Actinobacteriota, and Verrucomicrobiota. Microbial richness, was not drastically affected in the fish provided with dried yeast. The alpha-diversity, the Shannon and Simpson indices as well as the effective diversity and effective richness were increased, but not at a statistically significant level, while the composition of their microbial communities was significantly differentiated. Significant differences among treatments were observed in the genera of Acinetobacter, Cellulosilyticum, Clostridium, Corynebacterium, Cutibacterium, Enhydrobacter, Lawsonella, Micrococcus, Paracoccus, Phascolarctobacterium και Staphylococcus. Nine (9) OTUs exhibited significant differences between control and *Rhodotorula mucilaginosa* enriched treatments.

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Discussion

The results of the study demonstrated the existence of two groups of dominant phylotypes for both control and treatment groups. One of the groups tended to significantly differentiate in terms of bacterial strains. The dominance of the phylum Firmicutes in the intestinal microbiota of *Sparus aurata* is in accordance with previous studies. Firmicutes, Proteobacteria and Actinobacteria are considered as important for nutrition, the immune system, and metabolic homeostasis (Panteli et al., 2021, Moroni et al., 2021). Furthermore, Clostridia reduction is in line with a previous study in gilthead seabream in which diets containing garlic, carvacrol, or thymol essential oils provided effective antibacterial characteristics against fish pathogens, enhancing immune responses (Firmino et al., 2021). Beneficial bacteria in the gut of fish modulate fish innate immune system, compete for nutrients and antagonize other bacteria for adhesion sites. Concluding, *R. mucilaginosa* addition in seabream diets did not reduce microbial biodiversity and richness; any significant species differentiation seems that positively affect the intestinal microbiome.

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Rhodotorula mucilaginosa ENRICHED DIETS REGULATE GENE EXPRESSION OF THE POSTERIOR INTESTINE IN GILTHEAD SEABREAM *Sparus aurata*

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Introduction

The utilization of yeasts and their derivatives as additives in aquaculture fish feeds, can be an efficient way to promote fish health and robustness (Machuca et al., 2022). Yeast compounds may promote intestinal health and positively modulate the morphology of the intestinal mucosa (Warwas et al., 2023). Dietary supplementation of hydrolyzed *Rhodotorula mucilaginosa* at an inclusion level of 1% has been demonstrated to regulate nutrient composition, immune response and antioxidant capacity of juvenile Nile tilapia (*Oreochromis niloticus*) (Chen et al., 2019). The present study focuses on the effects of *R. mucilaginosa* enriched diets on the intestinal gene expression of gilthead seabream *Sparus aurata*.

Materials and Methods

Four experimental isolipidic, isoenergetic and isoproteic diets containing lyophilized *Rhodotorula mucilaginosa* strain ACA-DC 5340 obtained by ACA-DC collection of Agricultural University of Athens, were formulated by the Aquaculture Laboratory of the University of Thessaly. One hundred twenty (120) *Sparus aurata* juveniles were acclimatized to laboratory conditions, and were randomly distributed into 12 tanks (174L). The experiment consisted of four experimental groups (control: 0%, R1: 1%, R2:2% and R3: 3%) and each treatment included three replicates. Fish were hand-fed twice daily at a rate of 1,2% BW. At the end of the experiment, the hindgut of four (4) fish from each treatment (16 fish in total) was dissected and stored at -20°C. Total RNA was extracted from intestine tissue and samples were sequenced in an Illumina Novaseq platform (Novogene, United Kingdom). After sequencing (PE150, 6Gbp per sample), raw reads were mapped to *S. aurata* reference genome (fSpaAur1.1, GCA_900880675.1) using Hisat2. Raw reads were counted with htseq-count and differential gene expression was assessed using the DESeq2 library in R environment.

Results

Significant differences in gene expression were observed among treatments with 293 upregulated and 374 downregulated genes for R1 treatment, 474 upregulated and 419 downregulated genes for R2 treatment and 280 upregulated and 265 downregulated genes for R3 treatment. The three treatments exhibited 177 upregulated and 208 downregulated common genes, while the R1 and R2 treatments had the larger number of common differentially expressed genes. Hierarchical clustering of differentially expressed genes revealed two groups and principal component analysis (PCA) verified the existence of two clusters with 64% of total variance, while different R samples were not clustered per treatment. Gene Ontology (GO) analysis revealed that the major groups of upregulated genes are involved in lipid biosynthetic process (Biological Process), integral components of the membrane (Cellular Component) and iron ion binding (Molecular Function). On the other hand, no significant ontologies were identified for downregulated genes in all experimental treatments.

Discussion

Intestinal epithelium is of paramount importance for nutrient absorption (e.g., ions, peptides and lipids) and gastrointestinal status may change due to nutritional synthesis. Gene ontology analysis demonstrated an overrepresentation of genes with biological functions that are involved in the lipid biosynthetic process. For example, fish fed *Rhodotorula* enhanced diets exhibited increased expression of *lipid biosynthetic genes* (e.g. *desaturases, monoxygenases*) and *solute carrier 13* (*slc13*) a major carrier of citrate which is important for the regulation of lipid biosynthesis (Akhtar et al., 2023). The R2 group exhibited the highest number of counts in the aforementioned category. These results are in accordance with previous findings where dietary MOS affected the lipid metabolism of European seabass (*Dicentrarchus labrax*) (Torrecillas et al., 2015). Furthemore, iron ion binding genes (e.g. cytochromes P450, monooxygenases) were upregulated in all three experimental treatments. Iron ion binding genes were downregulated in *Salmo salar* fed with soy-derived substances, while simultaneously upregulated inflammation-associated genes (Kiron et al., 2020). These suggest that the supplementation of diets with *R. mucilaginosa* may positively affect lipid metabolism and iron binding in intestinal epithelium.

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THE EFFECT OF LIVE PREY FEEDING ON THE HEALTH STATUS OF RAS-CULTURED PIKEPERCH (*Sander lucioperca*) JUVENILES AS STOCKING MATERIAL FOR THE OPEN WATERS

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Introduction

Pikeperch (*Sander lucioperca*) is a top predator of European aquatic ecosystems. It is used as a complimentary fish species in polyculture stocks for bio-melioration (Adámek and Opacak 2005). Recirculating aquaculture systems (RAS), have emerged as vital for rearing pikeperch, with efficiency making it reliable source of the fish aimed for extensive aquaculture and restocking (Policar et al. 2019). The intensive culture in RAS, however, can be compromise the physiological balance and health status of reared fish (Ottinger, Clauss, and Kuenzer 2016).

Live prey feeding is a common practice in aquaculture to provide fish with a diverse and natural diet, promoting their overall well-being and physiological condition (Wang et al. 2009)a commercial dry feed (DD. However, the specific implications of live prey feeding on the health of pikeperch juveniles in RAS systems have not been extensively studied.

By examining the effects of live prey feeding on the health parameters of pikeperch, this study aimed to provide valuable insights into the potential consequences of different feeding practices on the health and welfare of RAS-cultured pikeperch juveniles. Ultimately, it supports the successful acclimatization and survival of pikeperch juveniles in open waters, contributing to the effectiveness of replenishment and conservation of wild populations.

Material and methods

Juvenile pikeperch of RAS origin were produced at the Faculty of Fisheries and Protection of Waters, University of South Bohemia (FFWP, USB). The production method involved a combination of pond culture during early ontogeny (4-50 days post-hatching; DPH) and culture in RAS (50-150 DPH) to facilitate weaning onto dry pelleted feed and subsequent ongrowing. At the time of the experiment, the RAS-cultured fish had a body weight (BW) of approximately 30.7 ± 2.64 g and a total length (TL) of 164 ± 7.86 mm. The experiment was conducted in a recirculating aquaculture system (RAS) equipped with an oxygen reactor, mechanical and biological filters. To evaluate the effect of live prey feeding after eight week feeding experiment with two groups: 1st negative group – fed with artificial pellets and 2nd positive group fed *ad libitum* with prey fish (*Pseudorasbora parva*). In total, 30 pikeperch juveniles were dissected at different stages of the experiment for the determination of growth rate, somatic indices, blood haematology, and biochemistry parameters. Ten fish were be sampled at the stocking time to assess the initial health status and the same number of fish at the end of the experiment from both positive and negative control groups.

Results and discussion

Pikeperch juveniles fed with pellets had comparable final body weight and SGR with the fish fed on live prey (Table 1). This indicates that the pellet feed provided similar growth results to the live prey. Similarly, the total length of fish fed with live prey was significantly higher compared to fish fed with pellets. This suggests that the fish fed on live prey experienced greater overall growth and development. Hepatic somatic indices and visceral fat indices were significantly lower in fish fed with live prey. This implies that the fish fed on live prey had lower liver and visceral fat content, indicating a potential difference in metabolism between the two feeding groups.

Analysis of biochemical indices showed significant differences between experimental groups (Table 1). Values of TP were significantly lower in juveniles fed on live prey. This suggests that the fish fed on live prey had lower levels of TP, indicating a potential difference in dietary protein intake and utilization between the feeding groups. Values of GLU were the lowest in the fish fed with pellet feed. This indicates a potential difference in carbohydrate metabolism and the baseline level of the stress between the two feeding groups. Fat metabolism was significantly influenced by live prey feeding. The fish fed on live prey had altered lipid metabolism, as evidenced by the differences in CHOL, ALB, and AST levels. The values of NH3 in the blood plasma of fish fed on live prey was the lowest, indicating a potential difference in protein metabolism and waste excretion between the two feeding groups.

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Table 1. The comparison of biometrical measurements, organ-somatic indices and biochemical parameters of blood plasma between pikeperch (*Sander lucioperca*) juveniles fed with live prey and pellet fed.

Parameters	Groups			Statistical analysis
	Initial	Pellet feeding	Live prey feeding	
Growth indicies				
BW, g	30.7 ± 2.64b	54.9 ± 7.03a	57.2 ± 6.42a	F(2, 27) = 66.0, P < 0.05
TL, mm	164 ± 7.86c	186 ± 7.84b	194 ± 6.06a	F(2, 27) = 47.2, P < 0.05
SGR	-	1.03 ± 0.19	1.11 ± 0.21	F(1, 18) = 0.66, P = 0.43
Somatic indicies				
HSI	1.45 ± 0.36a	1.91 ± 0.76a	0.59 ± 0.21b	F(2, 27) = 14.9, P < 0.05
SSI	0.56 ± 0.58	0.13 ± 0.01	0.14 ± 0.26	F(2, 27) = 0.85, P = 0.44
V(F)SI	4.15 ± 1.45a	3.90 ± 0.81a	0.83 ± 0.24b	F(2, 27) = 36.0, P < 0.05
RGL	0.39 ± 0.05ab	0.40 ± 0.03a	0.36 ± 0.03b	F(2, 27) = 3.81, P = 0.04
GaSI	0.79 ± 0.28a	2.56 ± 0.39a	1.78 ± 0.64b	F(2, 27) = 37.1, P < 0.05
Biochemical indicies				
TP	37.3 ± 6.53a	34.7 ± 2.95ab	30.7 ± 2.31b	F(2, 27) = 5.85, P < 0.05
LIPA	0.35 ± 0.07	0.35 ± 0.14	0.26 ± 0.11	F(2, 27) = 2.18, P = 0.36
AMY	16.0 ± 3.11a	12.5 ± 2.17b	7.45 ± 0.91c	F(2, 27) = 36.4, P < 0.05
GLU	9.66 ± 5.10a	3.42 ± 0.56b	7.51 ± 2.34a	F(2, 27) = 9.48, P < 0.05
CHOL	4.39 ± 0.82a	4.31 ± 1.35a	2.30 ± 0.48b	F(2, 27) = 15.3, P < 0.05
ALB	5.00 ± 1.63b	4.26 ± 0.89b	6.16 ± 0.70a	F(2, 27) = 7.06, P < 0.05
ALT	0.78 ± 0.43a	0.35 ± 0.19b	0.24 ± 0.09b	F(2, 27) = 10.7, P < 0.05
AST	3.92 ± 2.24	2.13 ± 1.87	2.13 ± 1.21	F(2, 27) = 3.21, P = 0.06
TG	4.80 ± 3.04	6.50 ± 3.39	6.25 ± 6.27	F(2, 27) = 0.60, P = 0.56
NH3	632 ± 220b	1241 ± 259a	496 ± 142a	F(2, 27) = 34.8, P < 0.05

SGR – Specific growth rate
HSI – Hepatosomatic index
SSI – Spleen somatic index
VSI – Visceral (fat) somatic index
RGL – Relative gut length
GaSI – Gastro-somatic index
TP – total protein, g/L
LIPA – lipase, µkat/L
AMY – amylase, µkat/L
GLU – glucose, mmol/L
TCHO – total cholesterol, mmol/L
ALB – albumin, g/L
GLOB – globulin, g/L
TG – triglyceride, mmol/L
NH3 – ammonium, µmol/L
ALT – alanine aminotransferase, µkat/L
AST – aspartate aminotransferase, µkat/L

In summary, the study findings indicate that feeding pikeperch juveniles with live prey resulted in improved growth (BW, TL, SGR), lower HSI and VSI, and potential differences in protein, glucose, and lipid metabolism compared to fish fed on pellet feed. Overall, the findings of this study suggest that transitioning pikeperch from pellet feed to live prey before their release into open waters can potentially lead to enhanced growth, and metabolism. These considerations are important for successful restocking programs and the overall sustainability of pikeperch populations into open waters.

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INFLUENCE OF CORTISOL- AND MICROALGAE-CONTAINING FISH DIETS ON NITRIFYING BACTERIAL COMMUNITIES AND THEIR ACTIVITIES IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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Introduction

Nitrification in biofilters of recirculating aquaculture systems (RAS) is an essential process in water treatment. In this process, ammonia produced by aquatic organisms, which is toxic to animals in higher concentrations, is oxidized to the less harmful nitrate. This process is catalyzed mainly by two groups of bacteria, firstly ammonia oxidizing bacteria (AOB) and secondly nitrite oxidizing bacteria (NOB). A stable and active nitrifying bacterial community is important for maintaining a good water quality and thus animal health and welfare. However, nitrification in RAS can be affected by several factors, especially fluctuating operating parameters. These include, for example, temperature, pH, oxygen content, or C/N ratio. Another potential influencing factor on the nitrifying bacterial community in RAS is cortisol. This hormone is released by fish in response to stress and can have negative effects on fish welfare at higher concentrations. Previous studies have shown that certain groups of bacteria can also be negatively affected by cortisol. Other factors can also positively affect fish welfare, such as microalgae as an important food source for fish, because some microalgae species contain valuable nutrients. However, knowledge about the influence of cortisol and microalgae on nitrifying bacteria is limited.

In this study, the influence of cortisol on the turnover rates and composition of the nitrifying community as well as a possible reduction of the stress response by microalgae as a dietary supplement was investigated.

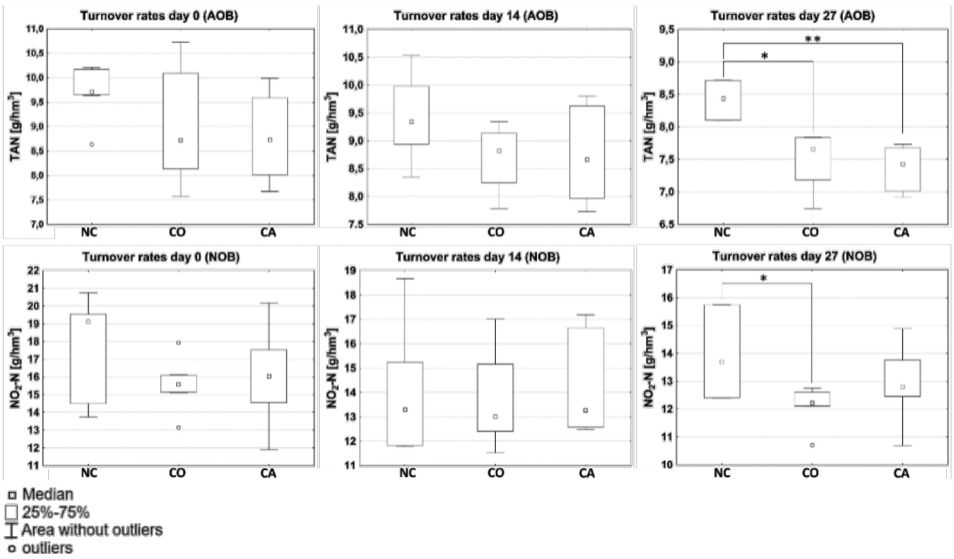

Fig. 1: Box plot of turnover rates of ammonia oxidizing bacteria **(a)** and nitrite oxidizing bacteria **(b)** after 0, 14 and 27 days with different treatments in RAS biofilters. **NC:** negative control (conventional feed); **CO:** cortisol containing diet; **CA:** cortisol and microalgae containing diets. ***:** p-value < 0,05; ****:** p-value < 0,01.

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Results

Mean values of nitrifying turnover rates showed a significant decrease in AOB and NOB activities in the biofilters after feeding fish (*Salmo salar*) with cortisol-containing feed for four weeks relative to the control group (conventional feed). In addition, an even more significant decrease in AOB activities was observed when fish were fed a combination of cortisol- and microalgae-containing diets, whereas NOB activities showed no significant decrease in the combined diet compared to the control group. However, it should be noted that the biological and technical replicates differ significantly in some cases (Fig.1). For a final assessment of the data, further investigations in this regard would be advisable.

Based on 16S rRNA amplicon sequencing, Bacteriodota, Pseudomonadota and Planctomycetota were identified as the most abundant phyla in all biofilters studied. Dependent on time and treatment, the relative abundance varied between 42% - 49% for Bacteriodota, 18% - 29% for Pseudomonadota, and 7% - 11% for Planctomycetota. *Nitrosomonas* and *Nitrospira* were the dominant nitrifying genera. The total percentage of nitrifiers varied between 3.3% (cortisol and microalgae containing diets) and 4.6% (conventional diet).

SIDESTREAM: SECONDARY BIO-PRODUCTION OF LOW TROPHIC ORGANISMS UTILIZING SIDE STREAMS FROM THE BLUE AND GREEN SECTORS TO PRODUCE NOVEL FEED INGREDIENTS FOR EUROPEAN AQUACULTURE

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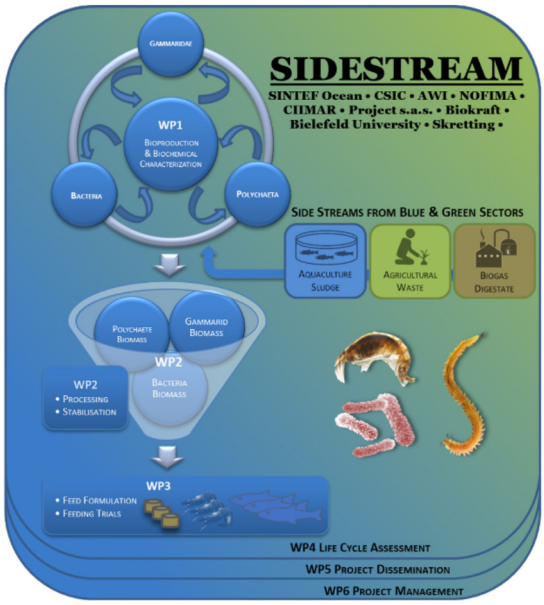
The goal of the BlueBio co-funded SIDESTREAM project was to evaluate new ways of recycling aquaculture and agriculture wastes to save resources and create value.

Aquaculture and agriculture produce vast amounts of wastes. The SIDESTREAM project regards these wastes as ‚side streams‘ and considers them a resource. We investigated to which degree marine organisms such as polychaete worms and gammarid shrimps, and also bacteria can be utilised to recapture nutrients, energy and biochemicals from side streams. Furthermore, we evaluated the suitability of the biomass as a fish and shrimp feed ingredient. Finally, we evaluated the sustainability and economic feasibility of these activities.

All side streams used as feed input for the projects model species were utilized as feeds, in some cases (e.g., liquid phase biogas side streams for bacteria) pre-treatments were necessary. Rearing protocols for the marine polychaete *Hediste diversicolor*, the gammarid shrimp *Gammarus locusta*, and the genetically modified *Corynebacterium glutamicum* have been established. These protocols have been developed to optimize biomass production, resource utilization and production of high nutritional value fatty acids (gammarids and polychaetes), and astaxanthin (*C. glutamicum*).

During the project we learned how to treat these biomasses to process and stabilize valuable compounds. Further, polychaete, gammarid and bacterial meals were formulated into fish and shrimp feed and tested for their suitability as replacement of either fish meal or fish oil, or as attractants.

Juvenile sea bass (*Dicentrachus labrax*) thrived on diets replacing 10%, 20%, or 40% of the fish meal with polychaete meals. None of the fish fed polychaete meals differed in growth, feed conversion, or protein digestion efficiency from those fed commercial sea bass diets.



Juvenile rainbow trout fed diets containing astaxanthin produced within the project showed less appetite for the diets compared to trout fed diets containing commercially available astaxanthin sources, which was also reflected in reduced growth rates. However, it shall be noted that the astaxanthin produced in the project was used as autoclaved bacterial biomass, not as extracted pigments, which clearly differs from using synthetically produced astaxanthin, and the result is hence to be treated with caution.

LCA analysis revealed that electricity and water usage is a main driver of costs and decreased sustainability when biogas digestate shall be used as feed input. The reason for this is the high ammonia content which needs to be removed from the

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solid phase. LCC comparing a high-tech and a low-tech scenarrio revealed that scaling effects in the low-tech scenario by far exceeds those in high-tech scarios. LCA on polychaete meals as a fish meal replacer revealed that suggests that the circular approach of rearing polychaete on aquaculture wastes activities may reduce the environmental impact by 23% as opposed to a linear approach.

SIDESTREAM covered several steps along the value chain – from biomass production to the provision of products through conversion and processing. SIDESTREAM enhanced our knowledge on the ability of invertebrates to produce high value compounds. The project lead to novel and refined production techniques and protocols leading to increased production of these compounds under highly controlled conditions.

BIVALVE GROWTH IN MULTITROPHIC AQUACULTURE AREAS OF GREECE

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Introduction

Aquaculture has a long history, but it is only in the late 20th and early 21st centuries that it has reached the point of providing 50% of the world's fisheries resources (*FAO, 2018*). As human population increases and aquaculture needs to be more and more effective, there is an on growing interest in Integrated Multi-Trophic Aquaculture (IMTA) which is considered a means for increasing aquaculture sustainability. IMTA is defined as the cultivation of two or more aquatic species from different trophic levels in the same area, imitating the energy flow of natural ecosystems (*Chopin et al., 2004*). The aim of the present study was to investigate the potential to cultivate bivalve species (*Mytilus galloprovincialis, Pinctada imbricata radiata* and *Pinna nobilis*) using IMTA methodology in existing fish farms of Greece and in the Allocated Zones for Aquaculture (AZAs) both existing and under development.

Material and Methods

The present study is based on bibliographic data, satellite data, as well as on data collected in the context of the "PINNA SOS" and IDMA projects. Fish farming sites and AZAs were recorded on a GIS map based on the study of Papageorgiou et al., (2021) and satellite data of chlorophyll-α (Chl-α), sea surface temperature (SST) and salinity (SAL) data from COPERNICUS were downloaded for the same sites. For those environmental variables, the ecological niche of each species was determined using data from the scientific literature and *in situ* data collected in the context of the above-mentioned research projects. Finding the appropriate value range of the studied variables (Chl-*α*, SST, SAL and juvenile presence), under which the conditions are more suitable for the growth of *M. galloprovincialis, P. radiata* and *P. nobilis,* a bibliographic review was performed focusing on their growth potential for each one of these variables. Therefore, this study identified a range of values where growth is maximized (optimal conditions) and two sets of values where the organism survives with little growth above and below the optimal conditions (experimental study). In the remaining values there is no potential for growth or reproduction. The juvenile presence records were based on spatial distribution data of *P. radiata* (Katsanevakis et al., 2008) and on existing mussel-farms from which juveniles (*M. galloprovincialis and P. nobilis*) are released in adjacent sites. Distribution data of natural populations were also used as a potential source of juveniles. Based on the values of each variable, a score was given (0 = unsuitable, 1=medium suitability, 2=high suitability) to determine whether each region was suitable for the growth of each species. The individual scores were then summed to determine the final score in terms of the probability of successful development of multi-trophic aquaculture in the existing units. Technically the minimum score equals 0 and the maximum score equals 10 points. The equation applied was:

$$SST + SAL + 2x\ (Chl\text{-}\alpha) + 2\ x\ (Juvenile\ Presence)$$

Due to the fact that chl-a and juvenile presence are significant parameters for the bivalve growth, they were multiplied by a factor of 2.

Results

From the above results, probability maps of the three studied species were produced, within the AZAs of Greece on an annual time scale. In the case of *P. radiata* and *M. galloprovincialis*, the derived maps reflect the current situation in Greek waters, as *in situ* observations (IDMA project) in most of the studied areas verify the presence probability of the species assessed. *P. radiata*, as it seems, has no specific ecological requirements conflicting with the existing habitat characteristics and as a result it spreads easily along the entire coastline of Greece. On the other hand, *M. galloprovincialis* is more sensitive to several environmental changes and thus, as the results of the survey indicated, the probability of its presence is increased in the northern, central and western part of Greece, while in the southeastern part the probability of its presence

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is almost absent, in other words, in oligotrophic waters. Although the environmental requirements of *P. nobilis* are not yet clear, it is considered that since it is an endemic fan mussel (i.e., a relative of the mediterranean mussel) (*Zotou et al., 2020*), it is also sensitive to environmental variables, as is the case with *M. galloprovincialis*. Thus, the extracted maps were expected to be similar for these two species.

Conclusions

P. radiata is one of a few invasive species that has a high commercial potential in the Mediterranean Sea. Due to its low feeding and ecological requirements, according to the produced maps it can be cultured in oligotrophic fish farms. The main inhibitor seems to be the distribution of natural population as no juveniles are commercially available. On the contrary, juveniles of *M. galloprovincialis* can easy be bought, however suitable IMTA locations require eutrophic waters, more commonly found in northern Greece. *P. nobilis* is a more complicated case since in the recent years, due to a mass mortality of the species, no natural population is available, as an infection by *Mycobacterium* and *Haplosporidium pinnae* has occurred causing the extinction of the species in a huge proportion throughout the Mediterranean region (PINNA SOS Project). The results obtained represent a theoretical scenario for the potential of growth, in order to find the most suitable IMTA locations, which *P. nobilis* could use as nursing ground for raising its juveniles. Although *P. nobilis* prefers *Posidonia oceanica* meadows for settlement, the environmental condition of an IMTA can cover their needs.

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CHROMATIN ACCESSIBILITY AND DIFFERENTIAL GENE EXPRESSION OF THE IMMUNE RESPONSE IN GILTHEAD SEABREAM *(Sparus aurata)*

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Introduction

The gilthead seabream is a widely consumed species in the Mediterranean and due to its extensive aquaculture, the pressure on the improvement of production has increased. One fundamental limiting factor of aquaculture production is the incidence of bacterial and viral infections. Deeper understanding of the underlying mechanisms of the immune response will help to prevent and treat these health problems. To this point, there have not been many studies on genome-wide profiling of the gene expression changes in response to specific pathogens on fish. As part of the AquaFAANG project (www.aqua-faang.eu), this work focuses on the development of genome-wide functional annotation maps ('ImmunoMaps') that represent host defense responses of the immune system in the presence of two distinct classes of disease agents, viruses and bacteria, in gilthead seabream (*Sparus aurata*) by using ATAC and RNA sequencing.

Materials and Methods

The experimental work involved first the standardization of experimental protocols for host immunity activation in the head kidney, the major lymphoid organ that plays a critical role in the generation of pro-inflammatory and anti-microbial responses. Two different pathogen associated molecular patterns (PAMP) were applied in order to study species robust innate responses, *in vivo* by injecting fish and *in vitro* in primary head kidney leukocyte cultures. A total of 12 gilthead seabream individuals were stimulated *in vivo* and 6 individuals *in vitro* with mimics of bacterial (neutralized *Vibrio*) and polyinosinic:polycytidylic acid (poly I:C) viral infections versus 12 control individuals. Sequencing was performed using Illumina technologies and library preparations were performed for transposase-accessible chromatin (ATAC-seq) and transcriptome (RNA-seq).

For both ATAC- and RNA-sequencing data, the bioinformatic analyses were performed using pipelines from the nf-core initiative (Ewels *et al*., 2020). The output of the nf-core pipelines was used to perform differential accessibility analysis as well as differential expression analysis with the use of DESeq2 (Love *et al*., 2014) in R. Then, the statistically significant elements (adjusted p-value < 0.1) were isolated and the differentially accessible peaks were separated into over-accessible peaks (Log2FoldChange>2) and under-accessible peaks (Log2FoldChange<2); similarly, the differentially expressed genes were separated accordingly into up-regulated (Log2FoldChange>2) and down-regulated (Log2FoldChange<2) genes. Differential accessibility and Differential expression profiles were created in the form of volcano plots and for the RNA-seq data analysis, gene set enrichment analysis was performed with the use of g:Profiler (Reimand *et al*., 2007) and a circus plot (Krzywinski *et al*., 2009) was created to represent the genomic distribution of differentially expressed genes (Figure 1). To provide additional insight on the way chromatin accessibility interacts with gene expression, both ATAC-seq and RNA-seq data were integrated: the differentially accessible peaks as well as the differentially expressed genes were mapped onto the genome and the 10 closest genes within 1 Mb from the peaks were selected and Log2FoldChange information was added to explore regulatory links between over- or under-accessible peaks with up- or down- regulated genes.

Results

These analyses revealed more generic immune-related and pathogen-specific responses. Firstly, RNA-sequencing showed 1,205 up-regulated genes for all *Vibrio* treatments (*in vitro* and *in vivo* together) and 1,235 up-regulated genes for all PIC treatments. Similarly, 2,427 genes were down-regulated in all *Vibrio* treatments and 720 genes for PIC treatments. The bioinformatic data analysis also revealed virus-specific (nfkbiaa, nod2, atf3, sting1, traf3, rsad2, tlr7, tnfrsf1a, irf7, traf6, tlr3) and bacteria-specific (pglyrp5, pglyrp6) up-regulated genes *in vitro* and *in vivo*. On top of the expression profiles, the association of differentially accessible peaks with differentially expressed genes allowed the assembly of a core immune related gene network, also unveiling a potential regulatory link between the up-regulated genes next to under-accessible peaks *in vivo* for all treatments (Vibrio-PIC, merged together) where an over-representation for interferon-gamma response terms was present.

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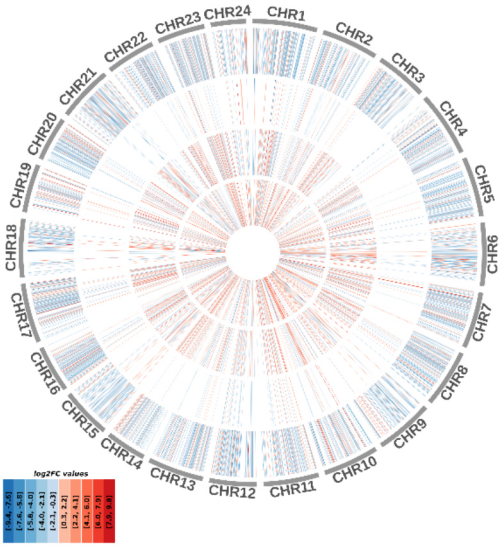

Figure 1: Genomic distribution of differentially expressed genes in *S. aurata* across different treatments visualized as a circos heatmap of log2FC values. Each circular plot represents a different set of experimental conditions and treatment, starting from the outer circle to the inner circle: *Vibrio in vitro*, *Vibrio in vivo*, PIC *in vitro*, and PIC *in vivo*. In each circular heatmap, gene loci across the *S. aurata* genome are represented as colored bars, with different colors representing log2FoldChange values color coded as shown in the respective legend.

Discussion

We present an overall image of the immune response map of gilthead seabream on bacterial and viral stimuli on epigenetic and gene levels. Our study revealed candidate genes for the study of immune response and specifically, an over-expression of virus-related genes (nfkbiaa, nod2, atf3, sting1, traf3, rsad2, tlr7, tnfrsf1a, irf7, traf6, tlr3) and bacteria-related (pglyrp5, pglyrp6) immune response genes that lays the background for further analysis. Furthermore, integration of ATAC-seq and RNA-seq data showed silencer activity on IFN response.

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TOTAL DIETARY REPLACEMENT OF WILD-CAUGHT FM, FO AND SOY USING BY-PRODUCTS FROM FISHERY AND AQUACULTURE AND MICROALGAE: IMPLICATION ON GROWTH, DIGESTIVE ENZYME ACTIVITIES, PLASMA BIOCHEMISTRY AND GUT HEALTH OF EUROPEAN SEABASS JUVENILES

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Introduction

The goal of sustainable aquaculture is to provide a continued supply of farmed aquatic food for human and animal consumption without compromising ecosystems. In the last twenty years soy was a valid alternative protein sources with positive results on growth and health of several aquatic species, including European sea bass. Depside that, plant ingredients may pose contrasts in sustainability and for this reason, in last years, was necessary to find other ingredients more sustainable, well represented by fishery and aquaculture by-product and microalgae as alternative protein sources. Microalgae are widely use in fish nutrition, especially for the high quantities EPA and DHA but are also extremely rich in protein content. Moreover, they can grow on different substrates, such as wastewater, making them the valid sustainable candidates as soy replacement. This study highlighted the possibility to total replace wild FM and FO and soy using by-product from fisheries and aquaculture and with microalgae as alternative protein source without affecting growth, digestibility, enzymatic activities and nutritional performance.

Materials and Method

Five experimental diets (C, 50FMFO, 50FMFO-50MIC, 0FMFO-50MIC, 0FMFO-100MIC) were formulated to totally replace wild fishmeal (FM), wild fish oil (FO) and soy protein using fisheries and aquaculture by-product and microalgae. Fifty fish per tank (initial body weight 46.66 ± 0.04 g), were reared in recirculated aquaculture system for 88 days. At the end of the trial, growth, feed intake (FI), proximate composition, nutritional index, apparent digestibility, somatometric indexes, blood plasma biochemistry were detected. The activity of major digestive enzymes as total alkaline protease (TAP), trypsin, chymotrypsin (CT), alkaline phosphatase (AP) and leucine aminopeptidase (LAP), was detected spectrophotometrically. The assessment of gut microbiota (GM) was made by Next-generation sequencing.

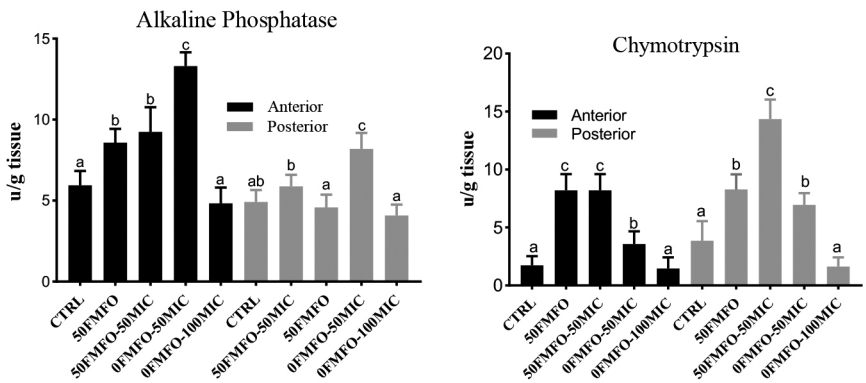

Figure 1. AP and CT activities in proximal and distal region of sea bass juvenile intestine

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Results

No statistical differences were evaluated for FI and growth parameters. TAP, trypsin and LAP activity did not show any statistical difference among treatments. Results of AP and CT are shown in Figure 1. The activity of AP in proximal intestine increased, reaching highest value in animals fed with 0FMFO-50MIC, then decreased in 0FMFO-100MIC diet. Even in distal segment, the activity of AP presented the highest value in diet with partial replacement of microalgae, 0FMFO-50MIC. The activity of Chymotrypsin was similar in proximal and distal part of juvenile's intestine. The values increased up to the highest value in 50FMFO-50MIC diet, then gradually decreased. Proteins body content presented lower value in diet 50FMFO-50MIC than in diet 50FMFO and 0FMFO-100MIC. Lipids body content were significantly higher in diet 50FMFO, 50FMFO-50MIC and 0FMFO-50MIC compared to control diet. No statistical differences were detected for apparent digestibility and all nutritional indexes considered. Considering plasma parameters, Creatinine, CHOL, HDL, TP and Fe values were statistically higher in diet C than in other treatments, while lower values were presented in diet with the higher percentage of replacement, 0FMFO-100MIC. On the other side ALP, Na and Cl values were lower in C diets and higher in 0FMFO-100MIC diet.

Discussion

This study highlighted the possibility to total replace wild FM and FO using by-product from fisheries and aquaculture without affecting growth, digestibility and nutritional performance of European sea bass juveniles. The enzymatic activities show the possibility to replace 100% of wild-caught FM and FO with fishery and aquaculture by-product and replace 50% of soy protein with microalgae, with positive effect on intestinal activity. These results make it possible to use by-products and microalgae as an alternative protein source, avoiding the use of soy and making aquaculture more sustainable.

Acknowledgement

This research was undertaken under the NewTechAqua (New technologies Tools and Strategies for a Sustainable, Resilient and Innovative European Aquaculture) which has received funding from the European Union's Horizon 2020 Programme under grant agreement No 862658, in collaboration within another Horizon 2020 European project, SABANA (Sustainable Algae Biorefinery for Agriculture and Aquaculture).

IN VITRO EFFECTS OF SEVERAL ANTIMICROBIAL PEPTIDES FROM GILTHEAD SEABREAM *(Sparus aurata)* ON HEAD KIDNEY LEUKOCYTES

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Introduction

Participating in the innate immune response host defence peptides, commonly known as antimicrobial peptides (AMPs), exhibit antimicrobial activity against a range of pathogens including Gram-negative and Gram-positive bacteria, viruses, and fungi. Beyond their direct antimicrobial effects, AMPs possess the capacity to enhance immunity and bridge the gap between adaptive and innate responses by serving as immunomodulators that act on host cells (Erdem Büyükkiraz et al., 2022). The majority of *in vitro* studies on AMPs have focused on investigating their direct effects on pathogenic bacteria. However, in this study, we aimed to examine the direct impact of AMPs on the viability of leukocytes and their ability to modulate the key immune activities performed by these cells. To do this gilthead seabream leukocytes were incubated with different AMPs (piscidin 1, piscidin 2, hepcidin H1, hepcidin H2C, hepcidin H2E, hepcidin H2H and hepcidin H2I) previously identified and characterized by our research group (Serna-Duque et al., 2022a, b).

Methodology

To assess viability and immune parameters, leukocytes were isolated from the cephalic kidney of seabream, adjusted to 10^7 cells/mL and incubated for 0 and 2 h at 25°C in Leibovitz L-15 culture medium containing one of the synthetic AMPs mentioned above, at a final concentration of 0 µM (control), 12.5 µM or 25 µM. Leukocyte viability was assessed using propidium iodide and flow cytometry (Cuesta et al., 1999) with flow cytometry (FACScan). Respiratory burst was studied using a chemiluminescence method (Bayne & Levy, 1991) and phagocytic activity was assessed using labelled bacteria as a phagocytic particle and flow cytometry (Rodriguez et al., 2003).

Results

The results indicated that the viability of most cells was not significantly affected by incubation with AMPs. However, the viability of cells incubated with piscidin 1 decreased when incubated with the highest concentration of piscidin 1. The respiratory burst of leukocytes was also significantly decreased when cells were incubated with hepcidin H1 and H2H for 2 h, compared to control cells. The phagocytic ability (percentage of cells with phagocytic activity) increased in leukocytes after incubation for 2 h with hepcidin H2H, compared to the values obtained in control cells.

Conclusion

The results suggest that AMPs generally have on the selected activities a minimal effect on gilthead seabream leukocytes. The immunomodulatory effect of seabream AMPs does not appear to be direct on leukocytes, immune effector cells, obtained from the same fish species, but rather appears to have an indirect effect on them as evidenced by *in vivo* assays.

Acknowledgments

Claudia Marín-Parra (PRE2021-098414) and Jhon A.Serna Duque (FPU19/02192) have PhD grants. This study was funded by the *proyecto de investigación* PID2020-113637RB-C21 *financiado por* MCIN/ AEI /10.13039/501100011033 and forms part of the ThinkInAzul programme supported by MCIN with funding from European Union Next Generation EU (PRTR-C17.I1) and by the *Comunidad Autónoma de la Región de Murcia-Fundación Séneca* (Spain).

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CRYOPRESERVATION OF SPERMATOGONIAL STEM CELLS IN FISH – A CRITICAL OVERVIEW

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Spermatogonial stem cells (SSCs) are the baseline cells of spermatogenesis. They have gained a lot of attention during the last few decades due to their great potential in biotechnology. Namely, as stem cells, SSCs can either self-renew or commit to differentiation through which they produce male gametes – spermatozoa. Okutsu et al. (2007) were the first to observe that when transplanted into recipients of a closely-related species, these cells incorporate into recipient's genital ridge, proliferate, and differentiate into donor-derived gametes ultimately giving donor-derived progeny. This commenced the research on a novel reproductive biotechnology known as the surrogate production technology.

The surrogate production technology entails the following steps: isolation of SSCs from the donor individuals, sterilization of recipient larvae, transplantation of SSCs into recipient larvae and rearing of recipients. The success of the method depends on the availability of donors and recipients of the appropriate life stage and the availability of good quality germ cells from the donor. To help synchronize the procedure, short- or long-term storage are necessary. The most used methodology for SSC storage is cryopreservation which enables their storage for theoretically indefinite time. SSC cryopreservation protocols have been developed for many fish species, and the aim of this study is to summarize the main conclusions of these studies.

SSCs can be frozen either within the whole testicular tissue, or as isolated cells within the cell suspension. Usually, they are cryopreserved within the testicular tissue, and Pšenička et al. (2016) and Marinović et al. (2017) displayed that freezing within tissues yielded higher viability. The tissue can then be either frozen through slow-rate freezing or vitrified. The choice of the approach should mostly depend on the tissue structure, i.e., whether the testis is mature and contains a lot of spermatozoa, or if it is immature and contains only early-stage germ cells. Most commonly, when the tissue is mature and contains a lot of spermatozoa, freezing is a much better option as vitrification gives very poor results. This is consistent with the poor performance of vitrification for cryopreserving spermatozoa in fish. Next, the cryomedium should be optimized. Both freezing and vitrification outcomes mostly depend on the type and concentration of the permeable cryoprotectant, while non-permeable cryoprotectants (sugars, BSA, FBS) affect the outcome to a much lesser extent. In most of the studies, dimethyl sulfoxide and ethylene glycol yield the most favorable SSC viability after freezing. During vitrification, much higher cryoprotectant concentrations are needed, therefore using equal amounts of two different cryoprotectants yields better result than a very high concentration of a single cryoprotectant. The cooling rate during freezing is mostly kept around -1 □/min, and several studies that have tested various rates have confirmed the superior performance of this rate.

Cryopreservation of SSCs is a very valuable method, however, without further application, the benefit of cryopreservation is only limited to creating gene banks and frozen zoos. Therefore, development of the surrogate production technology of cell culture technology is essential for the utilization of the cryopreserved SSCs.

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AQUAFEEDS SUPPLEMENTED WITH THE MACROALGAE A*sparagopsis taxiformis* IMPROVE THE IMMUNE RESPONSE OF JUVENILE *Diplodus sargus* IN A CONTEXT OF CLIMATE CHANGE

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Introduction

Aquaculture plays a key role in human nutrition and socio-economic development of many countries and its contribution is expected to increase in the future to fight hunger and undernourishment of a growing world population[1]. However, its sustainability and productivity can be compromised by climate change effects and occurrence of extreme weather events (e.g., marine heatwaves, MHW), that will be more frequent, intense, and long lasting[2,3]. Such changes will have implications for farmed fish , negatively affecting their physiology, namely, the immune responses and antioxidant mechanisms which, in turn, may compromise species survival and/or ability to cope with the presence of concomitant stressors, e.g. disease outbreaks[4].

Developing non-chemically based climate change adaptation strategies has recently become an urgent matter, as taking such actions will enable the aquaculture sector to overcome the challenges posed by climate change. The use of functional feeds enriched with natural immunostimulating ingredients (e.g., plants/seaweeds) has recently deserved some attention in the animal production sector, as it constitutes an eco-friendly alternative with lower costs and a wider range of efficacy than vaccines and antibiotics[5,6].

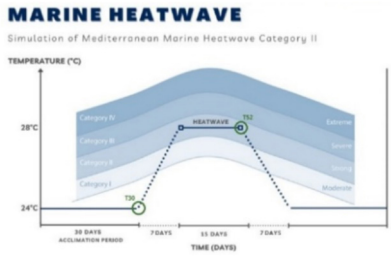

Figure 1 – Heatwave simulation.

In this sense, the present study aimed to explore the use of an invasive warm-water macroalgae species in the Mediterranean region, *Asparagopsis taxiformis*, as an eco-innovative adaptation strategy to improve the immune and antioxidant responses of farmed *Diplodus sargus* juveniles.

The outputs of this study will enable stakeholders to become aware of the potential impacts of climate change and to successfully apply adaptation/mitigation measures in the aquaculture sector.

Material and methods

<u>*Experimental diets:*</u> A practical, high-quality diet was used as the control (CTR), and three experimental diets based on the CTR diet were supplemented with *Asparagopsis taxiformis* (whole, dried, and powdered) at different percentages of inclusion: 1.5%, 3%, and 6%.

<u>*Fish Rearing Conditions and Feeding Trial:*</u> *Diplodus sargus* (n=165) were kept in seawater recirculation systems under optimal growth conditions (24°C, > 7 mg/L O2), for 30 days (T30), feeding on different experimental diets (CTR, AT-1.5%, AT-3% or AT-6%). After this period, a heatwave of category II in the Mediterranean area was simulated.

The temperature was slowly increased over 7 days (0.5°C/day) until it reached 28°C (i.e., $\Delta T = +4°C$) and remained at that level for 15 days (T52; Figure 1).

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Analytical approach: Blood and plasma from 9 fish/treatment was sampled at T30 and T52 (i.e., 3 fish collected from each of the 3 replicate tanks composed one treatment). A multi-biomarker approach combining haematological (blood cell counts, viability and haematocrit) and innate humoral parameters (IgM, antiprotease and peroxidase activity) was carried out to determine the immunostimulating potential of the different functional diets.

Results

Exposure to the MHW led to a decrease in the viability of erythrocytes and increase in cellular nuclear abnormalities in all treatments, though such effects were less pronounced in fish fed with supplemented aquafeeds, pointing out to a potentially positive effect due to the inclusion of *A. taxiformis*. No significant changes were observed in the peripheral leukocyte population in blood smears.

Fish humoral immune parameters were affected by both macroalgae supplementation and exposure to the MHW, alone or in interactive ways, depending on the analysed biomarker. Plasma antiprotease activity had no significant differences between treatments, whereas peroxidase activity increased in CTR, AT-1.5% and AT-3% treatments following exposure to the heatwave, indicating an enhanced immune response. Additionally, immunoglobin M (IgM) levels also increased in CTR, AT-1.5% and AT-6% treatments, thereby contributing to the prevention of autoimmune diseases, with the 1.5% supplemented diet exhibiting the most favourable outcome.

Conclusions

The inclusion of macroalgae *Asparagopsis taxiformis* in diets demonstrated a potential for enhancing the immune response of *Diplodus sargus* species when exposed to a MHW, with the 1.5% supplemented diet exhibiting the most cost-effective formulation.

These findings show that red macroalgae may be regarded as promising functional ingredients to be used in aquafeeds for carnivorous marine fish, contributing to a better welfare and resilience to environmental stressors, including those related with climate change.

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AN ATLAS OF REGULATORY ELEMENTS ACROSS DEVELOPMENTAL STAGES AND ORGANS IN THE TURBOT *Scophthalmus maximus*: IMPACT OF STRUCTURAL AND SNP VARIANTS ON GENE EXPRESSION

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A comprehensive characterization of regulatory elements of the genome across tissues and developmental stages represents an essential resource to understand the genetic basis of productive traits and to identify genetic variants potentially associated with valuable phenotypes to improve genomic predictions in breeding programs. Here, we identified and characterized regulatory elements in the turbot genome by integrating genome-wide ATAC-seq (open chromatin regions), ChIP-seq (H3K4me3-active promoter regions, H3K27ac-enhancer and promoter regions, H3K27me3-Polycomb repression, H3K4me1-enhancer and promoter regions) and RNA seq datasets from 54 pools of 6 embryonic stages (blastula, gastrula, early-segmentation, mid-segmentation, late-segmentation and prehatching) and 6 immature and 6 mature tissues (gonad, brain, liver, head kidney, gill and muscle) tissues. In total, we identified 10 distinct chromatin states among embryonic stages and tissues and annotated > 200,000 highly reproducible regions, including promoter, intergenic and intronic regions. Furthermore, 15x whole genome resequencing of 44 turbot adults from an experimental farm enabled to detect thousands of SNPs and structural variants on regulatory elements that are being checked for their impact on gene expression of the regulated genes.

UNDER THE ASTRAL EU PROJECT: MICROBIOME STUDY OF DIFFERENT FERTILIZATION TREATMENTS IN THE INTEGRATED PRODUCTION OF SHRIMP IN BIOFLOC SYSTEMS

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Introduction

Brazilian inshore Integrated Multi Trophic Aquaculture (IMTA) system functions in a closed system, relying on biofloc, processing waste and stabilizing water quality. Biofloc particles are composed of bacteria, flagellates, ciliates, and other organisms that use uneaten feed and faeces secretions to grow. Moreover, particles can be consumed directly by culture species such as shrimp, oysters, and tilapia, thereby making more efficient use of the nutrients contained within the feed and subsequently lowering total costs. The resulting, integrated, multi-trophic system is potentially more biologically efficient, more environmentally sustainable, and more economically competitive. However, research needs to be developed to optimise these systems and understand their microbiome role and dynamics of potential pathogenic bacteria. We aim to study the IMTA system in Brazil, by analysing two different fertilization methods - organic and inorganic -, as well as its microbiota impact on biofloc and shrimp gut (*Litopenaeus vannamei*) through different timepoints.

Material and methods

This trial was carried out in 6 independently IMTA systems located at the Marine Aquaculture Centre of Federal University of Rio Grande – FURG. Each production system consisted of three compartments/tanks where the water is continuedly re-circulating and different organisms are cultivated (Compartment 1: shrimp; Compartment 2: Oyster and tilapia; Compartment 3: seaweed). Thus, two fertilizations treatments were implemented in triplicates: Chemoautotrophic - with inorganic fertilization - and heterotrophic- with organic fertilization. Continuous recirculation of water between tanks occurred, with daily monitoring of environmental parameters. For this study, we mainly focused on the Compartment 1, where an amount of 400 shrimps were cultivated in tanks of 16m3 and the biofloc is generated. Three timepoints were deployed – T0 (initial), T1 (45 days) and T2 (86 days). Triplicates of shrimp gut and biofloc were collected per system, at the different timepoints and characterised for microbial populations. Bacterial diversity was assessed through 16S rRNA sequencing. Raw data was processed with QIIME2, and taxonomical analyses were performed with R software.

Results

Fertilization type significantly influenced the biofloc bacterial communities. A significant differentiation can also be seen in the microbiome systems at the different timepoints when comparing biofloc and shrimps' gut, which highly differentiate from T0 to T2 after both organic and inorganic fertilizations. Results showed that biofloc is influencing the shrimp gut microbiota, under both organic and inorganic fertilization. Comparison between biofloc and shrimp showed over 60% of shared ASVs. At genus level, in the inorganic fertilization, biofloc showed to be dominated by *Mycobacterium*, while in the organic fertilization, shrimp gut had the most abundant differential genus dominated by *Woesia*. Regarding pathogens, abundance of *Vibrio* and *Coxiella*, widespread disease-causing agents in aquaculture, decreased after biofloc implementation under both fertilizations.

Conclusions

Bacterial diversity results in biofloc IMTA systems showed a significant evolution during the aquaculture production. Presence of potential pathogens abundance decreased with time and better results were seen with organic fertilization. Furthermore, potential use of microbiota analyses could be used for pathogen detection and treatment.

EVALUATION OF WINE BY-PRODUCTS AS A POTENTIAL FUNCTIONAL INGREDIENT IN FEEDS FOR JUVENILE *Liza aurata*

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Introduction

Nowadays, a large part of aquaculture is based on the use of intensive systems characterized by increasing environmental stressors, reducing water quality and promoting the appearance of pathologies (Segner et al., 2012; Sneddon et al., 2016). Taking this into account, more attention is paid to the search for biologically active ingredients that can benefit the health and resistance of fish against the aforementioned adverse effects. In this context, it is worth noting the potential interest of looking for natural sources of polyphenols. These are abundant in wine by-products such as grape pomace, which has traditionally been used to feed terrestrial animals, but whose possible application in aquaculture is yet to be developed (Peña et al., 2020; Pulgar et al. 2021). Moreover, in the current situation it is unquestionable to consider the principles of the circular economy in any productive process related to agri-food, and therefore the integration of by-products or co-products generated by the industry in some phase/s of the production of the same or other foods. Considering all the above, the present study was oriented to evaluate the potential benefits of including grape pomace or red wine lees as functional ingredients in aquafeeds for juvenile golden grey mullet (*Liza aurata*) during a short-term feeding trial and a further hypoxic challenge.

Materials and methods

The wine by-products used in the experiment (red grape pomace and lees) were obtained from two artisanal wineries located in Almería and Cádiz (Spain), respectively. The experiment was carried out at the 'Servicios Centrales de Investigación en Cultivos Marinos' (SCI-CM) of the University of Cádiz. For the study, three experimental diets for golden grey mullet (*Liza aurata*) were formulated: i) control diet (C); ii) diet including 10% of grape pomace (GP); and iii) diet including 10% of lees from red wine (WL). Juveniles of *Liza aurata* of approximately 54 g were used in experimental triplicates (9 tanks in total), subjected to a feeding period of 47 days, with an average daily intake of 2% body weight. The study was carried out in two different experiments; the first one was focused on testing the general effects derived from the intake of bioactive compounds present in wine by-products on basal metabolism, immune status and intestinal microbiota. The second evaluated the potential protective effect against induced stress (medium hypoxia maintained for 3.5 h) evaluated by variations in the oxidative status. At the end of each sub-experiment, fish were anesthetized with a lethal dose of 2-phenoxyethanol prior to obtain the required samples of mucus, plasma, intestinal microbiota and liver. Samples were used to measure different metabolic parameters (glucose, lactate, triacylglyceride, cholesterol, glycogen and protein levels), welfare (cortisol), oxidative status (quantification of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase) and functionality of the intestinal microbiota.

Results and discussion

The results showed a positive effect of the inclusion of grape pomace in the feed for golden grey mullet on feed efficiency, as well as on different indicators of the metabolic and immunological status of the fish. In contrast, a negative effect of the inclusion of red wine lees in feed on the abundance and functional diversity of the intestinal microbiota of individuals was evidenced (Figure 1). In addition, after inducing stress by a short-term hypoxia, fish fed on feeds containing any of the two wine by-products presented significantly lower levels of cortisol (Figure 2), this pointing out a protective effect mediated by their content in phenolic compounds. Therefore, it seems clear that wine by-products, especially grape pomace, have great potential as functional additives and that future research should assess how to promote their practical application in aquafeeds.

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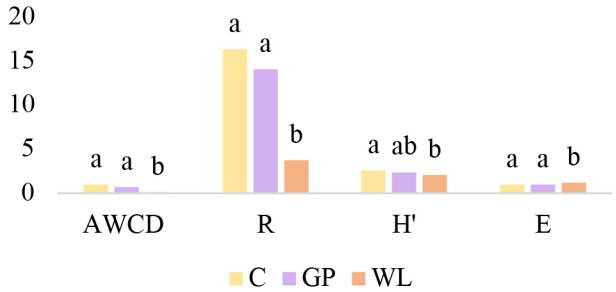

Figure 1. Variations in functional biodiversity of gut microbiota in fish fed on the different experimental diets. Values not sharing a common letter differ significantly with $p < 0.05$. AWCD: Average color development is an index of the total bioactivity; R: number of substrates oxidized (substrate richness); H′: Shannon index (H) is the functional biodiversity index; E: Shannon evenness.

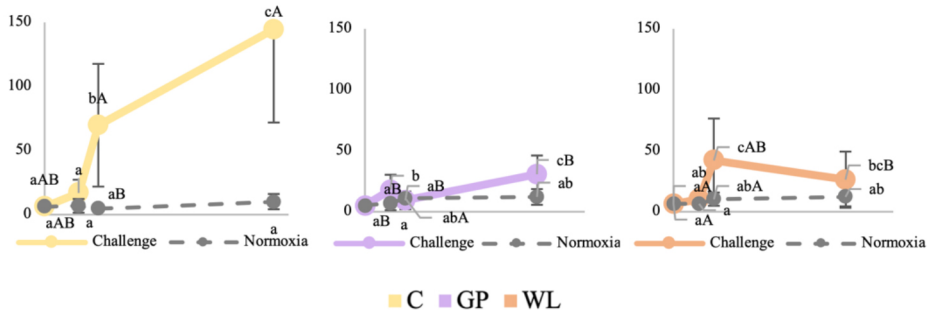

Figure 2. Effect of mid-hypoxia and recovery time on plasma cortisol levels measured in juveniles of *Liza aurata* fed on the experimental diets. Statistical comparisons same as above.

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GROWTH PERFORMANCE AND EFFECT OF FISH MEAL SUBSTITUTION IN *Mugil cephalus* UNDER RECIRCULATION AQUACULTURE SYSTEM

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Introduction

The increase in fish consumption nowadays has led to a growing need to explore new species for aquaculture that are more environmentally and economically sustainable. This has encouraged the search for low trophic level species with less dependence on fishmeal and fish oil for their diets, being *Mugil cephalus* one promising candidate.

Nevertheless, even with low trophic species, it is necessary a higher fishmeal substitution, but high substitution by alternative protein sources may have an impact in the intestinal status of rearing animals. Gut microbiota is fundamental in the regulation of digestion, immune response and resistance to disease or pathogen colonies in a fish, then alterations in its functionality can affect the fish health status (Estruch et al., 2015).

Thus, in this study the growth and gut microbiota of *Mugil cephalus* fed with two experimental diets containing 15% and 0% of fishmeal was to evaluate aiming to investigate the impact of different diets compositions on the fish performance and gut microbiota.

Material and Methods

The experiment was carried out from September to December 2022 at wet laboratory of Universitat Politécnica de Valencia. Sixty fishes (9.14 ± 1.52 g; initial weight) were randomly distributed in 6 tanks of 600 L (0.9Kg/m^3) under recirculation system. Two different diets, with 15% (HP15) and 0% (HP0) fishmeal inclusion, were tested in triplicate. The feeds were manufactured by extrusion cooking at the UPV feed factory. The fish were fed to satiety, three times a day. Monthly sampling was carried out to analyse fish growth and survival and instantaneous growth rate (SGR), daily feeding rate (FIR) and feed conversion ratio (FCR) were calculated.

Once was finished the trial, 5 fish from each treatment were slaughtered, beyond 5 initial fish, in order to assess the microbiome intestinal content by 16S ribosomal RNA analysis.

Results and discussion

Attending to growth performance, higher SGR and FCR were reported in HP15 diets (Table 1). Despite the omnivorous behaviour of this species, the results have showed that higher fishmeal inclusion provided better growth, then it seems that this species in juvenile state had less omnivorous behaviour.

A global view of *Mugil cephalus* intestinal microbiota shows 25 different phylos where it predominates of Proteobacteria (50.1% ± 9.93) and Firmicutes (34.17% ± 8.26) phylum, without any of two phylum predominant respect the diet or initial status (Figure 1).

In relation to the genus, 316 genus were identified where *Pseudomonas* (47.98% ± 10.33) was the most represented bacteria followed by bacteria of genus *Brevinema* (11.53% ± 4.73). Any significant differences respect the diet or initial status. A predominance of proteobacteria populations have been reported in fish fed with marine protein sources, meanwhile, firmicutes are typical from fish fed with plant protein sources. Therefore, the predominance of both phylum is according to its omnivorous behaviour. With regard to *Pseudomonas*, normally these bacteria can be found in omnivores fish, and they are associated with aid digestion (Egerton et al., 2018). In terms of species diversity between experimental groups, neither were found differences based on alpha Shannon index. A recent study performed by Bertini et al. (2023) in mugil, substituted the fishmeal content up to 10% and 20% by *Corynebacterium glutamicum* single cell protein, obtaining also as most abundant phylum Proteobacteria, but the predominant genus was *Streptococcus spp*. As in the current study, alpha Shannon index did not show differences. Thus, it seems that the fishmeal substitution does not affect importantly the intestine microbiote in *Mugil spp*.

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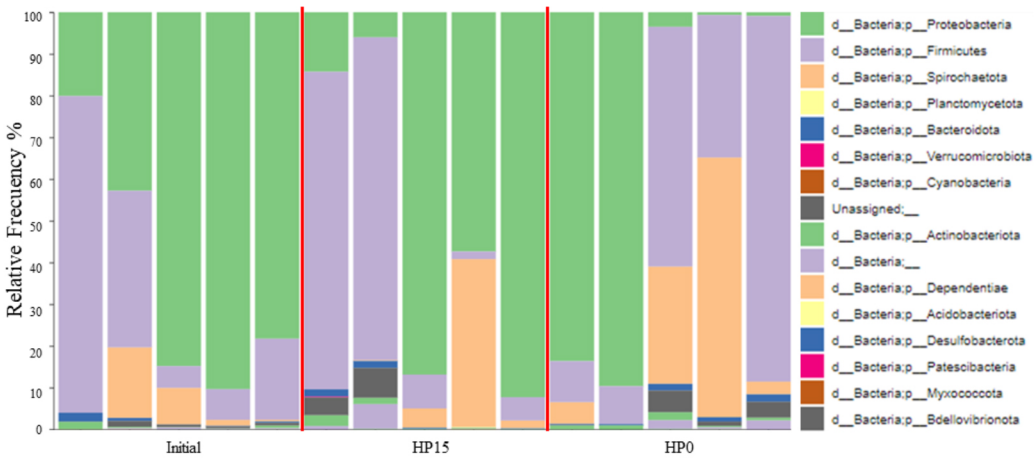
Table 1. Growth performance and index of *Mugil cephalus*. Values represent mean ± standard error.

Diet	Weight Gain (%) Mean ± SEM	Survival (%) Mean ± SEM	SGR Mean ± SEM	FIR Mean ± SEM	FCR Mean ± SEM
HP15	172.59 ± 33.55[a]	95.35 ± 3.55	0.99 ± 0.13[a]	1.31 ± 0.03	1.53 ± 0.19[a]
HP0	98.16 ± 4.76[b]	94.62 ± 3.29	0.68 ± 0.02[b]	1.34 ± 0.06	2.04 ± 0.13[b]

Different letters in the same column indicate significant differences at $p<0.05$. Newman-Keuls test.

Figure 1: Relative abundance (%) of gut microbiota composition at Phylum level in *Mugil cephalus* posterior intestine at initial status and after being fed with the experimental diet.



In conclusion, relative high fishmeal requirements seem to be necessary in *M. cephalus* at juvenile state, but no relevant alterations of intestinal microbiota were observed due to the total substitution of fishmeal.

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Acknowledgments

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STANDARDIZATION OF *IN SITU* HYBRIDIZATION TECHNIQUES AND ELECTRONIC MICROSCOPY FOR DISEASES DIAGNOSIS OF AQUATIC ORGANISMS

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Introductions

There are several etiological agents of acute, chronic, granulomatous, systemic or focal diseases in animals worldwide. In aquaculture, in fish, reptiles, amphibians and crustaceans, these microorganisms have caused large losses in production due to the death of infected animals or the bad appearance of sick animals, which makes commercialization unfeasible. Furthermore, some of these aquatic animal pathogens are zoonotic, with an impact on public health. Thus, from 2009 onwards, the standardization of diagnostic techniques began at the Interinstitutional Aquaculture Health Laboratory (Instituto Biológico/ São Paulo/Brazil), for the detection of pathogens of aquatic organisms as an additional diagnostic tool: *in situ* hybridization (HIS) in optical or photonic microscopy and immunoelectron microscopy (IEM) and immunocytochemistry as immunostaining with colloidal gold particles (IMCG) in transmission electron microscopy . Adaptations to the protocols initially developed for mammals were carried out, such as the removal of melanin from melanomacrophages. As there is a large amount of melanomacrophages (in the organs of ectodermal animals (frogs and fish), with brownish melanin granules, it was necessary to remove this melanin in order to facilitate the visualization of the DAB chromogen (diaminobenzidine) without interfering with the *in situ* hybridization technique in which specific nucleotide sequences were identified in the histological sections. This modification avoided false positive results. These techniques, after standardization, helped in the correct and efficient diagnosis of pathogens such as: white spot disease in shrimp, occurrence of

Material and methods

Material: organ fragments of in fish, reptiles, amphibians, and crustaceans

Methods: Electronic microscope

Negative Contrast Technique: The negative staining technique consists of a quick and easy preparation, presenting a result in a few minutes, being, therefore, the most productive approach in electron microscopy in terms of sample numbers. Particles from a suspension are adsorbed onto the surface of a specimen support, stabilized and counterstained usually by heavy metal droplets. By this approach, particles can be visualized down to subnanometer size and categorized based on their morphology. (Brenner, & Horne,1959; Curry et all, 2006)

Immunoelectron microscopy (IEM) is used when the number of viral particles in a sample is very low, when the virions are pleomorphic and difficult to identify due to the absence of typical viral morphology, such as a defined symmetry, presence of shape or spikes, particle size or number and arrangement of capsomeres, or when the samples are very electrondense and the aggregated complexes generated by the technique can facilitate identification [Lavazza et all. 2010]. It also allows the identification of the virus by the specific antigen-antibody reaction and such identification takes place through its morphology. It is also used to serotype morphologically similar (but antigenically distinct) particles.

Immunostaining with colloidal gold particles in the negative staining technique: In this technique, the antigen-antibody reaction is enhanced by labeling the antigen with protein A-associated colloidal gold particles using type- and genus-specific antibodies. The method also allows the detection and identification of virus-induced antigen structures and their localization in infected cells, serotyping viral strains [Kjeldsberg, 1986;] and determining antigenic variants in isolated strains. (Fig1B).

Technique of inclusion of fragments in resin: The technique of embedding in resin, followed by ultrathin sections is especially important to reveal fine details of the ultrastructure of all types of cells and tissues [Martins et all., 2013] and, in an infectious process, allows observing the pathogenesis of the infection and the agent identification [Fields,1996]. The ultrathin sections have the advantage of allowing the observation of the virus-cell interaction, revealing the site of viral replication and maturation in the host cells, pertinent information in the identification of unknown viruses [Fong ,19899]. The ultrastructural set of details not only determines the infection, but also the disease course in the herds [Catroxo& Martins, 2015].

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Optical microscope: In situ hybridization, probes are used to locate specific nucleic acid sequences at the subcellular level. Biotin is commonly used in probes for non-radioactive detection. Biotin can be visualized by numerous procedures, whether using avidin or streptavidin and both have high affinity for this amino acid. (FIG 1A)

Bleaching of Melanomacrophages from Tissues of Ectothermic Vertebrates for Later Use of Immunohistochemical and in Situ Hybridization Technique Due to the large amount of melanomacrophages in the organs of ectodermal animals, such as frogs and fish, with brownish melanin granules, it was decided to remove melanin in order to facilitate the observation of organ fragments under a direct light microscope when using biotinylated probes . These slides that we used for bleaching were immersed in 10% hydrogen peroxide (H2O2) in 0.2mol/L of Tris-HCl buffer pH 7.4 for 24 hours at room temperature. During this process the material was kept in the dark. (Fig 1C).

Results

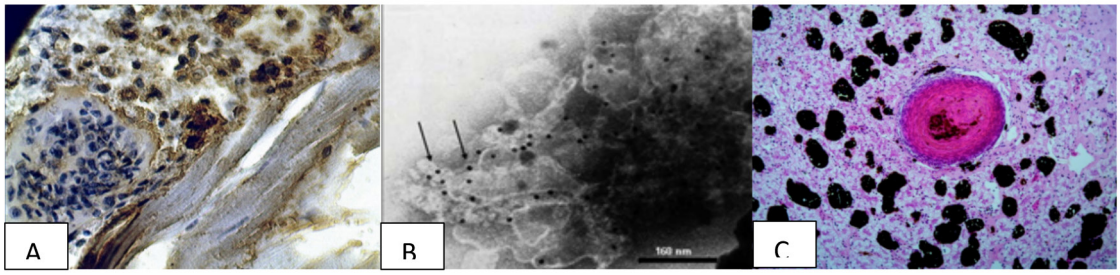

Fig. 1. (A)Dark brown positive result blue areas negative result (IHS) Heart and hemocytes x 630. (B) A strong antigen-antibody interaction was evidenced by the gold particles on the virion WSSV (arrows). (C) Granuloma with several melanomacrophages.

Conclusion

These techniques, after standardization, helped in the correct and efficient diagnosis of pathogens such as: white spot disease in shrimp, occurrence of *Mycobacterium* spp. and *Francisella* spp. in fish, and chytridiomyosis in frogs**.**

Thanks to FAPESP 2011 50009-9, FAPESP 2016/12930-0, FAPESP 09/14060-0

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QUEEN BEE LARVAE AND FISH HEALTH: AN *EX VIVO* APPROACH FOR TESTING THE INFLAMMATORY RESPONSE IN THE ANTERIOR INTESTINE OF EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

Nowadays there is a growing demand for new sustainable ingredients adhering to circular economy principles and improving animal performance and health. Within these, research has focused on exploring functional ingredients (FI) that act preventively against potential diseases or dysfunctions, thus fostering fish health status (Ruiz-Cano et al., 2022). Insects, such as honeybees (*Apis mellifera*), are gaining popularity as a possible FI. The queen bee larvae (QBL) are usually discarded during royal jelly production and are typically considered waste. However, recent studies have shown that QBLs are rich in protein, fatty acids, and essential amino acids, enhancing their nutritional value for use in food and feed (Addeo et al., 2021; Zhao et al., 2022). For evaluating new ingredients, animal nutrition research mainly relies on conventional *in vivo* feeding trials, which are time-consuming, expensive, and ethically challenging. The ethical concerns surrounding animal experimentation have prompted the adoption of the 3Rs principle to minimize the use of animals and reduce their suffering (Mukherjee et al., 2022). The *ex vivo* model is ethically advantageous as a first approach to evaluating novel ingredients, as it uses tissue slices obtained from an organism and thus substantially reduces the number of animals used in experimentation. The slices (explants) are kept under optimal conditions, mimicking the natural environment, maintaining intercellular connections and interactions, and ensuring that physiological processes more closely represent the *in vivo* situation (Wang et al., 2015). The purpose of this study was to apply an *ex vivo* approach to evaluate the viability and inflammatory response of anterior intestine explants of European sea bass juveniles after 4 hours of incubation with QBL immersed in royal jelly (raw ingredient, RAW), its extract (RAWex), and freeze-dried product (FD) and its extract (FDex) and after a lipopolysaccharide (LPS) challenge.

Materials & Methods

QBL extracts were prepared from the raw ingredient and freeze-dried using a sequential extraction with methanol (50/50) and acetone (70/30) solutions. Six European sea bass (*Dicentrarchus labrax*) juveniles with 106.4±15.7 g were euthanized with excess anesthesia (2-phenoxyethanol); thereafter, the anterior intestine was sampled and slices of 5 mm diameter were cut using a biopsy scalpel. Three slices were placed in each well of 24 well plates and incubated with 1 mL of pre-treatment medium (Penstrep 500 U, 5.5 mM glucose, 2 mM glutamine, and 10% FBS) for 1 hour. Then, the pre-treatment medium was removed, and 1 mL of treatment medium containing Penstrep 100 U, 5.5 mM glucose, 2 mM glutamine, and 10% FBS and, respectively, 0 (Control), 1, 2, 4, 8, and 10% of the RAW, RAWex, FD, and FDex were added to each well and incubated at 22 °C, 100 rpm, for 4 hours. Each treatment was tested in triplicate. At the end of the incubation time, one slice per well was collected and used for a viability test and another slice was stored in Trizol at -80 ºC until gene expression analysis. The remaining slice was challenged with 10 or 100 ng of LPS for 4 hours and then stored at -80 ºC until gene expression analysis. The slices' viability was assessed based on cellular metabolic activity using MTT (3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). The expression of proinflammatory genes, namely tumor necrosis factor α (TNF-α), cyclooxygenase 2 (COX2), and interleukin β (ILβ), was evaluated to assess the immune response.

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Results

After 4-hour incubation, the viability of the anterior intestine explants was not affected by the ingredients, independently of the type and concentration tested. However, compared to the Control, the inclusion of FD at 10% led to an increase in TNFα expression. After the challenge with 10 or 100 ng of LPS, no differences in the expression of immune genes tested were observed. Nevertheless, when exposed to a 10 LPS challenge, there was an augmentation in ILβ expression within 4% FD as opposed to the unchallenged 4% FD. Similarly, the application of 100 ng LPS led to a rise in ILβ expression within 10% FDEx compared to the unchallenged 10% FDEx.

Conclusions

This preliminary study aimed to assess the potential of the *ex vivo* approach as a preliminary screening of FIs for use in aquafeeds, using QBL as a potential functional ingredient. According to the present results, tissue viability was not affected by QBL, either as raw or as an extract, independently of the concentration tested. The explants responded to the treatments by increasing the pro-inflammatory cytokinin TNFα expression but only in the FD treatment, whilst differences were observed in 4% FD and 10% FDEx for the ILβ expression after the two LPS-challenging intensities. Overall, results suggest that QBL does not seem to enhance the immune response of European sea bass, but further studies are required to confirm these results and the potential of *ex vivo* assays as pre-screening tests of the tissue immune responses.

Acknowledgments

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MAINTENANCE REQUIREMENTS AND UTILIZATION EFFICIENCY OF METHIONINE AND CYSTEINE IN COMMON CARP

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Background

Common carp is the main carp species farmed globally with its annual production exceeding 4 million MT (FAO 2022). Protein is qualitatively the most expensive component in the feed and a more precise understanding of amino acid (AA) requirements will help formulating a cost-effective diet using alternative ingredients. Traditionally, dose-response study has been used to determine AA requirements, but this is quite time consuming and expensive. Recently, the factorial modelling approach was used to determine AA requirements in salmonids (Hua and Bureau 2019) - but for this approach, data on AA maintenance requirements and utilization efficiency are the prerequisites. Ration level technique has been proven to be useful in estimating maintenance requirement and utilization efficiency of AAs in fish (e.g., Helland et al. 2010; He et al. 2013). The objective of this study was to estimate maintenance requirement and utilization efficiency of AAs, specifically of methionine (Met) and Cysteine (Cys) in common carp, using ration level technique.

Materials and Methods

The trial was conducted in grow-out stage common carp with an initial body weight of 147.6 g at the Marine Aquaculture Centre of the Singapore Food Agency. A practical diet was formulated to meet the known nutritional requirements of common carp based on available data. The diet contained, on a dry matter basis, 31.5% crude protein, 18 MJ/kg gross energy, 1.90% Lys, 0.80% Met and 1.22% Met+Cys. The diets were fed to fish at 4 ration levels of 100%, 75%, 50% and 25% apparent satiation. A fifth dietary treatment was a non-protein diet fed to fish at 100% apparent satiation. The non-protein diets were formulated with protein-free ingredients but contained similar levels of energy, vitamins and minerals. The fish were hand-fed to two meals per day for 42 days. Quadruplicate groups of fish were randomly allocated to each of the experimental treatments. Water temperature was maintained at 24.9ºC. A simple linear regression model was fit between the AA gain versus intake and the requirement for maintenance was estimated as the amount of digestible AA to maintain zero AA gain, i.e. the intercept on the x axis of the linear regression and the marginal utilization efficiency of the AA above maintenance was estimated as the slope of the regression.

Table 1. Growth performance of common carp fed the experimental diet at increasing ration level

Diet fed at % apparent satiation	Body weight gain (g/fish)	Feed efficiency ratio	Specific growth rate (%/day)	Thermal growth coefficient
Protein-free, 100%	5.9[a]	0.08[a]	0.09[a]	0.007[a]
25%	11.2[a]	0.41[b]	0.17[a]	0.013[a]
50%	35.3[b]	0.65[c]	0.51[b]	0.037[b]
75%	58.1[c]	0.71[c]	0.78[c]	0.059[c]
100%	99.4[d]	0.71[c]	1.23[d]	0.094[d]
Pooled SEM	4.1	0.03	0.05	0.004

Values in the same column not sharing the same letter are significantly different by Tukey's test.

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Results

The increasing ration levels showed linear increase on growth rates of fish, with fish fed to apparent satiation showing the highest body weight gain, specific growth rate (SGR) and thermal growth coefficient (TGC) (Table 1). There were no significant differences in feed efficiency among the treatments of 50%, 75% and 100% ration levels. Protein free diet produced no differences in weight gain, SGR and TGC in comparison to the 25% ration level. For digestible protein retention efficiency, only protein free diet was significantly lower than other treatments. No significant differences in digestible Met retention efficiency were observed in all treatments. Maintenance requirement was estimated to be 9.20 mg/kgBW$^{0.8}$/d for Met and 16.1 mg/kgBW$^{0.8}$/d for Met+Cys with the marginal utilization efficiency being 50.5% and 45.7%, respectively for Met and Met+Cys. Data were further analyzed to determine maintenance requirements and utilization efficiency of other essential AAs. Data produced in this study can be used to derive AA requirements of common carp and formulate more precise diets for their different life stages.

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RESPONSE OF RAINBOW TROUT *Oncorhynchus mykiss* TO INCREASING LEVELS OF DIETARY GUANIDINOACETIC ACID SUPPLEMENTATION

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Introduction

Fish feed are produced primarily with plant ingredients with minimal amount of fish meal possible. While this helps reduce feed cost, it is equally important not to compromise on the nutritional quality of feed and thus, the fish performance. For example, animal ingredients contain certain amount of creatine or its precursor, whilst the plant based ingredients contain no creatine. Creatine and its phosphorylated form phosphocreatine (PCr) play a vital role in the cellular energy metabolism of animals. The adenosine triphospate (ATP)–creatine phosphate system transfers a high-energy phosphate from PCr to adenosine diphosphate (ADP) to regenerate ATP. Creatine is synthesized in body by its precursor guanidinoacetic acid (GAA) which is more stable under feed production process than the creatine itself. There are studies showing the positive benefits of dietary GAA supplementation in fish including tilapia (e.g., Aziza et al. 2020; Mabrouk et al. 2020) and carp (Yang et al. 2021). Borchel et al. (2014) showed the importance of creatine on the energy metabolism of rainbow trout. The objective of this study was to investigate the effects of increasing levels of GAA supplementation on the performance of juvenile rainbow trout.

Materials and Methods

Experimental basal diet (D1) was formulated with low levels of fish meal (5%) but still meeting the known recommended nutrient levels for rainbow trout. Diets 2-7 were supplemented with increasing levels of GAA at 0.04% increment levels (0.04-0.24%) whilst the Diet 8 was supplemented with the GAA source at 0.30%. All the 8 diets were allotted randomly to the 32 experimental tanks, containing 30 fish (~9 g, mean initial body weight) per tank and giving 4 replicate tanks per diet. Fish were fed 4 times a day over a 98-day study period, which included 90 feeding days and 8 no-feed days (fish were not fed during the weighing days).

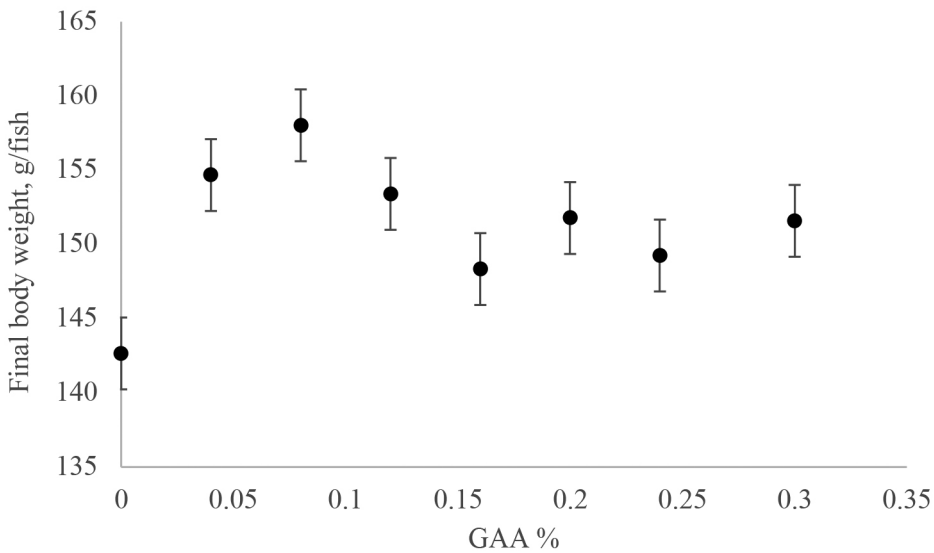

Fig 1. Effects of guanidinoacetic acid (GAA, %) supplementation on the body weight (mean ± SE) of rainbow trout at the end of 98 days of study period.

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Results

At the end of the study (day 98) mean body weight had increased to 143-158 g among the dietary groups and differed significantly ($p < 0.05$, ANOVA). Diets supplemented with 0.04 and 0.08 % GAA showed a significantly higher body weight gain compared with the basal group ($p < 0.05$, ANOVA). In terms of growth, fish fed diets supplemented 0.04-0.12% GAA showed significantly better thermal growth coefficient whilst only the 0.08% GAA supplemented diet produced significantly better specific growth rate compared with the basal. Feed conversion rate of fish fed different treatment diets ranged from 0.97 to 1.00 and showed no difference. On the initial and final day, no differences were detected on the whole-body proximate nutrient composition of rainbow trout, however, fish group fed with 0.08% and 0.12% GAA supplemented diet showed marginal increase in the body protein gain compared with the control group ($0.05 < p < 0.10$%, ANOVA) . Overall, our study results clearly indicate that supplementing low-fish meal rainbow trout feed at 0.08 % GAA significantly improves the fish growth performance.

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VALIDATING BIOLOGICAL EFFICACY OF METHIONINE SOURCES IN NILE TILAPIA

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Introduction

Soybean meal-based diets in fish are limited in methionine (Met) and need its supplementation in order to meet requirements of fish for optimal growth performance. DL-methionine (DL-Met) and methionine hydroxy analogue, DL-MHA (DL-2-hydroxy-4-methylthiobutyrate free acid, DL-HMTBa and its calcium salt, MHA-Ca) are the commonly available supplemental Met sources in animal feeds. In Nile tilapia, Teodosio et al. (2022), using labelled sources of DL-Met and MHA-Ca, demonstrated the better utilization of DL-Met versus MHA-Ca for body protein synthesis. While there debate exists in the literature, based on the published data, NRC (2011) concluded that it is reasonable to assume that the biological efficacy (BE) of MHA in fish is 75-80% that of DL-Met on an equimolar basis. This is equivalent to 63-67% for MAH-Ca on a weight basis considering its 84% product purity. This means 1 unit of MHA-Ca and 0.65 unit DL-Met elicit equal performance in fish. With this background, this study was undertaken to validate the 65% average bioefficacy of MHA-Ca versus DL-Met based on the growth performance and antioxidant status of Nile tilapia.

Materials and Methods

The trial was conducted at the experimental facilities of the University of Trás-os-Montes e Alto Douro (UTAD, Vila Real, Portugal) under the full responsibility of SPAROS. The trial comprised 7 dietary treatments (D1-D7) and all diets were based on a single basal formulation (NC) containing 0.47% Met and 1.00 % Met+Cys. The remaining 6 diets were supplemented with three Met sources (DL-Met, MHA-Ca and PROXYMet containing 65% DL-Met and 35% limestone). D2 and D3 with DL-Met at 0.1 and 0.2%; D4 and D5 with MHA-Ca at 0.154 and 0.308%; and D6 and D7 with PROXYMet at 0.15 and 0.31%. Diets were isonitrogenous (crude protein: 37.5 ± 0.5 %DM) and isoenergetic (gross energy: 19.2 ± 0.1 MJ/kg DM). Diets were fed three times daily over 92 days, to quadruplicate groups of Nile tilapia (50 fish per tank) with a mean initial body weight of 22.5 g (1.2 g, SD). The average water temperature during the trial was 26.5 ± 0.4°C and water dissolved oxygen levels were kept above 6.4 mg/L. Ten whole-fish from the initial stock (start of the trial) and a pool of 9 whole-fish from each replicate tank at the end of the trial were sampled and used for whole body composition analysis. Liver of 3 additional fish per replicate tank were sampled, and used for assessing antioxidant status criteria.

Table 1. Growth performance of Nile tilapia fed different diets over 92 days

Methionine sources; doses %	Final body weight g/fish	SGR %/day	Feed conversion ratio	Protein efficiency ratio
-	98.4 $^{a, x}$	1.60 $^{a, x}$	1.34 ^{b}	2.18 $^{a, x}$
DL-Met, 0.1%	113.8 $^{c, y}$	1.76 $^{c, y}$	1.17 ^{a}	2.50 $^{c, y}$
DL-Met, 0.2%	119.9 $^{c, z}$	1.82 $^{c, z}$	1.13 ^{a}	2.61 $^{c, y}$
MHA-Ca, 0.15%	109.8 $^{b, y}$	1.72 $^{b, y}$	1.21 ^{a}	2.42 $^{b, y}$
MHA-Ca, 0.31%	115.6 $^{b, z}$	1.77 $^{b, z}$	1.19 ^{a}	2.42 $^{b, y}$
ProxyMet, 0.15%	117.1 $^{c, y}$	1.79 $^{c, y}$	1.17 ^{a}	2.44 $^{bc, y}$
ProxyMet, 0.31%	119.2 $^{c, z}$	1.81 $^{c, z}$	1.14 ^{a}	2.53 $^{bc, y}$
P values				
Met Source	**<0.001**	**<0.001**	**0.022**	**0.003**
Met Dose	**<0.001**	**<0.001**	0.064	**0.019**
Met Source x Dose	0.084	0.209	0.821	0.279

Different superscripts within a column denote statistical differences (P<0.05).
Supercripts a, b and c for variable Met source. and x, y and z for variable Met dose.

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Results

Fish fed the Met supplemented diets (2 to 7) showed a significantly higher body weight, specific growth rate (SGR) and protein efficiency ratio (PER) and a significantly lower FCR than those fed D1, without Met supplementation (Table 1). Diets supplemented with Met sources DL-Met and PROXYMet led to a significantly higher final body weight, SGR, PER and a significantly lower FCR than those supplemented with MHA-Ca (Table 1). Met dose had also a significant effect on performance criteria, with higher FBW and SGR being found in fish fed diets with the highest supplementation dose. Dietary Met dose or supplementation had no significant effect on the whole-body composition of fish. Fish fed diets supplemented with Met sources DL-Met and PROXYMet showed also a significantly higher whole-body protein, fat and energy retention than those fed diets supplemented with MHA-Ca. Increase in Met supplementation doses led to a significant increase of the hepatic levels of the reduced glutathione form (GSH). Overall, all Met supplemental sources and doses generated significant gains on the performance criteria of Nile tilapia. However, these gains were significantly higher with DL-Met and PROXYMet products than with the MHA-Ca product, suggesting bioefficacy of MHA-Ca is even lower than 65% in Nile tilapia on a weight basis. As in our study, lower BE of MHA-Ca (43-52% on weight basis) versus DL-Met was also reported recently in common carp (Zhou et al. 2021). Results of this study need to be considered in formulating tilapia feeds for optimal production and economic performance.

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POLYCHAETE MEAL (*Nereis virens*) IN THE FEEDS FOR JUVENILE MEAGRE (*Argyrosomus regius*): EFFECTS ON NUTRIENT DIGESTIBILITY

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Introduction

In the pursuit of more sustainable fish feed ingredients, polychaete worms are emerging as a popular alternative to replace fish meal and fish oil (Pombo et al., 2020). The *Nereis* genus, specifically *N. virens* and *N. diversicolor*, is being investigated for its potential as a source of high-quality protein, fat, and essential fatty acids like EPA and DHA, which are beneficial for the growth and well-being of aquaculture species (Wibowo et al., 2020; Pajand et al., 2017; Bischoff et al., 2009). The objective of this study was to assess the digestibility of diets containing polychaete meal (*Nereis virens*, Sars 1835) as an alternative ingredient to replace traditional protein sources, such as fish meal and plant meals, in meagre.

Materials and methods

For the digestibility trial, nine groups of 15 fish (76.3±15.6g) were placed in 250L cylindroconical tanks equipped with a settling column. The fish were fed with a control diet and two experimental diets, which included 10% or 20% *Nereis virens* meal (ProChaete Innovations LTD, UK). The polychaete meal served as a replacement for up to 9g/kg of plant ingredients and up to 120g/kg of fish meal (Table I). An inert marker, celite®, was added at a level of 10g/kg. The proximate compositions of the feed and feces, as well as the apparent digestibility coefficients, were determined following the methods described in Gasco et al. (2016). The carbohydrate and energy content of the feed and feces were calculated based on the approach by Kounna et al. (2021)

Table I. Ingredients and proximate composition of the experimental diets and the polychaete meal

Ingredients (g/kg)	*N. virens* meal	0%	10%	20%
Fishmeal	-	450	390	330
Nereis virens meal	-	0	100	200
Corn gluten	-	145	130	110
Wheat flour	-	200	185	155
Soybean meal	-	100	90	90
Vitamins and minerals premix	-	5	5	5
Fish oil	-	90	90	100
Celite 545	-	10	10	10
Proximate composition (dry matter basis)				
Protein (%)	62.6	52.8	53.8	49.8
Fat (%)	2.4	15.5	14.0	13.7
Ash (%)	6.8	6.8	7.0	7.0
Carbohydrates (%)	28.0	25.0	25.9	29.5
Energy (MJ/kg)	20.6	22.9	22.6	22.3

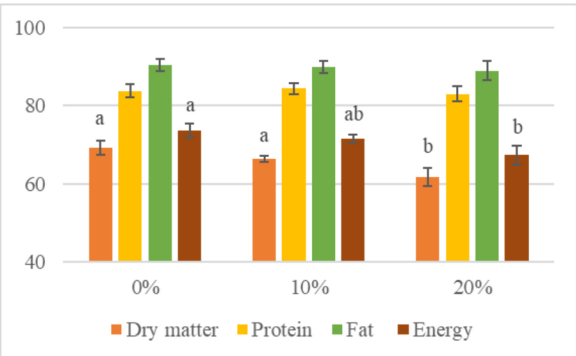

Figure 1: Apparent digestibility coefficients (ADC %) of nutrients and energy of diets containing 0, 10 and 20% of polychaete meal (*Nereis virens*.) in meagre *Argyrosomus regius*. In each ADC, different letters denote statistically significant difference ($p<0.05$) (mean ± standard deviation, n=3)

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Results and Discussion

Meagre demonstrates good tolerance for diets incorporating alternative ingredients like insects and algae, particularly when the replacement level of fish meal is low (Estévez et al., 2022). However, its ability to effectively utilize invertebrate meals, such as insect meals, is limited, and digestibility decreases when the inclusion exceeds 10% (Coutinho et al., 2021; Guerreiro et al., 2020). Similarly, in this experiment, the inclusion of polychaete meal resulted in a significant decrease in dry matter digestibility ($p<0.05$). However, protein digestibility remained similar across the experimental diets, ranging from 83.0% to 84.4%. Fat and energy digestibilities exhibited a tendency to decline with increasing levels of polychaete meal inclusion, although the decrease was only statistically significant in the apparent digestibility of energy ($p<0.05$).

In conclusion, meagre exhibits efficient utilization of polychaete protein up to a 10% inclusion level. However, beyond this threshold, there is a negative impact on the digestibility of dry matter and energy.

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FUNCTIONAL FEED FORMULATION: COMPARATIVE GROWTH, INNATE IMMUNITY, DIGESTIVE ENZYME INDICES AND BODY COMPOSITION OF NILE TILAPIA (*Oreochromis niloticus*) UNDER 75% FISH MEAL REPLACEMENT WITH SOYBEAN MEAL

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Introduction

Global aquaculture grew at a faster rate than other food crops, with an average annual growth rate of 4.6 % from 2007 to 2018. (FAO, 2020). The main challenge in growing aquaculture sector is to provide a constant supply of economical and eco-friendly feed in sufficient quantity. Traditionally fish meal is used as protein source in feed of various aquaculture practices. Due to its ever increasing demand, the supply of fish meal is not remained smooth beyond having high price and environmental concerns. In aquaculture, functional feed formulation is an optimal solution of reducing high feed cost in an environmental friendly way; by replacing animal origin ingredients with plant based sources and functional additives.

Material and Methods

An experiment was carried out to examine the effect of 75% replacement of fishmeal as protein source in diet with soybean meal (fermented/ non fermented; 2% probiotic/ 4% probiotic) on growth, immunity, proximate body composition and digestive enzyme status of *Oreochromis niloticus*. Five treatments of feeding were designated as (T0) containing 100% fishmeal, (T1) 75% fishmeal replacement with fermented soybean meal and 2% probiotics, (T2) 75% fishmeal replacement with non-fermented soybean meal and 2% probiotics, (T3) 75% fishmeal replacement with fermented soybean meal and 4 % probiotics, (T4) 75% fishmeal replacement with non-fermented soybean meal and 4 % probiotics. The comparative effect of these functional feed treatments were investigated with reference to growth response, innate immunity, digestive enzyme activity and body composition of Nile tilapia for 16 weeks in laboratory conditions.

Results

The observed weight gain was 14.1 g in T_0, 8.7g in T_1, 8.4 g in T_2, 10.38 g in T_3 and 9.96 g in T_4. The better value of FCR (1.88) was observed in T_0 group and the maximum value of SGR (1.9) was also observed in T_0. The WBCs count and IGM were highly reduced in non-fermented soybean groups. However, FSBM showed significant increase in WBCs count and IgM. Highest level of WBCs counts and IgM was observed in FSBM+ 4% probiotics. IgM level of *O. niloticus* was 10.13 mg/ml in T_0, 9.76 mg/ml for T_1, 9.30 mg/ml in T_2, 11.36 mg/ml in T_3 and 10.43 mg/ml in T_4. The results of the digestive enzyme activity with non-fermented SBM had negative effect on digestive enzyme activity. Fermented soybean groups showed an improvement in digestive enzyme activity in comparison to non-fermented groups but not comparable to control group. Lipase activity of *O. niloticus* was 196.6 U/mg for T_0, 190.30 U/mg for T_1, 186.30 U/mg for T_2, 190.21 U/mg for T_3 and 186.2 U/mg for T_4. Amylase activity was 4.46 U/mg for T_0, 4.40 U/mg for T_1, 4.56 U/mg for T_2, 4.26 U/mg for T_3 and 4.50 U/mg for T_4. Protease activity was 71.04 U/mg for T_0, 68.51 U/mg for T_1, 64.42 U/mg for T_2, 68.54 U/mg for T_3 and 64.54 U/mg for T_4. The results of present study indicated that FSBM had no significant effect on the proximate body composition with respect to moisture content, protein, fat and ash content, however crude protein and fat level were significantly decreased in non-fermented SBM groups. The levels of crude protein of *O. niloticus* were 16.05% for T_0, 16.05% for T_1, 13.54% for T_2, 16.02% for T^3 and 13.616% for T_4 and total fats were 3.66% for T_0, 3.65% for T_1, 2.24% for T_2, 3.64% for T_3 and 2.25% for T_4. The moisture contents were 78.26% for T_0, 78.16%, for T_1, 80.20% for T_2, 78.16% for T_3 and 80.55% for T_4 and total ash contents were 3.19% for T_0, 3.21%, for T_1, 4.57% for T_2, 3.21% for T_3 and 4.48% for T_4.

Discussion

Use of non-conventional (plant based) ingredients in fish feed due to presence of anti-nutritional factors and poor digestibility affects the fish performance adversely there by reducing overall production (Abowei and Ekubo, 2011). During the current experiment in comparison to the other groups, the growth in control group was higher, but there were slight differences in weight gain and length gain of Tilapia. Although fermented soybean meal was used with the addition of 2 % and 4 % probiotics through which anti-nutritional factors were removed which halted growth, but poor growth was observed in treatments. Study by Dan *et al*. (2017) showed similarity with these results as they found that fish growth was maximum in control group then followed by fermented soybean meal.

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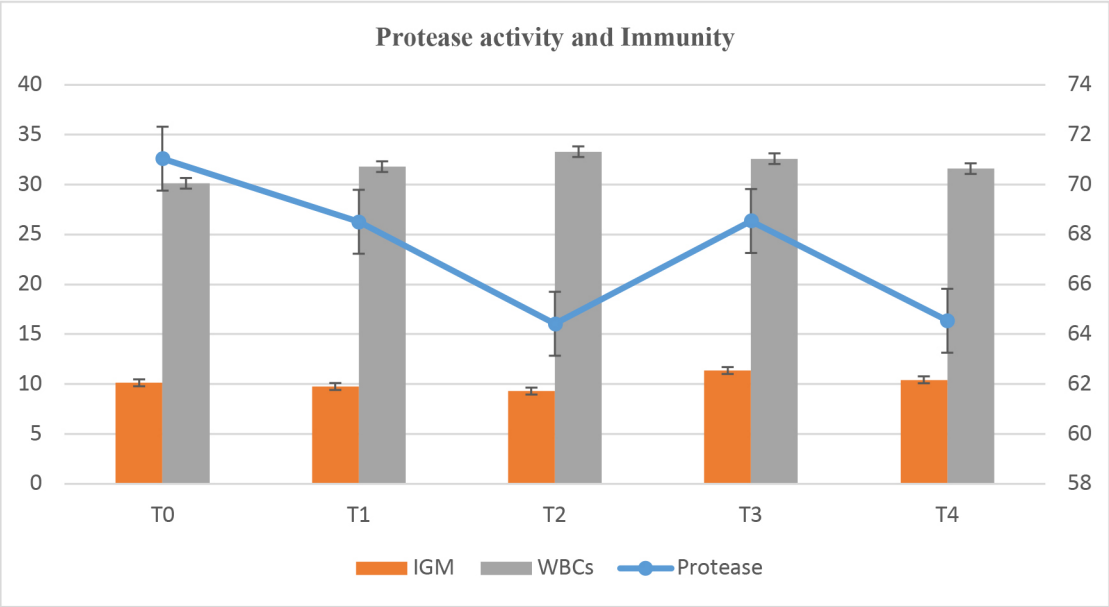

Figure: Interaction of protease activity and immunological response between different functional feed treatments in Nile tilapia.

The digestive enzymes play role in digestion and assimilation of feed by digesting and absorbing the nutrients properly (Deng *et al*. 2015). In the present study the digestive enzyme activity was significantly varied/ reduced in both fermented and non-fermented groups. The soybean meal contains the anti-nutritional factors which negatively affect the digestive enzyme activity. The protease activity was significantly reduced in non-fermented group due to the presence of protease inhibitor in SBM. Liu *et al*. (2020) reported that as the level of substitution of fishmeal with SBM increased there was significant reduction in digestive enzyme activity.

From the results of present study it is evident that high level fish meal replacement with soybean meal did not provide better growth, innate immunity, digestive enzyme activity or proximate body composition in Nile tilapia, however if we pretreat the soybean meal through fermentation along with probiotic inclusion then a comparable performance may be achieved.

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OCCURRENCE OF MICROPLASTICs AND METALS IN EUROPEAN SEABASS FROM DIFFERENT AQUACULTURE SYSTEMS: DIETARY EXPOSURE AND TOXICOLOGICAL RISK FOR HUMANS

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Introduction

Understanding the impact of microplastics (MPs, plastic particles < 5 mm) on aquaculture species has become a growing concern in food safety worldwide [1]. Indeed, MP occurrence has been reported not only in many wild-caught fish species but also in their farmed counterparts [2]. The interdependence between aquaculture production systems and reservoir environments, and their specificities related to plastic gear usage are likely to affect MP availability for uptake by fish [3]. Under the 'One Health' perspective, this work aimed to evaluate the MP occurrence in European seabass (*Dicentrarchus labrax*) produced in three different aquaculture systems by analysing water, fish feed and seabass tissue samples. Trace and non-essential metals in seabass muscle were also determined. Human dietary exposure and toxicological risk from consuming farmed seabass muscle were estimated using European Food and Safety Authority (EFSA) recommendations.

Materials and methods

Approximately 50 specimens were collected from each of the three selected aquaculture systems: a cage farm located in Turkey, and a pond farm and a recirculating aquaculture system (RAS) both located in Portugal. Particles suspected of being made of plastic were quantified in the gastrointestinal tract (GIT) and muscle and visually characterized according to their shape, colour, and size. The chemical identification was performed through Fourier Transform Infrared Spectroscopy (FTIR).

The concentration of trace (Cr, Ni, Cu, Zn) and non-essential (Cd, Hg, Pb) metals in the seabass muscle were determined through Atomic Absorption Spectrophotometry (AAS).

Human exposure to contaminants through seabass consumption was estimated based on European Market Observatory for Fisheries and Aquaculture Products (EUMOFA) data.

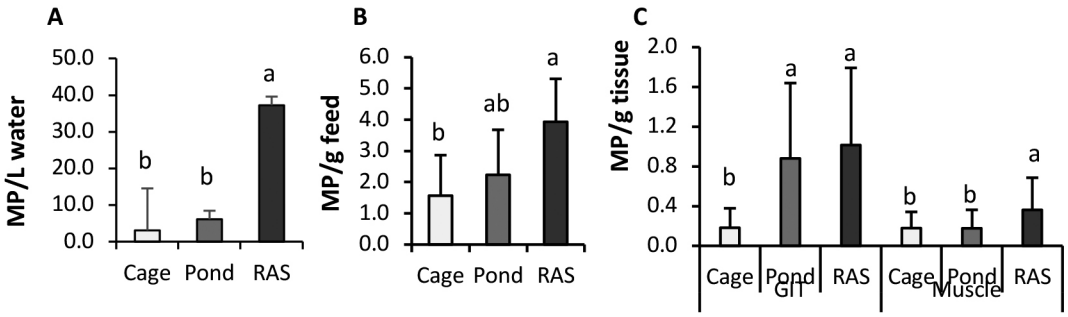

Fig. 1. MP occurrence per litre of water (A), and per gram of feed (B) and fish tissue, gastrointestinal tract (GIT) and muscle of European seabass (C) from three aquaculture systems. Different letters indicate significant differences among samples ($p < 0.05$)

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Results

Comparatively higher MP levels were detected in RAS water and feed than in the other systems (Fig. 1A and 1B). MPs with blue and black colour, fibre-shaped, and made out of cellulose/rayon and polyester, were the most common in all systems. MP characteristics were generally similar among fish tissues. Cage-farmed seabass had the lowest MP occurrence, with 89% of the fish having at least one MP recovered from a certain tissue. At the tissue level, RAS-farmed fish had the highest MP levels in muscle, while in GIT, the values were also higher in these fish but comparable to those observed in pond-farmed seabass (Fig. 1C).

Cr, Ni, Zn, Cd, and Hg concentrations detected in muscle were all below the maximum permissible concentrations established for this species. Cu and Pb were below detection limits. No significant differences were observed among systems, except for Zn with pond-farmed seabass displaying the highest value (5.5 ± 0.7 vs. 3.6-4.6 µg/g wet weight).

Based on the available health-based guidance values (HBGVs) provided by EFSA for metals and considering a 150 g meal of seabass fillet, no toxicological risk associated with farmed seabass fillet consumption was observed. A monthly human exposure to MPs ranging from 1.8-9.3 per kg of consumer's body weight was estimated, depending on seabass consumption habits in each country.

Discussion

Our findings indicate that water and feed are the primary pathways of MP exposure for farmed European seabass, which may potentially result in their retention in fish tissues. The presence of MP in muscle tissue indicates their potential availability to human consumers along with other environmental contaminants. However, the calculated human dietary exposure scenarios revealed low toxicological risks associated with consuming seabass produced from the three analysed production systems. Despite the findings, the controlled conditions in artificial systems such as RAS are more likely to provide better opportunities for minimizing MP contamination through the implementation of mitigation strategies (e.g., development of natural system components) in the systems. This work is in line with the One Health concept, which acknowledges the interdependence of human, animal, and environmental health.

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INNOVATION IN CRITICAL VARIABLES IN PULLET CARPET SHELL (*Venerupis corrugata*) IN THE HATCHERY PRODUCTION. STUDY OF THE BROODSTOCK QUALITY

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Introduction

The pullet carpet shell *Venerupis corrugata* is commercially exploited in southern Europe (Joaquim et al., 2010). The market demand and commercial value of this species are high. However, populations declined during the last decades due to overfishing and recruitment failure. Thus, it appeared crucial that an active intervention may be necessary to restore stocks aiming to attain reproductive viability. To reverse this declining situation, the hatchery production of larvae should be implemented. This study has the main objective to evaluate the evolution of the maturation state of *V. corrugata*, from different origins, in conditioning, contributing to the development and validation of a new protocol for obtaining larvae and maximizing hatchery production.

Materials and methods

Venerupis corrugata from three origins, Lagos coast (VCOF), Óbidos Lagoon (VCOB), located in Portugal and Ria de Arousa (VCRI), Spain, were induced to spawn at Oceano Fresco Biomarine Center in November 2021, and after, placed for conditioning to enhance the gonad development. Twenty individuals from each origin were sampled to evaluate the biochemical composition, gonadal stage and condition index. Sampling, spawning induction, and conditioning were repeated twice on 5 January and 9 February 2022. Larvae from each spawn and each origin were incubated. 50 larvae were periodically sampled to estimate the survival rate and growth.

Results and conclusions

Sexual maturation

In general, clams presented sexual synchronism in all populations except in Óbidos, where a slight advance in females' gonadal development was observed (Figure 1). In November, most individuals presented the gonad completely mature or in spawning and post-spawning phases. Only in Óbidos were found the early gametogenesis stage. The VCOF population had a higher gonadal index (4,5) when arriving from the natural environment. After a month and a half of conditioning, most individuals from all origins recovered their pre-spawning condition, reaching gonadal index values between 4.7 and 4.9. The first conditioning was more efficient for the VCOB population. The second conditioning was less effective than the first one once most individuals remained in post-spawning (stage 5), and the gonad recovery was not so effective.

Biochemical composition

Proteins were clams' predominant dry tissue constituent (Figure 1). The glycogen content was the biochemical compound that revealed higher variations along conditioning periods. Clams from VCOF presented the highest value of this compound in November, suffering a pronounced decrease in January. In VCOB, glycogen decreased in the first conditioning and slightly increased in the second. In the VCRI population, contrary to the others, glycogen content increased in the first conditioning, followed by a decrease in the final of the second conditioning in February. Total lipids content decreased in the second conditioning for the VCOF population. In VCOB, this compound increased in the first conditioning. In clams from VCRI, total lipids don´t suffer significant alterations.

Condition Index

In general, the condition index of all tested origins decreased along conditionings, compared to its initial condition. This decrease was more pronounced in the second conditioning for VCOF clams, and in the first one, for VCOB and VCRI clams.

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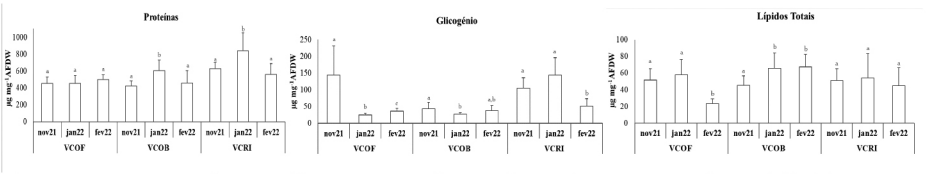

Figure 1. Mean values (±SD, n=10) of proteins, glycogen, and total lipids (µg mg^{-1} AFDW) of clams *V. Corrugata* from VCOF, VCOB, and VCRI, in November 2021 and at the end of each conditioning, in January and February 2022. (a,b) significant differences between dates ANOVA, P<0.001).

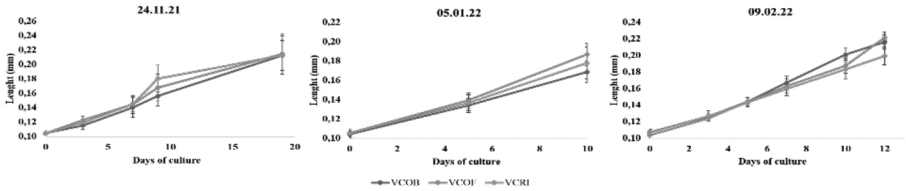

Figure 2. Variation in length (mm) (mean±SD, n=50) in *V. Corrugata* from VCOF, VCOB, and VCRI, from three spawnings (24.11.21; 05.01.22 and 09.02.22) during larval culture.

Larval viability

The highest average growth was recorded in VCOF larvae in February on the 12th day of culture (Figure 2). In November, no significant differences were found between the origins at the end of the larval culture. However, differences were visible in January, being the VCOF larvae having the highest average length. The broodstock origin did not have a significant influence on larval growth.

Acknowledgements

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5
AQUAVOLTAICS IN LIGHT OF FISH DEPENDENCE ON LIGHT

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Fish, light and aquavoltaics

Global environmental concerns and increasing energy demand, combined with steady progress in renewable energy technologies, are opening up new opportunities for the use of renewable energy sources. Solar energy is the most abundant, inexhaustible, and cleanest of all renewable energy sources (Parida et al., 2011). Photovoltaic conversion is the direct conversion of sunlight into electricity without the interposition of a heat engine. All solar cells require a light-absorbing material present in the cell structure to absorb photons and produce free electrons through the photovoltaic effect.

'Floatovoltaic' represent an emerging power-generation technology utilizing idle water and solar energy systems—comprising floating photovoltaic (FPV) panels over water. They are an attractive source of low-carbon energy because they free up land for other uses and produce greater electricity yields compared to land-based systems. FPV designs for freshwater are evolving as the technology matures and primarily include PV panels mounted on individual floats, on racks attached to floating pontoons, or on poles fixed to the water's bottom (Fig. 1) (Liu et al 2018).

However, to date little is understood of the impacts of FPV on the hosting water body. Anticipating changes to water body processes, properties and services owing to FPV deployment represents a critical knowledge gap that may result in poor societal choices and water body governance (Armstrong et al., 2020). As the world's population increases and competition for land rises, dual-use approaches are becoming essential solutions in the agriculture and aquaculture sectors. With the constantly growing aquaculture industry and the increasing demand for eco-friendly production processes, the necessity for employing optimized aquaculture systems, supplied by renewable energies, gaining progressively attention in the global food production sector. Teaming up photovoltaic with agriculture or aquaculture, namely, the agrivoltaics and aquavoltaics (AquaPV), create novel energy-food (land or water) nexus offering mutual benefits potentially (Jing et al., 2022). AquaPV is a concept emerged with combining electricity production and aquaculture. The goal of AquaPV is the efficient use of water with the dual use for both food and energy generation. The AquaPV approach aims to maintain parameters such as water and air temperature, light availability, water pH, dissolved oxygen, feeding system, and predator pressure, and improve the system by exploiting synergies between aquaculture and FPV systems. While solar panels above the water or on its surface provide the electrical energy, the aquatic organisms living within the water below provide a sustainable food source.

Light characteristics are very specific in an aquatic environment and light is extremely variable in nature. 'Receptivity' of fish to light profoundly changes according to the species and the developmental status (Beouf and La Bail, 1999).

Fish move within their environment and often their environment moves around them, affecting the light that the fish receives (Sumpter, 1992). Moreover, light shows interesting characteristics in the aquatic environment. In fact, 'quality' (the different wavelengths which are absorbed by water to various extents), 'quantity' (different intensities) and 'periodicity' (daily cycles, which vary seasonally according to latitude) should also be considered. Fish behaviour can be affected even by artificial light stimuli. A common reaction of fish groups to the presence of artificial light is to school and move towards the light source (Ben-Yami, 1976). Functional explanations for such a reaction include predator avoidance and enhancement of feeding efficiency (Pitcher and Parrish, 1993). In terms of aquavoltaics, light emitting diodes (LEDs) can be installed on the bottom of the pontoon structures in the aquavoltaic system, powered by the PV portion of the system to affect the photoperiod of aquatic life. This design provides a powerful tool for the aquaculturist to increase and further optimize production for specific aquatic species but needs to be tested further, while the effects of energy conversion need to be considered, also. (Pringle et al., 2017). Normally, fish are either more active in light and less active in darkness or vice versa, and this can be altered by daily changes in factors such as temperature or oxygen. Growth of aquatic organisms is linked to light, but it is not unique because species vary in their growth conditions. Fish and larvae, for example, must be reared in specific light ranges depending on the species and stage of development (Boeuf and Le Bail, 1998). Aquavoltaics provide shade on the fish pond water surface, and the blocked light is absorbed by solar panels and converted into usable energy. If uncontrolled, an increase in shading decreases algal growth, general plant life, and microbial density are reduced, affecting the entire food chain down to the fish intended for breeding. With a suitable system approach, aquavoltaics can contribute to sustainable water use and fulfil the concept of the food-water-energy nexus. More research is needed to understand the effects of direct contact with pontoon structures and solar arrays on aquatic life.

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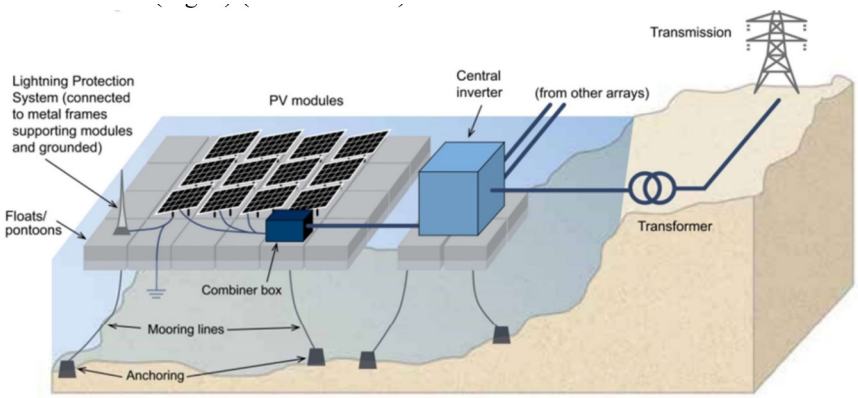

Fig. 1. Schematic representation of a typical large-scale FPV (Source: Solar Energy Research Institute of Singapore (SERIS) at the National University of Singapore)

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USE OF LEVAMIZOLE FOR GROWING OF THE JUVENILE STURGEONS

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Introduction

The jump-like processes of sturgeon fish development in early ontogeny create conditions for the use of immunostimulants in order to effectively overcome the stress caused by their cultivation in recirculating aquaculture systems (RAS). The synthetic substance levamisole is used in aquaculture. It has been reported that the use of this substance in the diet of juvenile pacu during stress altered circulating cortisol levels and also improved the innate immune response to infection with *Aeromonas hidrophila* (Pahor-Filho E., 2017).

The immune-stimulating properties of this substance added to carp and sea bass food have also been described (Guesta et al., 2004, Rymuszka et al.,2005). However, most data on the effects of levamisole relate to mature fish, and information about its effect on juveniles, especially sturgeons, is insufficient. Therefore, the purpose of this study is to evaluate the effects of the levamisole on the growth, development, and viability of juvenile sturgeon fish.

Material and methods

Larvae and juveniles of the hybrid bester *(♀ Huso huso X ♂ Acipenser ruthenus*) and freshwater sterlet (*Acipenser ruthenus*) (BSS) were used in the study. Experiments were performed at RAS on larvae of Bester sturgeon, immediately after their transition to exogenous nutrition and lasted 28 days at temperature 18°C (n=1000). In the first month of rearing, live food, Artemia nauplii (Artemia salina), was used. Live Artemia nauplii were enriched with levamisole according to the method used to create a concentrate for the enrichment of rotifers and nauplii (Watanabe, 1983). In the experiment, a levamisole concentration of 14.6 mg per 1 g of live nauplii was used in 100 g of enrichment substrate for 8 hours. The main indices of fish were studied: weight, body length, growth rate, mortality.

Results and discussion

The addition of levamisole to the diet during the first week of exogenous feeding had a positive effect on the growth of young sturgeon. The average weight of fish in the experimental group increased by 3.96 times for a week, which is 9% higher than the control values. Average daily weight gain in the experimental group was 24.9%, in the control group - 22% per day. During levamisole application during the first and second day the growth rate of fish was 19 and 20%, after that it increased to 27.3% and by the end of the seventh day it remained at 27%. In the control group, the growth rate was 17 and 18% per day during the first two days, after which it also reached the level of 27%.

After 21 days of the experiment, it is noticeable that the average body weight of young sturgeons in the group consuming levamisole increased to 0.794 g statistically reliably ($p < 0.05$), which exceeds the control values by 50.5%. The average growth rate was 9.3% and 11.3% in the experimental and control groups, respectively. Thus, the positive difference in the average weight of the experimental fish is a consequence of the prolonged action after levamisole application during the first week of the experiment. However, analyzing the daily growth rates, the negative effect of repeated application of Levamisole is observed, which leads to a decrease in the growth rate relative to the control group.

Conclusions

Thus, levamisole in an amount of 14.6 mg/g of Artemia nauplii is reasonable to use during the first week when larvae switch from mixed to exogenous nutrition. Its repeated use after three weeks is inexpedient because of the lack of positive indicators in fish. Finding and developing safe and effective ways to apply new substances that positively affect the survival of juvenile sturgeon is a promising area of research. Our work will continue, and in the future we plan to extend the results obtained to other sturgeon species.

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Introduction

The jump-like processes of sturgeon fish development in early ontogeny create conditions for the use of immunostimulants in order to effectively overcome the stress caused by their cultivation in recirculating aquaculture systems (RAS). The synthetic substance Levamisole is used in aquaculture. Thanks to its use in the diet of the juvenile paku during stressful situations, the circulating cortisol level was modulated, and the innate immune response to *Aeromonas hidrophila* infection was also improved (Pahor-Filho E., 2017). Also, the immunostimulating properties of this substance in the process of feeding it to carp and seabream have been described (Guesta et al., 2004, Rymuszka et al.,2005,). Most of the information on the action of Levomizole concerns mature stages of fish, but there is insufficient information on its effect on juveniles, in particular sturgeon. This abstract presents the effect of the Levamisole on the growth, development, and viability of juvenile sturgeon fish.

Material and methods

The object of the study were larves and juveniles of the bester hybrid (♀ *Huso huso* X ♂ *Acipenser ruthenus*) and freshwater sterlets (*Acipenser ruthenus*) (BSS). The experiments were carried out in RAS on small bester sturgeon larvae, immediately after their transition to exogenous nutrition and lasted for 28 days at a temperature of 18°C (n=1000). In the first month of cultivation live feed was used - artemia nauplii (*Artemia salina*). Live artemia nauplii were enriched with Levamisole according to using the method used to create a concentrate for enrichment of rotifers and nauplii (Watanabe T., 1983) In the experiment, a concentration of Levamisole was used in the amount of 14.6 mg per 1 g of live nauplii, in 100 g of enrichment substrate for 8 hours. The main fish indicators were studied: mass, body length, growth rates, mortality.

Results

The addition of Levamisole to the diet during the first week of exogenous feeding has a positive effect on the growth of juvenile sturgeons. The average weight of experimental fish in the experimental group increased during the week by 3,96 times, which is 9% higher than the control values. The average daily weight gain in the experimental group was 24,9%, in the control group - 22% per day. In the process of using Levamisole during the first and second days, the growth rate of fish was 19 and 20%, after which it increased to 27,3% and by the end of the seventh day it was still at the level of 27%. In the control group, the growth rate was 17 and 18% per day for the first two days, after which it also reached the level of 27%.

After 21 days of the experiment, it is noticeable that the average body weight of young sturgeons in the group that consumed Levamisole increased to 0,794 g was statistically significant ($p < 0.05$), which exceeds the control values by 50,5%. Average growth rates were: in the experimental group – 9,3%, in the control group – 11,3%. Thus, the positive difference in the average weight of the experimental fish is a consequence of the prolonged action after the use of Levamisole during the first week of the experiment. However, analyzing daily growth rates, a negative effect of repeated use of Levamisole is observed, which leads to a decrease in growth rates relative to the control group.

Conclusions

Thus, it is advisable to use Levamisole in the amount of 14,6 mg/g of artemia nauplii during the first week during the transition of larvae from mixed to exogenous nutrition. Its repeated application after three weeks is impractical due to the lack of positive fishery indicators. The search for and development of safe and effective ways to use new drugs that positively impact the survival of juvenile sturgeon is a promising area of research. Our work will continue and we plan to extend our results to other sturgeon species in the future

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THE SALTBOX PROJECT: WEIGHT-MORPHOMETRICS RELATIONSHIPS FOR INTENSIVELY EUROPEAN SEABASS *Dicentrarchus labrax*

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Introduction

The SaltBox project aims at designing and manufacturing a fish size/biomass estimator by using Coded Structured Light and adapted to the two major Mediterranean aquaculture species, gilthead seabream and European seabass. For the image captured by the estimator to be "translated" to actual body weight it is necessary to establish accurate weight-morphometrics relationships from traits that the estimator can accurately "see". The aim of the present study is to report these relationships for European seabass.

Materials and Methods

A total of 2933 specimens were individually weighted and photographed with embedded ruler. Targeted body weight ranged from 50 to 1000 g and special attention was paid to obtain at least 75-100 observations in a range of 25 g weight classes. Photographs were analyzed by digital image analyses and several morphometric traits were obtained. Spearman rank correlations were calculated, regression analysis was performed, and several models were applied to identify the most accurate one predicting weight from one or more of the morphometrics traits.

Results

The strongest correlation was obtained between weight and body monolateral area, followed by body lengths (i.e. total, fork, standards length, body height). The best model to describe weight (W)-morphometric traits (MT) relationships was of the type: $W = aMT^b$, with the exception of W-eye diameter and W-eye area relationships, which were neither strong nor well fitted. Multiple or polynomial regression also produced inferior estimations and the same result was obtained when the ratios of several combinations between traits examined were used. Also, when data were analyzed separately for body weight ranges of productive interest (i.e. 50-100 g, 100-500 g and 500-1000 g), relationships obtained were weaker and did not offer any advantage over those of the full body weight range examined.

Conclusion

Results obtained indicate that body monolateral area can be best used to accurately estimate actual body weight. The size/biomass estimator resulting from the SaltBox project can be tuned towards the precise estimation of this morphometric trait.

Aknowledgements

The project is co-funded by the European Maritime and Fisheries Fund and the Greek government.

THE EFFECT OF INSECT-BASED EXTRUDED DIETS ON GROWTH PERFORMANCE AND FEED UTILIZATION IN ATLANTIC STURGEON (*Acipenser oxyrhynchus*) JUVENILES

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Introduction

The Atlantic sturgeon is one of the largest and most endangered fish in Europe. The last records of the natural population are dated to the 1970s and currently, the species is recognized as extinct in the wild in Poland, while the last specimen in the Baltic Sea was recorded in 1996. Its reintroduction projects are based on hatchery material and fish raised in captivity. Thus maintenance, as well as nutrition guides, must be developed for successful species propagation and restocking.

Materials and methods

1800 juvenile Atlantic sturgeons (10,3 g each) were used in the 50-day-long feeding trial. They were divided into 3 treatments using 6 tanks per group and 100 fish per tank. The control diet (CON) contained 300 g/kg of fish meal. In 2 experimental treatments 150 and 300 g/kg of *Hermetia illucens* partially defatted meal (BSFM) was introduced. After 50 days of feeding growth performance and somatic indices were measured, and all the data were analysed using analysis of variance and post hoc Duncan's test.

Results

There was no fish mortality recorded. Among all treatments, there were no significant differences in final body weight, body weight gain, specific growth rate as well as feed conversion ratio. Condition factor and viscerosomatic index were not differentiated between insect meal inclusion levels. However, in treatment with 300 g/kg of *Hermetia illucens* meal the lowest hepatosomatic index was observed. There were no differences in fin length indices.

Conclusions

Hermetia illucens meal may be used in Atlantic sturgeon nutrition even with high inclusions of up to 300 g/kg of feed with no negative effects on growth performance, somatic indices or animal welfare. It may be concluded that insect meals are suitable feed materials for wild fish rearing for restocking purposes.

This study was carried out as part of the project entitled: "Innovative technology of highly adaptative juvenile sturgeon fish rearing for the natural and seminatural environment", no. 00001-6521.1-OR1500001/20, Task 2.1 "Innovations" according to EU Regulation No. 508/2014, Priority 2 – "Supporting environmentally sustainable, resource-efficient, innovative, competitive and knowledge-based aquaculture" realized in the Operational Program "Fisheries and Sea".

EFFECTIVE STIMULATION OF OVULATION IN SELECTED RHEOPHILIC CYPRINIDS SPECIES

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Introduction

Rheophilic cyprinids populations decrease in European rivers during the last 50 years due to human activity and its effect on the environment. As an important element of trophic systems, they may be considered an „umbrella species" for the entire ecosystems of lowland rivers. Thus methods of their reproduction and rearing of juvenile stages must be developed and improved for the effective production of restocking material.

Materials and methods

Adult females of common barbel (*Barbus barbus*), vimba bream (*Vimba vimba*), ide (*Leuciscus idus*) and European chub (*Squalius cephalus*) were obtained from a long-term captive population maintained in the Experimental Station of Feed Production Technology and Aquaculture in Muchocin. In the experiments, the possibility of stimulation for egg production was assessed. 80 fish per species were divided into four treatments (20 females each) CON – control with no stimulation, E – with environmental stimulation by prolonged photoperiod and increased water temperature, H - with hormonal stimulation (ovopel) and EH - with both environmental and hormonal stimulation.

Results

In CON treatment ova were obtained from 20% of common barbel only, while environmental induction did not increase the number of females producing eggs. In the case of vimba bream, Ide and European chub environmental stimulation did not induce ovulation. When hormonal stimulation was applied 40% of Ide, 60% of common barbel and European chub and 100% of vimba bream produced eggs. EH treatment increased ovulation success up to 80% in common barbel and European chub as well as 100% in vimba bream and Ide.

Conclusions

Despite the highest increase in ovulation due to hormonal stimulation use the application of environmental stimulation of females in rheophilic cyprinids seems to be an important element of effective and sustainable breeding in this group.

This study was carried out as part of the project entitled: "Innovative system of rheophilic cyprinid fishes reproduction and rearing in biologically effective and low emission conservative aquaculture", no. 00001-6521.1-OR1500001/22, Task 2.1 "Innovations" according to EU Regulation No. 508/2014, Priority 2 – "Supporting environmentally sustainable, resource-efficient, innovative, competitive and knowledge-based aquaculture" realized in the Operational Program "Fisheries and Sea".

INSIGHTS INTO INTEGRATED MULTI-TROPHIC AQUACULTURE IN A CLOSED RECIRCULATING SYSTEM FOR PRODUCTION OF NORTH SEA MACROALGAE, BIVALVES, AND SHRIMP

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Introduction

Infectious diseases are a common and on-going problem for reared aquatic species. Microbial management tools allow for increased control in aquaculture production systems, reducing the spread of pathogenic microbes while improving the health of cultured organisms. Recirculating aquaculture systems (RAS) are one such tool that is used to select for neutral and beneficial microbes that competitively exclude specific and opportunistic pathogens. However, the high investment costs and complexity of RAS inhibits its widespread implementation. Integrated multi-trophic aquaculture (IMTA) holds the potential to reduce or replace some of the expensive and sophisticated units that comprise RAS, making this technology more widely available while contributing value-added product, a proposition that may contribute to global food sovereignty. This project focuses on the co-culture of three species from different trophic levels in a closed cold-water multi-trophic marine RAS: nori (*Porphyra umbilicalis*), Pacific oyster (*Crassostrea gigas*), and North Sea prawns (*Palaemon* sp.). The effect of different types of nutrient bioremediation (traditional bacterial biofiltration versus macroalgae) on nutrient uptake efficiency, the microbiome, and the growth and survival of all trophic levels is evaluated.

Materials and Methods

P. umbilicalis was obtained from the Ghent University Phycology Research Group*, C. gigas* was obtained as T6 spat from Aquacultuur Oostende bvba (Ostend, Belgium), and *Palaemon* post-larvae and adults were wild-caught via dip nets from rocky tidal pools in coastal Belgium and Northern France. Three closed RAS comprised the experimental set-up and were operated at the Laboratory of Aquaculture and Artemia Reference Centre (ARC), Ghent University. Each up-flow RAS had a total fill volume of 500L, composed of three cylindroconical poly-ethylene culture tanks (100L; 90L fill volume) arranged in parallel with outflow to a drum filter (40 µm-mesh size) and protein skimmer before reaching the nutrient bioremediation tank (Figure 1). All systems were filled with filtered natural seawater, operated at a flow rate of 70L/h, a 12:12 L:D light regime, and with an ambient room temperature of 18°C. Three approaches to nutrient bioremediation were compared: a traditional biofilter (system 1), a traditional biofilter in combination with *P. umbilicalis* (system 2) and *P. umbilicalis* alone (system 3). The biofilter volume was dimensioned to treat the expected nutrient release from the *C. gigas* and *Palaemon* sp. as well as the residual nutrients in the microalgae supernatant fed to the *C. gigas*. *P. umbilicalis* were stocked based on the volume of the bioremediation tank and optimization for light exposure.

In each cylindroconical tank, a flat base was placed mid-way to provide a horizontal surface area for the *Paelaemon* prawns. Two cylindrical PVC baskets (80 mm height and 300 mm diameter) with a 2 mm mesh bottom were stacked in the tank and elevated with Bio-Net blocks in which aeration was fixed. The Bio-Net support consisted of small crevices, providing shelter places for the prawns. Each tank was stocked with 25g (0.28g/L) of *Palaemon* sp. and 115g (1.28g/L) of *C. gigas,* the oyster biomass being distributed equally over the two baskets in each tank. All culture tanks received 2% of oyster wet weight in microalgae dry weight in a 1:1:1 ratio of *Tetraselmis chuii*, *Chaetoceros muelleri* and *Isochrysis galbana,* All microalgae were obtained from Proviron (Hemiksem, Belgium). *Palaemon* prawns are fed with 1.5-2 mm shrimp pellets.

The total biomass of each trophic level was kept constant in all systems by removing the excess biomass produced over the course of a week. Microbial samples were taken weekly from each nutrient bioremediation tank. Water quality and nutrients (TAN, nitrite, nitrate, phosphate, calcium, and alkalinity) were measured three times per week in each bioremediation tank prior to the administration of feed. Temperature, pH and dissolved oxygen were assessed daily in each nutrient bioremediation tank and all culture tanks. The wet weight of all trophic levels was recorded weekly, as well as the dry weight and ash weight of *P. umbilicalis* and *C. gigas*, and the shell length and width of *C. gigas*.

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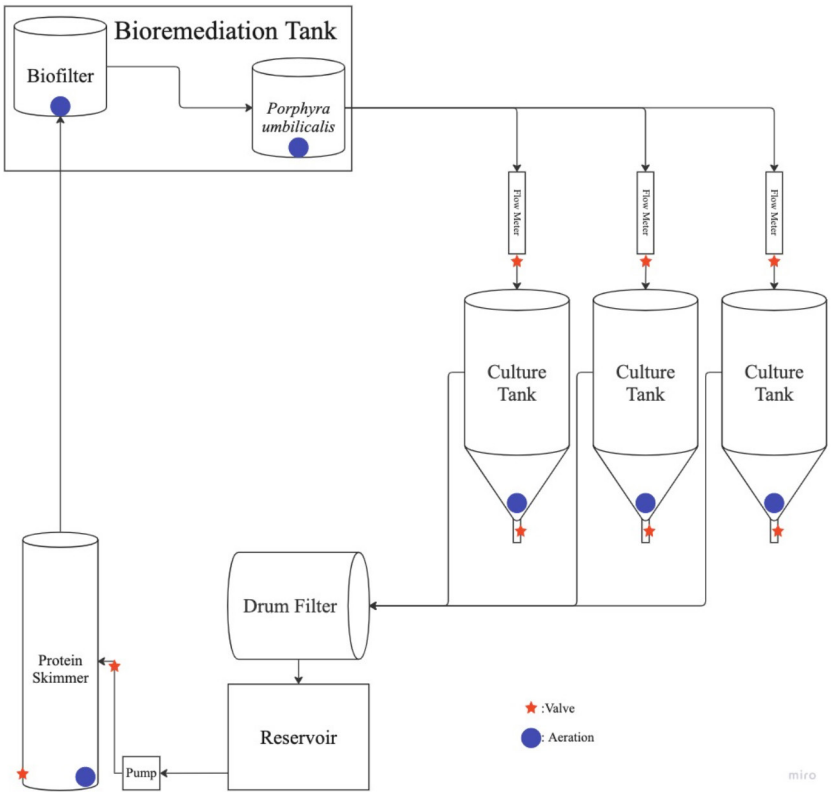

Figure 1: Flow scheme of the closed recirculating systems used during the experiment (not to scale). System 1 contains biofilter alone, system 2 contains biofilter and P. umbilicalis, and system 3 contains P. umbilicalis alone.

Results

This experiment represents pioneer research into co-cultivation of multiple trophic levels in a closed RAS. Despite this being the first time *P. umbilicalis*, *C. gigas*, and *Palaemon* sp. have been reared in such a system, there have been no detrimental effects on the health and welfare of the organisms. Furthermore, preliminary results indicate no significant differences in growth for *C. gigas* and *Palaemon* sp. between the three systems, and the different forms of nutrient bioremediation tested in this experiment have effectively kept all water quality parameters within a biologically suitable range.

Conclusion

Initial data from this innovative research suggests that conventional recirculating aquaculture farm designs may be effectively modified by replacing the traditional biofilter unit with macroalgae, reducing the upfront costs associated RAS production while contributing value-added biomass.

Acknowledgments

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DO EFFECTS OF NUTRITIONAL PROGRAMMING IN FRESHWATER IMPACT GENE REGULATION DURING SEAWATER REARING OF ATLANTIC SALMON (*Salmo salar*)?

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Introduction

The Scottish farmed salmon industry continues to expand and there is a requirement to reduce the level of finite marine origin raw materials used in traditional feed formulations. One alternative is to produce feeds with higher proportions of plant-derived oils and proteins, but these diets can lead to reduced feed utilization and also have lower levels of essential omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). A novel solution to this problem is a concept referred to as "nutritional programming". This involves applying an early nutritional intervention or "stimulus" where fish are fed a predominantly vegetable-based diet for a short period, to induce more efficient uptake and utilization of nutrients from a similar diet when fed later in life. Previously, it was demonstrated that Atlantic salmon (*Salmo salar*) fed such diets during a three-week stimulus phase from first exogenous feeding, resulted in significantly greater growth and nutrient retention efficiency during freshwater grow out, associated with upregulation in key pathways of intermediary metabolism (Clarkson et al. 2017; Vera et al. 2017). The current study aims to elucidate whether the effects of nutritional programming are sustained during post-smolt rearing in seawater at a transcriptomic level.

Materials and methods

Four experimental dietary groups were established in triplicated treatment tanks (Figure 1). Two treatments were fed an experimental vegetable-based diet for a three-week "stimulus" period from first feeding (V), while remaining tanks were fed a standard marine-based diet (M; Figure 1). Following stimulus, all groups were then fed a standard marine ingredient diet until week 36. Thereafter, two treatment groups were re-fed a vegetable-based diet for four weeks prior to sea transfer (MVV, VVV), while remaining tanks were maintained on marine-based feeds (MMV, VMV). Following a two-week acclimatization post- sea water (SW) transfer, when fish were fed a standard marine based diet, all fish were transferred to a vegetable-based diet for a 14-week period. Liver and pyloric ceaca tissue were collected from six fish per tank immediately prior to SW transfer and at the start and end of SW challenge for molecular analysis. RNA was extracted from tissues, reverse transcribed and sequenced on an Illumina NovaSeq platform, producing an average on 49 million reads per sample, before quantifying expression based on read alignment to the *S. salar* genome.

Results & Discussion

Our hypothesis was that fish subjected to a vegetable-based stimulus diet would better utilize dietary nutrients in later development. Growth and retention results presented previously (McMillan et al. 2021) suggested no conclusive evidence, by those measures, that this was the case. However, there was evidence that groups fed a combination of both diets in FW adapted to the SW environment more rapidly. We also saw some evidence, from fish fed both diets in FW, that fish may better regulate their metabolism according to requirements when the n-3 LC-PUFA levels are running low. This study presents RNAseq results indicating whether there is evidence to support a continued nutritional programming effect at a transcriptomic level during the SW rearing phase. Furthermore, functional regulatory pathways will be discussed relating to the FW dietary regime, with primary focus on regulation of fatty acid metabolism at the end of FW and subsequent SW stages. Empirical evidence of nutritional programming regulating fatty acid biosynthesis to trigger endogenous production of essential EPA and DHA could determine how we formulate more resource-efficient aquafeeds and feeding strategies for Atlantic salmon in the future.

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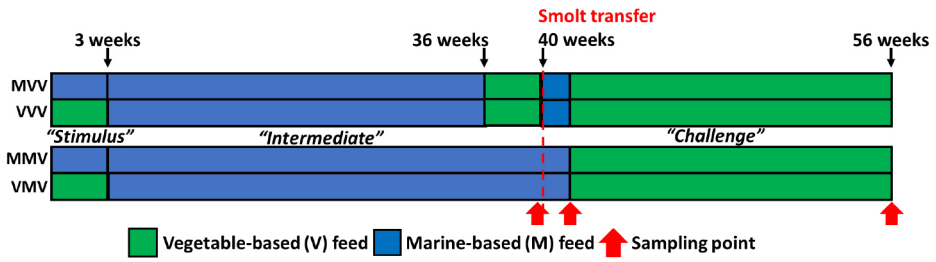

Figure 1. Schematic of trial showing when specific diets were fed and sampling points where tissues were collected for gene expression. All timepoints are cumulative.

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Acknowledgements

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University of Aberdeen and BioMar.

IMPACT OF OXYTETRACYCLINE EXPOSURE ON FISH SKIN MICROBIOMES IN AN EARTHEN POND POLYCULTURE SYSTEM

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Introduction

Fish farming is striving to maximise sustainable production to meet the needs of a growing global human population, however, disease is a major barrier to achieving this (Stentiford *et al*., 2017). To address the problem of disease in aquaculture there has been, and continues to be, widespread use (and misuse) of antibiotics for both disease treatment and prevention (Cabello *et al.*, 2016). Globally, antibiotic use is particularly prevalent in freshwater finfish culture (Schar *et al.*, 2020) and per capita consumption is increasing faster than meat and dairy consumption. Reports have documented antimicrobial use in the rapidly expanding aquaculture industry, which may contribute to the rise of antimicrobial resistance, carrying potential consequences for animal-, human-, and ecosystem-health. However, quantitative antimicrobial use across a highly diversified aquaculture industry is not well characterized. Here, we estimate global trends in antimicrobial use in aquaculture in 2017 and 2030 to help target future surveillance efforts and antimicrobial stewardship policies. We estimate antimicrobial use intensity (mg kg −1 , however, it is unclear what influence antibiotic exposure may have on the fish microbiome under normal culture conditions, which is a critical component for maintaining health and resilience against disease. Here we assessed the effects of exposure to oxytetracycline (OTC) on the fish skin and pond water microbiomes; antibiotic residue fate; and development of antimicrobial resistance. This was performed as a mesocosm study in Khulna, Bangladesh to provide realistic environmental and microbial conditions that directly translate to aquaculture practices.

Materials and methods

Tilapia and carp (*Oreochromis niloticus* and *Labeo rohita*) were polycultured in earthen aquaculture ponds (3x control and 3x treatment ponds) and exposed to a typical medical treatment dose (100 mg/kg) of OTC via the diet for five days. Sampling of water and fish skin swabs were collected prior to treatment, and subsequently at 2, 9 and 23 days following the 5 day antibiotic exposure. To characterise the prokaryotic and microeukaryotic communities, amplicon sequencing libraries of pond water filters and fish skin swabs were prepared as previously described (McMurtrie *et al.,* 2022). PCR-free metagenomes of pond water were also prepared to characterise the diversity of antimicrobial resistance genes selected in the resistome following antibiotic exposure. Sequencing of 16S V4 amplicons, metagenomes and 18S V9 amplicons was performed on the Illumina Novaseq SP-250, S1-150 and Illumina MiSeq V2-150 respectively. To assess antibiotic residue fate, pond water was concentrated by Solid Phase Extraction and quantified by the multi-residue UPLC-MS/MS validated methodology of Holton and Kasprzyk-Hordern (2021). Generalised linear mixed effects modelling was performed to statistically assess OTC residue fate and fish growth.

Results and discussion

Tilapia grew over the experimental period by 0.61 ± 0.31 g (95%-CI) $p < 0.001$ per day, with no observed difference between control and treatment groups $p = 0.756$, illustrating no toxic or beneficial effects of the antibiotic treatment on growth (Fig. 1A). Throughout the experimental period, concentrations of OTC in water of control ponds remained low at 0.045 ± 0.018 µg/L (95%-CI) (Fig 1B). Two days after the completion of exposure, OTC concentration reached 9.6 ± 2.11 µg/L (95%-CI) in dosed pond water, and the overall concentration of OTC in treatment ponds was significantly elevated compared to control ponds $p < 0.001$. At the final sampling point, 23 days post-exposure, the concentration of oxytetracycline in treatment ponds had returned to pre-exposure levels.

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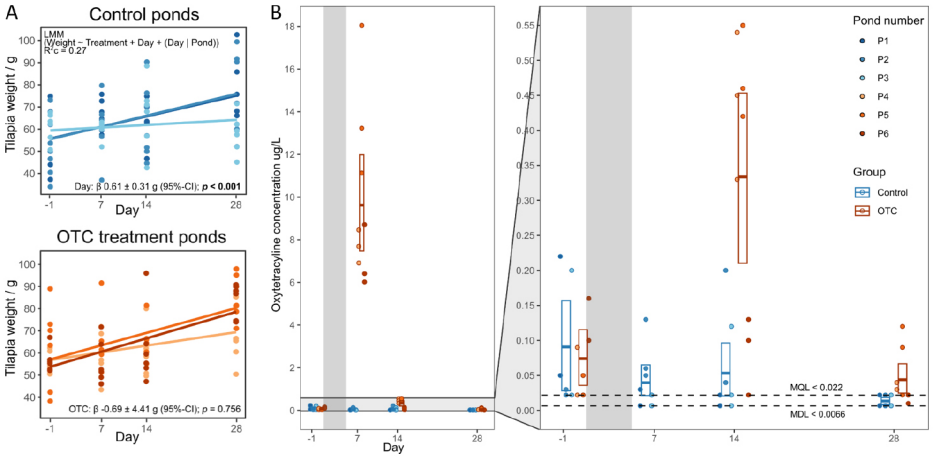

Figure 1. A) Tilapia growth during the experimental period assessed by a linear mixed effects model, with random intercept and slope for replica ponds. B) Oxytetracycline concentration throughout the experimental period. Boxes show estimated population mean and 95% confidence intervals. Dashed lines indicate Method Quantification Limit 0.022 µg/L and Method Detection Limit 0.0066 µg/L.

Ultra-deep sequencing of 16S V4 amplicons for 384 samples of fish skin and pond water yielded a median sequencing depth of 168,440 paired-end reads per sample. Additionally, 104 samples of pond water were sequenced as 18S V9 amplicons, resulting in a median sequencing depth of 56,184 paired-end reads. We will report the impact of antibiotic exposure on fish skin microbiome community structure and will correlate specific taxonomic shifts to measured oxytetracycline concentrations. In parallel, to assess the development of antimicrobial resistance following therapeutic use of antibiotics in aquaculture we performed shotgun metagenomic sequencing on 12 pond water metagenomes of control and treatment ponds. Sequencing generated a total of 750 million paired-end reads. Ongoing analysis is exploring the diversity and abundance of antimicrobial resistance genes, in addition to mobile genetic elements selected in the resistome, that will be reported upon at the meeting.

By characterizing animal and environmental health, as well as the development and spread of antimicrobial disease resistance, these analyses aim to provide a comprehensive understanding of the implications for antibiotic (mis)use in aquaculture within the one health framework.

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EFFECTS OF DIFFERENT PHOTOPERIOD SALINITY AND SMOLT SIZE ON SMOLT PHYSIOLOGY IN RAS

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Introduction

The salmon farming industry has invested in larger and more technologically sophisticated RAS facilities over the last decade. Current practices favour constant high temperatures, use of continuous light and increased salinity in RAS to accelerate the growth of Atlantic salmon smolts from 50-80 grams a few years ago to approximately 500-600 grams today. The anadromous salmon goes through parr-smolt transformation (PST), which entails a series of morphological and physiological changes preparing them for seawater. However, industry reports several biological traits, such as increased silvering, elevated gill NKA activity and ability to ion regulate when challenged with SW, associated with PST to occur independent of applying standard photoperiod protocols. This have made it increasingly difficult to accurately time the "smolt window" and evaluate quality of large smolts (>250 gram). Thus, there is a need to better understand the physiological responses of larger smolts produced under intensive conditions in RAS.

Material and method

Here we present the effects of rearing protocols using four different photoperiod regimes on three different sized (150g, 350g, 800g) in freshwater or brackish water (12 ‰). The photoperiodic regimes were: No winter signal (NW), 6 weeks LD 12:12 winter signal given early (EW), 6 weeks LD 12:12 winter signal given late (LW) and 20 weeks LD 12:12 winter signal given late (LLW). We analysed how these protocols affect smolt development, measured as Na^+; K^+ -ATPase (NKA) enzyme activity in the three major osmoregulatory organs, gills, intestine, and kidney. Smolt development was also evaluated with a short-term seawater performance challenge test.

Results and discussion

Our findings suggest that a more holistic approach to evaluate overall smolt quality in modern aquaculture would be beneficial, in particular when producing large smolts and post-smolts. Our findings were also analysed in the context of long-term seawater performance, health and welfare.

EFFECT OF DIFFERENT TRANSFER TEMPERATURE SHIFT ON THE STRESS RESPONSE, NEURAL PLASTICITY AND ROBUSTNESS OF THE ATLANTIC SALMON (*Salmo Salar*)

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Introduction

The successful transfer of post-smolts depends on the fish' abilities to mount good stress responses, initiate neural adaptations and maintain cognitive properties (neural plasticity) to sustain resilience during the sensitive SW phase. However, current year-round transfer strategies can expose farmed post-smolt Atlantic salmon (*Salmo salar*) to a broad range of thermal stressors that may challenge these functions, with negative implications regarding fish allostasis, health and welfare.

Material and methods

To investigate the effect of various transfer strategies, SW adapted post-smolts at 10-13ºC were transferred to low (10ºC, 7ºC, and 4ºC) and high temperatures (13ºC, 16ºC, and 18ºC), with thermal differences consistent with off-(winter) and on-(summer) season transfer strategies. Differences in allostatic loads, altered physiological states, and capacities to overcome these thermal challenges were evaluated based on the stress response, neural plasticity and ability to withstand stress: Fish were examined for modulations in primary stress responses (plasma cortisol levels) and telencephalic neural plasticity genes important for cognition (neurod, bdnf, pcna, c-fos, mr, gr1, gr2 and hsd11b2) following the transfer, acclimation and in response to an acute challenge test (ACT) using confinement stress.

Results and discussion

Our findings show that elevated allostatic loads are present at both ends of the thermal profile, however, post-smolts clearly have a higher ability to withstand low temperatures compared to high ones. Accordingly, post-smolts retained robust abilities to maintain brain plasticity and initiate key neural adaptations against low thermal challenges. In contrast, post-smolt transfer to elevated temperatures adversely inhibited stress responses and neural functions, pointing to allostatic overload and poorer capacities to deal with increasing thermal challenges. In all, these findings demonstrate thermal differences in the production environment is associated with stresses that can alter key neural processes important for good stress management, abilities still conserved at low temperatures, but not at higher ones. This conforms to the natural life-history strategies of Atlantic salmon and highlights the importance of satisfying low thermal requirements of post-smolts during production, but also points to the potential future challenges of climate change.

EFFECTS OF MICROALGAE SUPPLEMENTATION IN THE DIET OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Microalgae are a large of diverse group of microorganisms producing a variety of valuable compounds: They are the richest source of polyunsaturated fatty acids in the environment and can harbour a wide array of other compounds with beneficial health properties, including antioxidant; anti-inflammatory, antimicrobial, or anti-virulence/quorum quenching. Despite these advantages, little is known about the application of microalgae as feed adjuvant in the diet of farmed fish.

Material and methods

To investigate the potential benefits of dietary microalgae, young rainbow trout (*Oncorhynchus mykiss*), of approximately 30 g; 15 cm length were allocated in between 6 feeding groups (fishmeal diet; diet enriched in soy protein; diet enriched with *Chlorella sorokiniana*; diet enriched with *Tetradesmus obliquus*; diet enriched with intact *Haematococcus pluvialis*; and diet enriched with leftover *H. pluvialis* following extraction of the astaxanthin). Each diet was represented in triplicates 3x15 fish per treatment. The fish were maintained that way for ten weeks. Afterwards, sera were harvested for metabolite analysis and the composition of the intestinal microbiota was analysed by amplicon sequencing using primers for the V1-V3 region of the 16S rRNA.

Results and discussion

No significant effect was found on the growth of the fish. However, the diets were associated in changes in the concentration of serum's metabolite and in the composition of the fish' intestinal microbiota. These results suggest that incorporation of microalgae can have potential health benefits as dietary supplement in the diet of farmed fish.

AQUAE STRENGTH, AN INTERNATIONAL COOPERATION PROJECT FOR PROMOTING SUSTAINABLE AQUACULTURE

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As the global population grows, the increasing food demand has boosted the search for sustainable and innovative food sources. Several countries worldwide rely on aquatic environments as a source of livelihood. Within this context, aquaculture is one of the fastest-growing livestock productions and holds a huge potential to meet the food demand. The AQUAE STRENGTH project, "Strengthening Capacity on Aquatic Animal Health and Epidemiological Surveillance," is financed by the Italian Ministry of Health and supported by the World Organisation for Animal Health (WOAH). Aligned with WOAH priorities for the Aquatic Animal Health Strategy, the project seeks to elevate epidemiological surveillance and responses to aquatic animal diseases while advocating for sustainable practices such as the responsible use of veterinary medical products in aquaculture. Based on a multifaceted approach, it strives to foster capacity building and knowledge sharing among professionals, fortify the management of aquaculture production, and implement advanced Geographic Information System (GIS) techniques. Additionally, it aims to provide and refine diagnostic methods, thus strengthening mechanisms for detecting and controlling aquatic animal diseases. The project is implemented by a consortium that comprises seven Italian Governmental Institutions (Istituto Zooprofilattico Sperimentale), four beneficiary countries (Morocco, Tunisia, Israel, and Cambodia), and three advisory technical bodies from Norway, Denmark, and the UK. One of the primary tools used was the production of online Webinars, which were designed to provide theoretical insight into the project topics and level the background knowledge of the participants. To evaluate the state-of-the-art and identify areas that required intervention, the project employed assessment questionnaires addressed to beneficiaries. These questionnaires were customized and catered to the project's topics and allowed a comprehensive understanding of existing conditions, thereby facilitating the interventions. The project highly values collaborative discussions and the engagement of each involved partner therefore several meetings and round tables were organized to foster collaboration. These events helped to outline project activities, aligned efforts and provided insights into the needs of beneficiaries. Regular field visits to the beneficiary countries were planned during the project as valuable opportunities to interact in person. Beyond facilitating questionnaire reviews and infrastructure evaluations, these visits created a space for interactions and allowed the project staff to better understand beneficiary needs. Furthermore, the project provides tailor-made training sessions to beneficiaries within consortium institutions and laboratories. While aquatic ecosystems substantially sustain populations, their swift degradation due to human activities mandates immediate action. The comprehensive approach of the AQUAE STRENGTH project tackles this challenge, aligning with responsible and sustainable practices. Empowering nations to advance their aquaculture and fishery sectors through optimal management of aquatic resources is essential in fortifying food security and promoting socioeconomic progress.

CANECO-EFFICIENT,CIRCULARECONOMY-DRIVENANDORGANICFORMULATIONS IMPROVE THE PERFORMANCE AND ROBUSTNESS OF NILE TILAPIA?

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Introduction

Aquaculture is facing challenges in terms of environmental sustainability of the feeds used and susceptibility of the farmed organisms to diseases. Fish meal and fish oil are still commonly used in farmed species' nutrition because of their nutritional value. Although most of these ingredients in the European Union are derived from well-regulated wild fish stocks and are certified, they are at the limit of sustainable exploitation. Moreover, particularly in some developing countries (e.g., China) with high levels of production, the use of these ingredients makes aquaculture practices associated with a high carbon emission. Given the high density in intensive farming systems, fish can be exposed to stress, which may have negative impacts on their immune system. Consequently, this effect will potentially deteriorate fish health and well-being, increasing the possibility of infection with pathogenic invaders, eventually leading to mortality. Functional ingredients possess bioactive compounds that regulate physiological processes and exhibit biological activities, which could improve weight gain, feed efficiency, and disease resistance in fish while reducing the environmental impacts of the sector. Accordingly, through the inclusion of novel functional ingredients in diets, such as the microalgae spirulina and the plant quinoa, may offer a potential solution to mitigate these issues.

This study aims to assess the effects of functional feeds on key growth performance indicators (e.g., weight gain, feed conversion ratio, feed intake), health (e.g., gut epithelium integrity, immune condition and oxidative status) of juvenile Nile tilapia (*Oreochromis niloticus*). Feeds were formulated with eco-efficient, circular economy-driven and organic frameworks.

Methods

Three experimental diets were formulated and produced by SPAROS Lda (Olhão, Portugal). A practical (PD) diet as control mimicking a commercial-type feed, without fish meal and fish oil. Organic (ORG) based on ingredients compatible with organic certification (including spirulina and quinoa). Eco-efficient (ALT) using circular economy-driven subproducts (e.g., poultry and feather meal) and emergent (e.g., spirulina, insect meal, quinoa) ingredients. Spirulina had inclusion levels of 10% and 2.5% and quinoa 5% and 2.5%, in ORG and ALT, respectively. All diets were formulated to be isonitrogenous (crude protein 39.4% wet weight (WW))and isoenergetic (gross energy, 19.2 KJ/g WW). The trial was performed in 12 tanks of 300 L (3 diets x 4 replicates) with an average temperature of 24.4 ± 1.2 ◦C (mean ± SD). Each replicate tank contained 30 Nile tilapia with an initial mean weight of 31.0 ± 0.5 g. Fish were daily fed with the experimental diets until visual satiation for 61 days. Growth performance indicators were assessed, including weight gain, relative growth rate, feed conversion ratio (FCR) and voluntary feed intake (VFI). Fish were sampled for whole-body composition analysis and dissected for liver, anterior and posterior intestine for assessment of immune condition, oxidative status and gut health. Diet apparent digestibility was also determined. Statistical analyses were performed using SPSS version 23.0 by one-way ANOVA, followed by Tukey's post-hoc.

Results

Although fish were fed until satiation, the VFI and FCR significantly differed across diets (Fig. 1 and 2; $p<0.05$). Both indicators were within normal values for PD and ALT, but not for ORG, showing that fish exhibited a negative response, which was reflected in the performance indicators.

Fish that were fed diet PD exhibited higher weight growth (3.5 – fold increase), ALT roughly doubled their initial body weight, while ORG almost did not show any growth (Fig. 3). Similarly, the final body weight of PD was also 1.7-fold and 3.3-fold higher than diet ALT and ORG ($p<0.05$), respectively.

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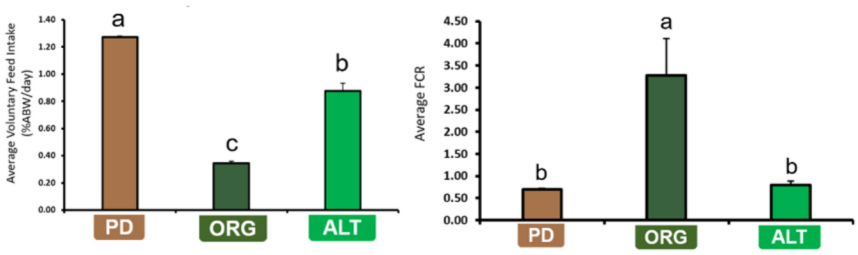

Figures 1 and 2 – Average Voluntary Feed Intake (VFI; %Average Body Weight/day) and Feed Conversion Ratio (FCR) of fish exposed to three different dietary treatments: i) Practical (PD), ii) Organic (ORG), iii) Eco-efficient (ALT), over 61 days. Bars represent the mean counts ± SD (n=4 tanks per treatment, except for FCR of diet ORG where n=3). Different letters (a, b and c) indicate significant differences (one-way ANOVA; $p<0.05$).

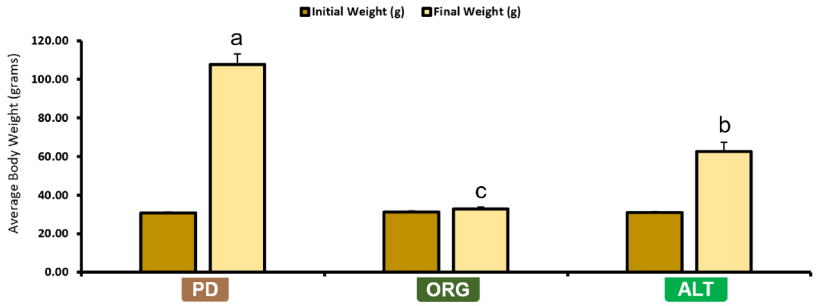

Figure 3 – Average initial and final body weight (grams) of fish exposed to three different dietary treatments: i) Practical (PD), ii) Organic (ORG), iii) Eco-efficient (ALT), over 61 days. Bars represent the mean counts ± SD (n=4 tanks per treatment). Different letters (a, b and c) indicate significant differences (one-way ANOVA; $p<0.05$).

Conclusions

The three aquafeed formulations demonstrated significantly distinct impacts on juvenile Nile tilapia performance. Diets ALT and specifically diet ORG provided worse growth performance indicators than PD. The evaluation of the impacts of the diets on fish health (e.g., gut epithelium integrity, immune condition and oxidative status) is underway. In any case, this study reinforces the notion that novel formulations and ingredients need to be thoroughly evaluated.

Acknowledgements

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BEYOND NUTRITIONAL VALUE: MYCOPROTEIN *Paecilomyces variotii* IMPROVES GROWTH PERFORMANCE AND OVERALL HEALTH RESPONSES IN ATLANTIC SALMON

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Introduction

Farmed salmon will play a significant role in bridging the demand gap for high-quality food. However, salmon farming is faced with several challenges including access to high quality feed resources, and multi-stressor conditions such as suboptimal environmental conditions and diseases leading to mortalities and economic losses. Therefore, the need for sustainable novel feed resources to replace the conventional feed resources currently used in salmon feeds has never been more urgent. Novel aquafeed ingredients should be sustainable and address both nutritional and health related challenges in salmon farming. Microbial ingredients (MIs) such as yeast and fungi provide a viable alternative to address both challenges due to their high nutritional value [1] and bioactive components with proven health benefits [2]. *Paecilomyces variotii* (PEKILO®) is a filamentous fungus with high crude protein content (60-70%) and it contains bioactive components such as β-glucans, mannans and nucleic acids which induce health benefits for fish. Considering this, our study under the NordForsk-funded NordicFeed project was designed to characterize the immunomodulating effects of *P. variotii* in Atlantic salmon (*Salmo salar*) using *in vitro* and *in vivo* techniques. We hypothesized that the functional bioactive components in *P. variotii* will improve overall health and welfare in Atlantic salmon.

Materials and methods

The immunomodulatory effect of *P. variotii* fermented on sulphite stillage as substrate was characterized using adherent mononuclear leucocytes isolated from head kidney (HK) and spleen of Atlantic salmon (*ca*. 2 kg) and incubated for 6, 24 and 48 hours with or without the presence of heat inactivated *Moritella viscosa* (Figure 1, left panel). For the *in vivo* assessment, vaccinated Atlantic salmon pre-smolts were fed diets with *P. variotii* replacing 0, 5, 10, or 20% of crude protein from fishmeal and soy protein concentrates for 4 weeks in freshwater. Growth and health parameters were analyzed (Figure 1, right panel).

Results

Results from the *in vitro* studies (Figure 1, left panel) indicated that *P. variotii* induces a strong immune response in the HK of Atlantic salmon at all time-points evaluated, but most strongly after 6 h. Co-stimulation of the spleen and HK leucocytes with both *P. variotii* and *M. viscosa* induces an even stronger immune response, particularly in the HK. Upregulation of regulatory cytokine in the HK and downregulation in the spleen was observed when the bacteria alone or a combination with *P. variotii* was added to cell cultures. In addition, *P. variotii* enhanced the antimicrobial activity of leucocytes with higher transcript levels of β-glucan receptors, demonstrating recognition of this bioactive compound in the feed ingredient. Our results from the feeding trial (Figure 1, right panel) indicates that all inclusion levels of *P. variotii* resulted in feed intake, specific growth rate like the control diet and a dose-dependent linear decrease in the feed conversion ratio with increasing inclusion of the mycoprotein. The immune responses induced by *P. variotii* were systemic. Low and high doses of *P. variotii* modulated a strong T cell response, innate responses, and enhanced antimicrobial activity. Medium and high dosage also modulated a Th1 response as well as receptors and signaling molecules characteristic of β-glucan recognition (Figure 1). Further, inclusion of *P. variotii* in diets for Atlantic salmon increased specific antibody titres against *V. anguillarum*.

Conclusion

P. variotii can replace up to 20% of crude protein in the diet of Atlantic salmon without compromising growth performance of the fish. In addition, *P. variotii* has significant health benefits in Atlantic salmon beyond its nutritional value including enhancing the natural killer cells, the complement system, antimicrobial activity of leucocytes, modulated type 1 and type 2 responses and improved humoral responses.

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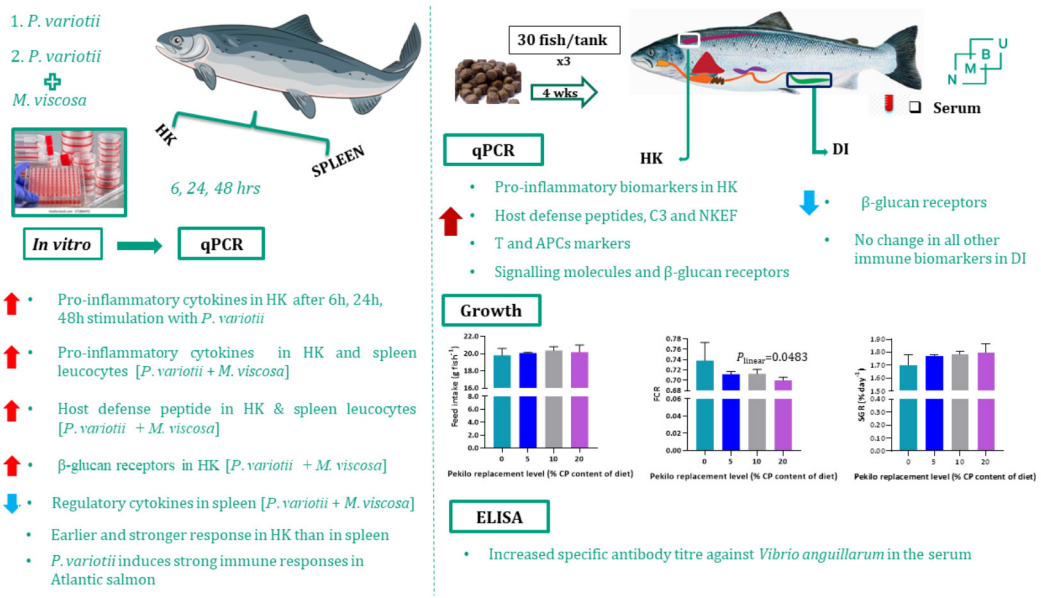

Figure 1. *In vitro* and *in vivo* health modulating effects of *P. variotii* in Atlantic salmon.

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MICROBIAL STRUCTURE OF *Sparus aurata* FED INNOVATIVE DIETS

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Introduction

Innovative ingredients in fish diets are used to improve fish growth performance indicators and the ecological footprint of aquaculture activities with the benefits of improving fish health. Thus, the replacement of fishmeal and fish oil in fish diets with novel ingredients that can be sustainable produced is of much interest. It is widely accepted that under experimental rearing or aquaculture farming conditions, the fish gut microbiome can be manipulated by changes in the fish diet. Gut microbiota may play significant role in fish nutrition, producing enzymes (Ray et al. 2012) that promote host growth and health. However, the extent to which microbial function varies with host demand and the underlying mechanisms are largely unknown. The aim of this study was to investigate the effect of dietary inclusion of insect meal, tunicate meal and algae meal on *Sparus aurata* midgut microbiota.

Materials and methods

The experimental trial took place at the Department of Ichthyology and Aquatic Environment, University of Thessaly, Greece. Briefly, 1080 individuals *S. aurata* (initial mean weight 6.52±0.03g) were distributed randomly to eighteen 250l tanks. Six experimental diets were formulated to be isolipidic. The Control (26.55% fishmeal- FM), the 0%FMFO (total replacement of FM and fish oil with algae meal, *Schizochytrium limacinum* and *Phaeodactylum tricornutum*, insect meal *Hermetia illucens* and tunicate meal *Ciona intestinalis*), the IM (68.09% replacement of FM with insect meal *H. illucens*), the TM (45.91% replacement of FM with tunicate meal *C. intestinalis*), the PA (18.41% replacement of FM with *P. tricornutum*), and HA, (100% replacement of FO with *S. limacinum*) diet. Each diet was assigned to triplicate groups of 60 fish per group. The trial lasted 45 days, after 15 days of acclimatization. The fish fed three times per day *at libitum*. At the end of experiment, fish were starved for 24 hours and aseptically the midgut was dissected. After DNA extraction bacterial diversity was assessed by amplification of the V3-V4 region of the bacterial 16S rDNA gene using IlluminMiSeq with universal bacterial primers. Raw sequence reads were processed and analyzed, using the MOTHUR software (v. 1.45.3) and SILVA database was used to classify the operational taxonomic units (OTUs).

Results

Several bacterial Families were identified with different distribution in the midgut of fish fed with the different diets (Figure 1). The most abundant Family was LWQ8 in fish fed the control and IM dietary treatment and Burkholderiales was in fish fed the 0%FMFO dietary treatment. In the midgut of fish fed the TM and HA diets Rhodobacteraceae had an increased relative abundance and in midgut of those fed PA diet Bacillaceae was most abundant.

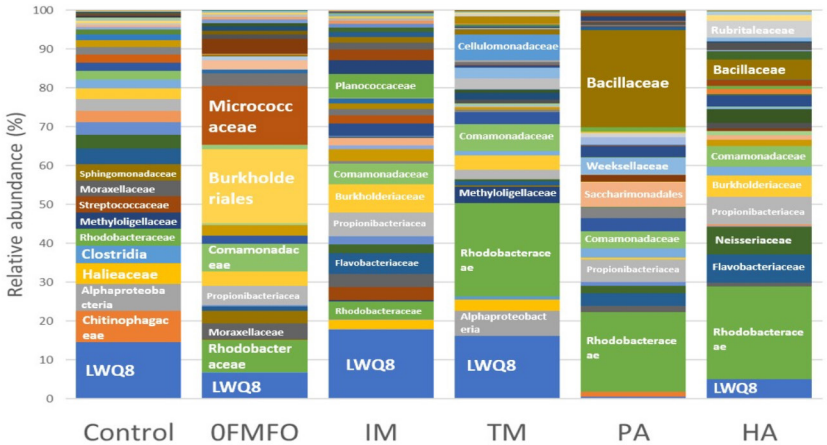

Figure 1: Relative abundance of bacterial Families in *S. aurata* midgut of different experimental dietary treatments.

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Discussion

Fish fed the TM diet had increased their bacterial Taxa in their midgut, an indicator of fish health, implying a normal gut physiology. Saccharimonadales, (LWQ8 bacteria) were found in all dietary treatments. It is associated with the degradation of sugars and glucose (Albertsen et al. 2013). Increased abundance of Burkholderiales in fish fed the 0%FMFO diet was related to immune and stress response (Sehnal et al. 2021). Rhodobacteraceae were a common family among the dietary treatments and were found to assist in the fermentation of complex polysaccharides (Gupta et al. 2019). Bacillaceae produce amylase, cellulase, phytase, protease, lipase, and chitinase (Askarian et al. 2012), enzymes that could contribute to better fish growth, performance. Bacterial Families that were beneficial to the host were found in the midgut of fish fed the six experimental diets associated with better growth performance.

Acknowledgement

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TRANSFORMATION SCENARIOS FOR BOOSTING ORGANIC FARMING AND AQUACULTURE TOWARDS THE FARM-TO-FORK TARGETS

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Introduction

With the European Green Deal, the Farm-to-Fork Strategy and the Biodiversity Strategy, the EU has set targets of reaching at least 25% of the EU's agricultural land under organic farming by 2030 and significantly increasing organic aquaculture. In the EU, the total organic aquaculture production is only accounting for 6.4% of the total production (EUMOFA, 2022). There is no concrete Farm-to-Fork target for organic aquaculture, but a three times growth rate as for farmland to a 5% share would require enormous efforts. Reaching these ambitious goals demands a balanced upscaling of both production and consumption, implying a huge transformation in farm structures and value chains. This transformation needs to be supported by research and innovation, strong advisory services, knowledge exchange and training opportunities for all organic operators and related professionals. The overall objective of OrganicTargets4EU (https://www.organictargets.eu/) Horizon Europe, research project is to support the achievement of the organic farm-to-Fork targets.

Materials and methods

OrganicsTarget4EU combines EU-level assessments for all Member States, with detailed analyses in selected focus countries: seven for agriculture (DE, HU; DK, IT, RO, AT, FR) and two for aquaculture (DE, GR covering freshwater and marine species respectively). While market data on organic aquaculture are not available, the share of organic aquaculture production was 21% and 1% (tonnes live weight) in 2018, in Germany and Greece respectively (https://ec.europa.eu/eurostat/databrowser/view/org_aqtspec/default/table?lang=en).

The project will identify the current and past developments of the organic sector by examining the institutional framework for organic farming and aquaculture and conduct a foresight based on scenario analysis. Participants will be recruited through interviews and a survey. These include knowledge providers, organic farmer organisations, organic aquaculture sector, certification bodies and policy makers. Workshops will be held aiming to: i) foster mutual learning processes and initiate transformation of existing advisory services; ii) stimulate the initiation of viable new conversion/organic advisory services in countries where they are not yet available, iii) explore possible funding mechanisms or public-private partnerships to develop or scale up advisory services, iv) foster relationships for a viable professional network beyond the project lifetime.

A recent review has shown that whilst availability of subsidies, environmental concerns, profitability, and uncertainties about the stability of the organic market are recurring as influential factors in the decision process of becoming organic, the most recurring factor is supportive social networks. The project will initiate Communities of Practice in which conventional farmers and aquaculture producers learn together what is involved in organic production and in conversion. Communities of practice are social learning groups of people, who share a concern and who deepen their expertise in this area by interacting on an ongoing basis (https://hbswk.hbs.edu/archive/cultivating-communities-of-practice-a-guide-to-managing-knowledge-seven-principles-for-cultivating-communities-of-practice).

The Community of Practice in Greece for aquaculture has been established. Group members in Greece for organic aquaculture are 5 conventional fishfarmers that farm sea bream, sea bass, rainbow trout and mussels.

Results/Discussion

OrganicTargets4EU supports the Farm-to-Fork Strategy in achieving the targets of at least 25% of the EU's agricultural land under organic farming and significantly increasing organic aquaculture by 2030. To achieve these ambitious goals, 18 partners coordinated by IFOAM Organics Europe will set up a multi-actor process to create possible scenarios for reaching these targets and provide in-depth knowledge of the key drivers and lock-ins affecting the organic sector's development. The Farm-to-Fork Strategy fully acknowledges the role of aquaculture in creating more sustainable food systems. Therefore,

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it aims at a significant increase of organic aquaculture. The project will produce results that will support the growth of both marine and freshwater organic aquaculture. While organic farming has passed the niche phase, organic aquaculture is still in very early stages of development. Barriers exists at the technical, market and regulatory level. The project will address these for organic aquaculture. By helping to achieve the organic Farm to Fork targets, the project supports sustainable aquaculture systems, for marine and freshwater conditions, that are climate-resilient, environmentally-friendly and combine low impact on aquatic ecosystems with high animal welfare.

Preliminary results from the Community of Practise group in Greece for organic aquaculture have showed that the main obstacles for expansion of the organic aquaculture are the high cost of the organic feed production, the slower fish growth rates on the on-growing phase, the high cost of the certification and the luck of specialised advisory government service for organic aquaculture.

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FISH WASTE FROM AQUAPONICS TO PRODUCE NUTRIENT-RICH INSECT MEAL

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Introduction

Freshwater aquaculture contributes to 20% of the aquaculture production in EU, producing ca. 275'000 tonnes fish per year with a first sale value of 910 M€. Productivity in this sector must grow to contribute to the global demand for protein, expected to more than double by 2050 to 500 million tonnes (FAO, 2021). The sector employs 20626 people in 7582 companies (EUMOFA, 2021). The main production methods are extensive fishponds systems (33%) and intensive tank and raceways (65%). A small percentage (2%) of intensive systems are Recirculating Aquaculture Systems (RAS). Aquaponics is an integrated multitrophic aquaculture system involving farming fish and growing plants together. It requires no soil and uses water from fish production to support plant growth, which leads to a dramatic improvement in sustainability and efficiency of aquaculture practices. The practice of aquaponics in Europe is limited, with less than 50 commercial aquaponic enterprises (Turnsek et al., 2020). The aim of this study which is part of the AWARE (Horizon Europe, https://www.aware-eu.eu/about/) research project is to increase the circularity of fish waste and create fish species-specific diets for aquaponics.

Materials and methods

Fish waste produced in aquaponics will be used as substrate to produce nutrient-rich insect meal. The fish waste composition will be evaluated, particularly its fatty acid and amino acid profile, and investigation will take place on how it affects the nutritional value of the insects grown on it and the insect meal that will be produced in terms of crude protein, ash, moisture, fatty acids, amino acids, vitamin D content and minerals. The insects will then be used to produce insect meal, which will in turn be fed to the fish grown in aquaponics. New aquaponic fish species-specific diets will be formulated and tested in aquaponics systems to produce fish and vegetable for human consumption. The AWARE project will create a new farm-to-fork value chain and demonstrate technical solutions for efficient and sustainable aquaponics.

Results/Discussion

Aquaponics generally involves Recirculating Aquaculture Systems (RAS) recirculating more than 90% of the water. The fish production and hydroponics can be linked, or decoupled, with periodic water transfer between the two. Aquaponic systems benefit from high levels of production with extremely low land and water resource requirements, along with relative biological security and excellent direct access to urban markets. Aquaponic systems are currently limited by high capital cost, high energy requirements, and limitations in customer willingness to pay for ecosystem services. RAS and multitrophic aquaponics have been identified by The Strategic Working Group on Fisheries and Aquaculture of the Standing Committee on Agricultural Research (SCAR-Fish) as two of the issues that urgently need to be explored by research and addressed by innovation.

Aquaponic farming requires knowledge in geochemistry (nutrient cycling and other physico-chemical parameters), veterinary (animal welfare), fishery, horticulture and physiology (fish and plants farming), microbiology and biotechnology (study of the microbiome, pathogens and viruses). The AWARE project will innovate aquaculture practices in Europe by improving and increasing the uptake of RAS multitrophic aquaponics. This will be achieved by a) producing fish species-specific diets from fish waste, b) integrating aquaponics with wastewater treatment, using reclaimed wastewater as a water source; c) advancing the technology to improve resource efficiency; d) creating a regulatory and policy roadmap to facilitate its scale-up and market uptake. AWARE sets out to increase capacity to produce fish for human consumption at zero km in every European city, with no impact on natural habitats, no dependence from natural freshwater availability, and high resiliency to climate change. This goal could be met by farming fish locally using reclaimed water. Europe lacks the regulatory and policy framework to allow such a value chain to take root, due also to the absence of compelling scientific evidence of safety, quality, economic feasibility, and social acceptability.

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EFFECTIVENESS OF EXOGENOUS ENZYMES AND MICROORGANISMS IN LUPIN MEAL: *IN VITRO* EVALUATION

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Introduction

Forage legumes are considered to be promising candidates to fulfil the gap of the rapid growth in aquafeed production. In particular, lupin seed, with its diversity of species, high protein content and ability to be grown in different soil and climate types, can even be considered as a potential competitor to soya bean. Although lupin seeds contain moderately high levels of protein, calories, certain minerals and vitamins, their use in food and feed is still limited by the presence of several antinutritional factors that reduce enzyme activity and the absorption of minerals and other nutrients (Krogdahl et al., 2001). In animal nutrition, the digestibility and absorption of nutrients can be improved through biotechnological processes such as solid-state fermentation (SSF). The aim of this study was to improve the nutritional value of lupin meal (*Lupinus albus*) by using SSF with different microorganisms, conditions of humidity and temperature and by comparing it with a different biotechnological process such as the addition of exogenous enzymes.

Materials and methods

The whole seeds of *Lupinus albus* have been ground to produce lupin meal and have been analyzed for the level of phytic acid and free phosphorus. Then lupin meal was treated under different processes (moisture, temperature, time and pH) and enzyme combinations. Briefly, four commercial enzyme products were assessed i.e. xylanase, phytase and two multienzymes. Two different moisture levels (8 and 45%) and thermal treatments (25 and 50°C) were evaluated. Process duration was either 25 or 240 minutes and in cases pH was reduced citric acid was added. Three microorganisms were selected for SSF: *Aspergillus niger*, *Saccharomyces cerevisiae* and *Bacillus subtilis*. Each micro-organism was cultured on appropriate agar for optimal growth and inoculated on lupin meal under optimum humidity and temperature conditions for the number of days required to achieve optimal growth. Lupin meal products from both biotechnological processes were evaluated for levels of phytic acid and free phosphorus. Analysis was determined using Megazyme kits.

Results and discussion

Phytic acid hinders the activity of enzymes, which is necessary for protein degradation in the intestine and stomach (Kies et al., 2006). Phytic acid normally constitutes 0.2%–2% of the dry weight in cereals and legumes with 60%–90% of the total phosphorus present bound to phytate (Feizollahi et al., 2021). Biotechnological processes increase digestibility of nutrients and bioavailability. Additionally, cooking of pulses is a commonly used process that highly improved the nutritional value of foods by reducing their ANFs (Patterson et al. 2017). The results showed positive effects of microorganism SSF and exogenous enzyme treatment in the reduction of phytic acid and increase of free phosphorus. Lupin meal in combination with multienzyme product, high moisture content (45%) and higher thermal treatment (50°C) resulted in lower phytic acid content (0.23%) and increased free phosphorus levels from 0.08 to 0.34%. Fermentation by *Aspergillus niger* showed the greatest reduction in phytic acid content and increased free phosphorus levels compared to the other microorganisms. However, treatment with exogenous enzymes showed higher efficacy in phytic acid and free phosphorus in relation to fermentation with microorganisms.

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Table 1: Results of phytic acid and free phosphorus in lupin meal after biotechnological processes.

Treatment	T (°C)	Moisture (%)	pH	time	Phytic acid (%)	Free P (%)
Lupin meal Untreated	-	-	-	-	1.314	0.075
Aspergillus niger	37	50	5.5	7 days	0.427	0.326
				10 days	0.449	0.316
		60		7 days	0.376	0.331
				10 days	0.412	0.329
Saccharomyces cerevisiae	30	50	4	1 day	0.728	0.084
			7		0.802	0.089
			4	2 days	0.782	0.083
			7		0.711	0.085
		70	4	1 day	0.738	0.089
			7		0.773	0.086
			4	2 days	0.692	0.096
			7		0.707	0.091
Bacillus subtilis	40	40	4	1 day	0.651	0.087
			6		0.722	0.117
			4	2 days	0.670	0.119
			6		0.712	0.115
		60	4	1 day	0.698	0.118
			6		0.701	0.110
			4	2 days	0.657	0.125
			6		0.581	0.103
Multienzyme	25	8	5.5	30 min	0.110	1.180
Xyl-prot-amyl					0.100	1.240
Phyt-xyl					0.190	0.960
Multienzyme	50	45		240 min	0.340	0.230
Xyl-prot-amyl					0.200	0.740
Phyt-xyl					0.280	0.540

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COMPARATIVE GROWTH, PERFORMANCE AND SURVIVAL OF THE EUROPEAN FLAT OYSTER *Ostrea edulis* IN INTERTIDAL AND SUBTIDAL CULTIVATION

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The European flat oyster *Ostrea edulis* has been harvested for centuries but production continues to be significantly affected by stress and diseases impacting oyster growth and survival. Whilst production remains active in some areas, supplying a high-value product to a niche market, cultivation practices continue to be adapted to meet the demand. These include exploring sites further away from the shore as well as the potential for co-cultivation with other species groups such as shellfish and seaweeds.

This study explores the feasibility of growing oysters subtidally at an existing seaweed cultivation site in comparison to a traditional intertidal oyster monoculture. Juvenile *O. edulis* were deployed in baskets at either site and monitored for two years (Nov 2020 – Oct 2022) for their progression in growth, survival, and marketability (meat yield and condition index), respectively. Ultimately, the shelf life of the harvest-size oysters was determined, and sites tested for the presence/absence of the diseases-causing *Bonamia ostreae* using a qPCR approach.

Oyster growth was rapid in the first year of culture and progressively slowed, following different seasonal dynamics and average growth in shell height of 1.09 and 1.55mm mth^{1} in intertidal and subtidal culture, respectively. Meat yield and condition index varied throughout the study period and with season, being highest in early Summer and lowest in late Winter, and coinciding with the seasonality in oyster reproduction and environmental conditions (water temperature, food availability).

Most notably however, oyster survival differed dramatically across sites and seasons with re-occurring mass mortalities during hotter summer months at the intertidal but not the subtidal site. With mortality rates exceeding 70% compared to 1% in subtidal culture, and without confirmed presence of *B. ostreae* at either site, *O. edulis* appears to perform better in subtidal (i.e., suspended) culture.

This study provides long-term multi-parameter data on the conditions and performance of *O. edulis* in commercial culture. Our findings highlight that suspended culture can improve overall performance and most importantly survival, providing new opportunities for exploration of mono- as well as co-cultivation operations offshore.

EFFECTS OF TIRE PARTICLES AND ASSOCIATED-CHEMICALS ON THE PACIFIC OYSTER *Crassostrea gigas* PHYSIOLOGY, REPRODUCTION AND NEXT-GENERATION

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Introduction

The mass of tire particles in the ocean is expected to represent 93% of the aquatic plastic contamination by 2040. This might have consequences on organisms, like physical injuries and health repercussions notably due to chemical risk following the leaching of tire-associated chemicals. In such context, it is pivotal to assess the risk posed by this stressor in marine living organisms and aquaculture activities in the context of long-term chronic exposure scenarios. In the present study, a multidisciplinary and multigenerational approach was implemented in the Pacific oyster *Crassostrea gigas,* a species having a high economic value and an important ecological role in the ecosystem functioning. To assess the effects of particles and leachates, several endpoints reflecting different organization levels were investigated.

Material and Methods

The effects of tire particles, tire leachates and natural particles were investigated at individual scale (generation G0) over a 6 weeks exposure in a flow-through system using endpoints reflecting different organization levels (e.g. transcriptomic analyses, gut microbiota, tissue alterations, feeding activity, growth, reproductive outputs) up to offspring performance (e.g. development, fertilization rate, larval growth). Secondly, the G1 offspring performance (development, survival and growth of larvae and juveniles) was monitored over one year up to their ability to reproduce (G2 larval progenies), providing unprecedented views about the potential long-term risks from exposure to tire particles at environmentally relevant concentrations for marine organisms.

Results

No significant differences in ecophysiological parameters and haemocytes features were observed between exposed parental conditions whereas molecular analyses revealed the disruption of energy metabolism and stress response following leachates exposure. Oocytes had the highest number of differentially expressed genes; among these, many were associated with endocrine disruption and demonstrated that oocytes are mostly targeted in case of prolonged exposures of the broodstock during gametogenesis. Microbiota analysis revealed the over-representation of *Tenacibaculum* spp., a gram-negative and motile bacterial genus often associated with mortality events of marine animals, which suggested the onset of dysbiosis following exposure to high concentration of both tire particles and leachates. Parental exposure had an impact on gamete quality with a 22% reduction of motile spermatozoa in the leachate conditions but had no consequences on the fertilization success nor lead to long-term effects on offspring growth and reproductive outputs. Considering the concept of energy-limited tolerance to stress, it would be of great interest to test tire risk in the context of harsher natural conditions (e.g. food limitation, water quality).

Overall, while our results bring a positive note on the apparent resilience of Pacific oysters to tire particles exposure, caution should be taken when extrapolating these results to other rubber materials or to natural – harsher – conditions occurring in coastal ecosystems, bearing in mind that the first answer to plastic pollution is to reduce its production and usages whenever possible and favor durable materials.

MEDITERRANEAN AQUACULTURE: FUTURE DEVELOPMENTS MUST ADDRESS FARMED FISH WELFARE

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Introduction

Europe is one of the largest seafood markets and the second largest trader of seafood products. Regional consumption keeps increasing (estimated 24 kg per capita in 2019), with almost 60% of EU seafood's total supply covered by imports, making it highly dependent on them, e.g., a noticeable amount of Turkish seafood exports end up in the EU market. Within the Union, aquaculture production mainly concentrates in four top countries (i.e., Spain, France, Greece and Italy) and three finfish species (i.e., European seabass, gilthead seabream and rainbow trout) (EC, 2022; STECF, 2023).

However, despite the relevance of these species, Mediterranean-basin aquaculture is frequently overshadowed by salmon farming, e.g., a large portion of scientific publications within the last decade were dedicated to Atlantic salmon. Therefore, the objectives of this presentation are: (i) to identify knowledge gaps concerning Mediterranean finfish welfare and link them to production levels and investments made by the EU, and (ii) to assess the current scientific knowledge on the welfare and farming practices of top predator species, planned to be intensively farmed on a large scale.

Material and methods

A thorough literature review was conducted on the available aquaculture scientific publications in Spain, France, Greece, Italy, and Turkey. Articles, reports, guides and manuals were analysed to gain knowledge on each aquaculture sector at a national level, the presence and definition of animal welfare in these documents, data related to species-specific good farming practices, and financial support available from the EU.

Results

The European Maritime, Fisheries and Aquaculture Fund (EMFAF) has allocated over €2,000M to fostering aquaculture during 2021-2027. However, welfare receives very little money, e.g., Spain (1.9%), Italy (1.2%), and Greece (0.6%), and the planned measures are mainly related to animal physical health. In the case of France, the funds allocated to animal welfare are unspecified. Also, despite the variety of EU projects working with finfish carnivorous species (e.g., FutureEU Aqua, Impaqt, MedAid, PerformFish, and Seafood Tomorrow), their welfare remains to be adequately addressed if compared with the available scientific knowledge.

Similarly, the reports, guides and manuals analysed follow the same trend (Mendeley, 2023), except for Canada, Ireland, and UK's salmonids guides. Animal welfare is cited without a precise definition, and when defined, it references mainly to physical health (disregarding mental aspects). The lack of species-specific scientific knowledge and guidelines is also evident for those new large-scale intensive-rearing top predator farms (i.e., bluefin tuna, greater amberjack) which are planned in Spain, and expected to appear in other Mediterranean countries.

Discussion and conclusions

Aquaculture is quickly developing and evolving, and it is often portrayed as a sustainable alternative to wild fisheries and a means to achieve food security. However, it remains to be seen how further developments based on rearing current and new carnivorous species can be sustainable. Also, Mediterranean finfish species are usually neglected because most resources are allocated to salmon farming, mainly located in Norway and the UK.

The EU actively promotes aquaculture development and diversification in two opposite directions: a) by farming low-trophic species, integrating multitrophic systems and limiting the use of Fish Meal Fish Oil as fish feed (EC, 2023a, 2023b), and b) by rearing top predator species Mylonas *et al.* (2018). The latter has diverse animal welfare issues, including the lack of scientific knowledge and farming guidelines.

In this context, following a precautionary approach, more fish welfare research is needed before the sector further diversifies. The opening of large-scale intensive-rearing farms should be postponed unless high animal welfare standards are guaranteed, especially regarding all carnivorous species, of which little is known regarding good farming practices and welfare. By doing so, animal welfare would be at the core of aquaculture and in alignment with the EU Strategic Aquaculture Guidelines 2021-2030 (EC, 2021).

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MULTI-OMICS INVESTIGATIONS OF THE ROLE PLAYED BY EMBRYONIC TEMPERATURE IN PROGRAMMING LONG-TERM IMMUNE FUNCTION IN ATLANTIC SALMON

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Atlantic salmon represent a major component of the aquaculture industry with an annual production value of 16.7 billion USD in 2017, the largest for any finfish and the second highest of all marine species (Houston et al, 2020). This industry suffers from a high mortality rate. In Scottish aquaculture, salmon production losses remained stable at around 20% between 1990 and 2014 (Murray and Munro, 2018). However, this has increased in recent years with the latest smolt input to harvest loss rate standing at 25.6% (Munro, 2022). High mortality in farmed salmon production is not specific to UK aquaculture with approximately 20% of Norwegian farmed salmon also failing to reach the end of the production line (Directorate of Fisheries, 2019).

This is the result of low robustness, with viral diseases including cardiomyopathy syndrome and pancreas disease, bacterial diseases including tenacibaculosis, and parasites (especially salmon lice) accounting for much of this loss during the seawater production phase. The increased mortality among salmon is indicative of impaired immunity.

The environmental and production factors contributing to this effect are not properly understood. One potential cause of low robustness and impaired immunity may be attributed to suboptimal temperature during embryogenesis. As poikilothermic organisms, temperature influences essentially all biochemical and physiological processes of fishes like Atlantic salmon. It has been shown in many organisms including Atlantic salmon, that exposure to external stimuli such as temperature during early development may permanently alter metabolism and physiology, even long after the stimuli has been removed (Ziqiang et al., 2019). This phenomenon is known as metabolic programming. Higher embryonic temperature has been shown to accelerate growth of Atlantic salmon (Austreng et al, 1987) resulting in larger sized muscle fibres containing significantly more myofibrillar material. Muscle cell size increase was also found to proceed at a greater pace in higher embryonic temperature groups relative to lower temperature groups (Stickland et al, 1988). Additionally, higher embryonic temperature was linked to decreased promotor methylation in the Atlantic salmon myogenin gene, a transcription factor essential for muscle tissue development, which was in turn correlated with increased myogenin expression (Burgerhout et al, 2017).

However, growth of embryos at higher temperatures has been shown to result in greater rates of mortality and abnormal development (Gunnes, 1978). It is possible that metabolic programming of an embryo towards fast growth may impair energetic allocation into other functions and processes. For example, in coho salmon, fast-growing transgenic fish overexpressing growth hormone showed evidence of decreased immune function compared with wild-type fish (Alzaid et al, 2018). This suggests a fine balance is normally maintained between various physiological systems, which may become disrupted when the organism is pushed too strongly towards growth.

The COOLFISH project, in which my PhD is embedded, seeks to fully understand the effects of such metabolic programming by early rearing temperature on Atlantic salmon development and health throughout the production cycle. Three groups of Atlantic salmon were grown under different temperatures (4, 6 and 8°C) during embryogenesis (solely from fertilization to the eyed stage). After the embryos reached the eyed stage, these groups were reared under a common temperature for the rest of their life (until the parr stage, when a final disease challenge was performed using *Yersinia ruckeri*). Fish were sampled at start feeding, early in development and after the immune challenge.

My PhD aims to understand the functional mechanisms driving temperature differences in immune function, taking a multi-omics approach. At the conference single nucleus sequencing data of livers taken at start feeding sampling will be presented. Analysis is currently at an early stage but will be completed before September. Preliminary results show hundreds of differentially expressed genes found in hepatocytes and immune cell clusters between the temperature groups. This will be paired with freshly generated proteomics data (n=9 per temp group) from matched samples confirming the link between transcription levels and the actual abundance of proteins, strengthening the link between the cell-specific transcriptome and phenotype.

COCULTURE OF GRAZERS AND OYSTERS IN THE UNITED STATES: A LOCAL NATURE-BASED SOLUTION ADDRESSING FOOD SECURITY

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Introduction

Aquaculture can potentially adhere to the standards of the Nature-based Solutions (NbS) framework and contribute to the scaling up of NbS. Thus, this industry may take advantage of ideas that are both environmentally friendly and provide the best social-economic outcomes. The farming industry has recently been in search of added sustainable practices and new possible farming species, which have the potential to increase the availability of nutritious seafood and also promote higher food security. One such approach is the coculture of different species with a focus on positive socio-environmental outcomes, in addition to increased seafood provision.

The US is the top seafood importer in the world and a big consumer of shellfish. Given this opportunity for domestic production, growers, and related seafood businesses (e.g. restaurants) have expressed interest in innovative forms of aquaculture that bridge farming efficiency and marine conservation spheres (e.g. restorative aquaculture, regenerative aquaculture). At the same time, aquaculture management that increases shellfish quality, and is less time-consuming and less costly for biofouling control is required. Within that context, this work explores the feasibility of coculture of native grazers, Atlantic Purple Sea urchins (*Arbacia punctulata*), and the Common periwinkle (*Littorina littorea*), with suspension feeder Eastern oysters (*Crassostrea virginica*) as two separate projects, and discuss how the approach can fit the framework of NbS.

Methods

Using traditional bottom culture cages, and farming bags, different treatments were deployed in selected locations in the Chesapeake Bay area, where oyster culture is common, using a randomized design. Oyster stocking density in farming gear was maintained constant and similar to standard commercial densities in all treatments. Experimental designs involved the collection and use of two different sizes and stocking densities for urchins; whereas periwinkles were deployed in different coastal culture zones with varying stocking densities. Treatments within projects are being compared in terms of potential effects on farming efficiency, farming management improvement, and species' conditions and survival. We additionally discuss how the design of grazer-oyster coculture can contribute directly or indirectly as a NbS based on IUCN's 'Global Standards for Nature-based Solutions' (Fig.1).

Results

Preliminary results show positive effects of the coculture choice upon oyster production relative to the monoculture, such as less biofouling attached to farming gear (Fig. 2), and improved target bivalve species quality (e.g. higher condition index, shell cleanliness), with additional potential environmental outcomes (less use of freshwater in operations, energy).

Discussion

Although this research is ongoing and projects are still in the initial phase, grazers could potentially be a useful tool to improve shellfish farming management and bivalve shellfish product quality. Several technical aspects and economic considerations to bring grazers to the seafood market still need to be carefully assessed, but broadly, we expect the advantage of deploying cocultures to be at least two-fold for the farmer: coculture can increase the revenue of the farms with a secondary product and lower farming management costs; besides potentially being a suitable strategy to small-scale farmers. Moreover, coculture with these grazers uses the same in-use farming space and can be designed to link with marine conservation initiatives, resulting in additional positive environmental outcomes.

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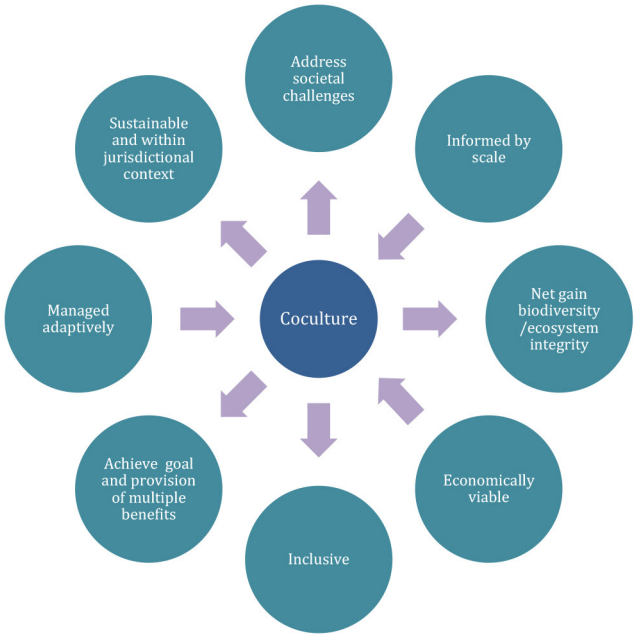

Figure 1: NbS standards (IUCN, 2020) and coculture approach.

Figure 2: Purple urchins deployed in farming bags with Eastern oysters in a coculture design, and biofouling comparison example.

CHANGES IN PROTEIN, FAT, ASH AND DRY MATTER IN DIFFERENT WEIGHTS OF BIGHEAD CARP (*Hypophthalmichthys nobilis,* Richardson, 1845)

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Introduction

Given the increasing demand for fish and fish products, it is necessary to determine the quality of fish fillets, as quality and nutritional value are considered as a major factor in fish sales and acceptance by consumers (Naeem and Ishtiaq, 2011; Naeem et al., 2016; Shirmohammadli and Mohammad Nejad, 2022). The amount of chemical composition in the aquatic body depends on the type of nutrition, living environment, age and sex of the living creature (Kochekian Sabour and Yasimi, 2011). Therefore, in the present study, changes in the composition of bighead carp meat, which is a valuable bone fish that is bred in many parts of the world, were studied at different weights.

Materials and Methods

For this research, 2 year old bighead carp were used in 4 weight groups as follows: Group A: Average weight 1000 gr, Group B: Average weight 2000 gr. Group C: Average weight 3000 gr and Group D: Average weight 4000 gr. To evaluate the quality of bighead carp meat, 5 fish were separated from each weight group after checking for health and abnormal symptoms and disease in the fish. After biometrics and measurement of fish length and weight, 100 grams of muscle was isolated from each fish sample to evaluate meat quality. The AOAC (1990) method was then used in the Food Analysis Laboratory to evaluate and analyze meat quality. SPSS 22 software was used for data analysis (Shirmohammadli and Mohammad Nejad, 2022).

Results and Discussion

Table 1 shows changes in protein, fat, ash and dry matter quality of bighead carp in different weight groups. The results showed that the amount of protein changed significantly in different groups and a significant difference was observed between different weight groups ($p<0.05$). So that the highest amount of protein was observed in 2000 gram fish. Changes in the fat of bighead carp showed that there are significant changes in fish with different weights ($p<0.05$). The lowest amount of fat was observed in the weight group of 2000 grams. But overall results showed that there was no statistically significant difference between different weight groups in dry matter and ash ($p>0.05$).

The results of this study showed that the change in fish weight has an effect on the quality of the fillet, so that the 2000 gram bighead fish had more fillet protein and less fat compared to other weights. The results of this study are not consistent with the study that was conducted on 500-1600 g silver carp and common carp. Their results showed that fish weight had no effect on fillet quality (Shirmohammadli and Mohammad Nejad, 2022). The reason for this difference can be physiological characteristics, species differences, nutritional conditions, etc. The results of this study showed that 2000 grams of bighead fish has more fillet protein than other weights. Therefore, it is suggested to present fish of this size to the consumption market and for consumers to use it.

Table 1: Changes in protein, fat, ash and dry matter at different weights of bighead carp

Factor (%)	Protein	Fat	Ash	Dry matter
1000 (g)	9.82 ± 0.73 [a]	8.99 ± 0.48 [b]	1.38 ± 0.13 [a]	20.21 ± 0.38 [a]
2000 (g)	12.54 ± 0.9 [b]	5.68 ± 1.53 [a]	1.43 ± 0.16 [a]	19.67 ± 0.47 [a]
3000 (g)	8.85 ± 1.32 [a]	9.18 ± 0.43 [b]	1.39 ± 0.05 [a]	19.42 ± 0.78 [a]
4000 (g)	9.98 ± 0.61 [a]	9.17 ± 0.24 [b]	1.43 ± 0.11 [a]	20.58 ± 0.57 [a]
Average	10.29 ± 0.89	8.25 ± 0.67	1.4 ± 0.11	19.97 ± 0.55

❋The small Latin letters show that there are significant differences among different weight

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Based on the results of this study, the average protein of bighead carp was 10.29 ± 0.89 %, which is much less than fish such as silver carp (14.18) (Shirmohammadli and Mohammad Nejad, 2022), *Liza auratus* (17.69%), *Clupea harengus* (18.45), *Tilapia zilli* (18.80 %) (Olagunju et al., 2012), *Orcynopsis unicolor* (22%), *Euthynnus affinis* (24%) (Aberomand, 2012), *Otolithes ruber* (19.64 %), *Scomberomorus guttatus* (19.9%), *Scomberomorus commerson* (19.5%) (Velayat-Zadeh and Askari Sari, 2013). Fat is a component of the chemical composition of the muscle that represents the largest difference in the amount of fish muscle (Askari Sari et al., 2016). The average fat of bighead carp in this study was 8.25 ± 0.67 %, which was higher than the amount of fat in *Liza auratus* (0.74%), *Liza dussmieri* (0.25%) (Aberoumand, 2012), *Otolithes ruber* (1.23 %), *Scomberomorus guttatus* (2.1%), *Scomberomorus commerson* (3.4 %) (Velayat-Zadeh and Askari Sari, 2013), as silver carp (14.18 %) (Shirmohammadli and Mohammad Nejad, 2022), and much less than fish such as as common carp (9.42 %) (Shirmohammadli and Mohammad Nejad, 2022), *Clupea harengus* (11.14 %), *Scomber scombrus* (12.33 %), *Tilapia zilli* (18.80%) (Olagunju et al., 2012), *Orcynopsis unicolor* (16 %), *Euthynnus affinis* (14 %) (Aberoumand, 2012). According to the present study and its comparison with other studies, the bighead carp can be considered among the fish with medium protein and fat. In addition, due to the high value of protein, 2000 grams of bighead fish can be suggested as an acceptable weight for the consumer.

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DIFFERENT METHODS OF OVULIN HORMONE INJECTION ON STRESS INDICES (GLUCOSE AND CORTISOL) OF *Cyprinus carpio* var. Sazan BROODSTOCKS

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Introduction

Glucose and cortisol are important indicators in assessing the level of stress in living organisms. Blood glucose is the most variable index that is greatly affected by handling, transportation, environmental stress, seasonal changes, nutritional status and maturity (Hosseini Fard et al., 2013). Cortisol in bony fish affects reproductive function and gamete growth. Cortisol, an important corticosteroid produced during the spawning season, can suppress fish immunity (Odhiambo et al., 2020). Inducing sexual maturation in fish can be stressful and can have an adverse effect on fish reproduction. Hormone injections into fish for the purpose of inducing reproduction, in addition to affecting the levels of sex steroids, can cause changes in fish's blood stress indices (Falahatkar et al., 2016). Therefore, it is important to use a method that puts the least stress on the fish. In this study, different methods of injecting the Ovulin hormone on glucose and cortisol of *Cyprinus carpio* var. Sazan were studied.

Materials and Methods

12 *Cyprinus carpio* var. Sazan were injected in three methods in one time: injection under the pectoral fin, injection under the dorsal fin and injection under the ventral fin at a dose of 0.6 ml / kg of Ovulin hormone. Blood samples were taken from male and female broodstocks with 5 cc syringes and from fish tail arteries in two stages (before hormone injection and 12 hours after hormone injection). In the hematology laboratory and after centrifugation at 5000 rpm, serum was isolated from blood cells for 5 minutes and glucose and cortisol levels were measured by automated devices. All statistical analyzes were performed using SPSS software version 26. Shapiro-Wilk test was used to investigate the normal distribution of data in groups and for all variables. One-way analysis of variance (Oneway ANOVA) was used for statistical comparison between groups, and after performing the Test of Homogeneity of Variances and Duncan test was used to compare groups with each other (Mohammad Nejad, 2022).

Table 1: Stress indices of female broodstocks of *Cyprinus carpio* var. Sazan in different methods of Ovulin hormone injection

Stress index	Injection area	Before injection	After injection
Glucose	Pectoral fin	79.5 ± 5.5 [a]	125 ± 4 [a*]
	Dorsal fin	72.5 ± 4.5 [a]	113.5 ± 4.5 [a*]
	Ventral fin	68 ± 6 [a]	108.5 ± 5.20 [a*]
Cortisol	Pectoral fin	8.84 ± 0.43 [a]	114.5 ± 7.5 [a*]
	Dorsal fin	7.88 ± 0.47 [a]	21.25 ± 1.15 [b*]
	Ventral fin	9.39 ± 3.84 [a]	108 ± 4 [a*]

Non-common Latin letters for each index in each column indicate the difference between the injection methods and the * in the row indicates a statistically significant difference before and after the injection ($p < 0.05$).

Table 2: Stress indices of male broodstocks of *Cyprinus carpio* var. Sazan in different 0methods of Ovulin hormone injection

Stress index	Injection area	Before injection	After injection
Glucose	Pectoral fin	49.5 ± 3.5 [a]	91 ± 3 [ab*]
	Dorsal fin	55.5 ± 4.5 [a]	121 ± 5 [a*]
	Ventral fin	48.5 ± 6.5 [a]	83.5 ± 10.5 [b*]
Cortisol	Pectoral fin	16.15 ± 0.75 [a]	27.7 ± 0.5 [a*]
	Dorsal fin	13.55 ± 0.77 [a]	17.95 ± 3.25 [b]
	Ventral fin	9.03 ± 0.89 [b]	28.8 ± 1.4 [a*]

Non-common Latin letters for each index in each column indicate the difference between the injection methods and the * in the row indicates a statistically significant difference before and after the injection ($p < 0.05$).

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Results and Discussion

The results of glucose and cortisol analysis in male and female broodstocks of *Cyprinus carpio* var. Sazan showed that in both sexes and in all three injection methods, its amount increased after hormone injection (Tables 1 and 2). The amount of stress increase due to hormone injection in both male and female broodstocks in the method of pectoral fin and dorsal fin was more than the method of injection under the ventral fin. The comparison between the two broodstocks also showed that the increase in stress in the female broodstocks was more than the male broodstocks. These results were consistent with the study of changes in stress indices of *Cyprinus carpio* in different stages of artificial reproduction (Mohammad Nejad, 2022). The results of this study show that hormone injection is effective in increasing stress in Caspian carp. Consistent with the results of this study, injection of various hormones in artificial reproduction increased glucose and cortisol levels in some fish (Mohammad Nejad, 2022).

Almost any type of stress (physical and neurological) causes an immediate and pronounced increase in ACTH secretion and within minutes leads to a sharp increase in cortisol secretion from the cortical part of the adrenal glands. One of the many effects of cortisol is to increase the body's resistance to stress by reducing glucose uptake, so as cortisol increases, blood glucose levels also increase rapidly (Saha et al., 2003), which was also proven in the current study. Due to the injection of Ovulin hormone in male and female broodstocks, the amount of cortisol increased due to stress and then the absorption of glucose from the blood decreased and as a result, the amount of glucose in the blood serum increased. However, the relationship between stress and reproductive hormones of Caspian carp was not investigated in the present study but, the relationship between stress and its indicators with sex hormones in fish has been proven. The effect of cortisol on reducing estradiol and testosterone has been reported in some fish (Falahatkar et al., 2016).

Therefore, using a method that can put the least stress on the fish during the breeding process is important and necessary. The results of the present study show that hormone injection by injection method under the dorsal fin can cause less stress to the *Cyprinus carpio* var. Sazan, so it is recommended compared to the other two methods in the reproduction of this fish.

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THE HIGHER STOCKING DENSITY THE BETTER PHYSIOLOGICAL STATUS OF *Seriola dumerili* UNDER RAS CONDITIONS

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Introduction

The diversification of species can be a good strategy for the economic success of the aquaculture industry. This strategy has been followed in Europe with international projects like DIVERSIFY, which highlights the role of some species, as *Seriola dumerili*, for its great potential, both taking biological and economic issues (Robles & Mylonas, 2017). For this purpose, it is necessary to determine optimal culture conditions for this species and one of these basic conditions is the stocking density. A first approach in this field was made by Jerez et al. (2017), who evaluated the effects of different stocking densities on this species. This study reached a maximum density of ~7 Kg/m^3 for the high-density group, which showed a lower growth performance. However, our study was aimed to evaluate the metabolic orchestration, intestinal well-being and welfare reaching stocking densities higher than 30 Kg/m^3 for the high-density group.

Material and Methods

Greater amberjacks were obtained from natural spawning at the Marine Scientific and Technological Park of ECOAQUA Institute of the University of Las Palmas de Gran Canaria (Las Palmas, Canary Islands, Spain) and transferred to the Centro Tecnológico de la Acuicultura (CTAQUA, El Puerto de Santa María, Cádiz, Spain). Then, a total of 588 individuals (~ 24 g initial body mass) were distributed in a RAS system with 9 tanks of 400 L, which constituted the 3 experimental groups (in triplicate): i) Low Stocking Density (LSD), with a final estimated density of 8 Kg/m^3; ii) Medium Stocking Density (MSD), with a final estimated density of 16 Kg/m^3 and iii) High Stocking Density (HSD), with a final estimated density of 32 Kg/m^3. After 72 days of an *ad libitum*-feeding trial, a biometric sampling was done, and samples from plasma, liver and muscle were taken. Somatic and zootechnical indices were calculated, and samples from each tissue were analysed.

Results and Discussion

No significant differences were observed in the final body mass of the fish in any experimental group. In addition, the Fulton's condition factor (K) did not show any differences either, so fish in the three densities grew allometrically. Therefore, the results in terms of growth performance are positive and, moreover, parameters such as the specific growth rate (SGR) show normal values in all three groups (Jerez et al., 2017; Fernández□Montero et a., 2018; Monge□Ortiz et al., 2018). In terms of somatic indices, only the mesenteric index (MSI) showed significant differences, with an increase in perivisceral fat of the MSD group, concomitantly with i) higher lipid deposition in the liver, and ii) the lowest intestinal selectivity denoted by epithelial resistance (Rt). Finally, regarding intermediary metabolism, the results show a higher energy mobilisation with increasing density, which can be invested in growth performance. Interestingly, the MSD and HSD groups had significantly lower cortisol levels than the LSD group, which can be the cause or the consequence of the gregarious nature in juveniles of this species (Mazzola et al., 2000).

Results obtained in the present study, focussed on the combination of different analytical approaches, indicated that a higher stocking density than the highest currently used for *Seriola dumerili* herein is possible, since neither the growth performance nor the welfare of the individuals was negatively affected. Furthermore, these findings are highly transferable to the aquaculture sector, making the culture of this species more profitable, where the recommended stocking density could be set, at least, at 30 kg/m^3 without impact on fish performance parameters.

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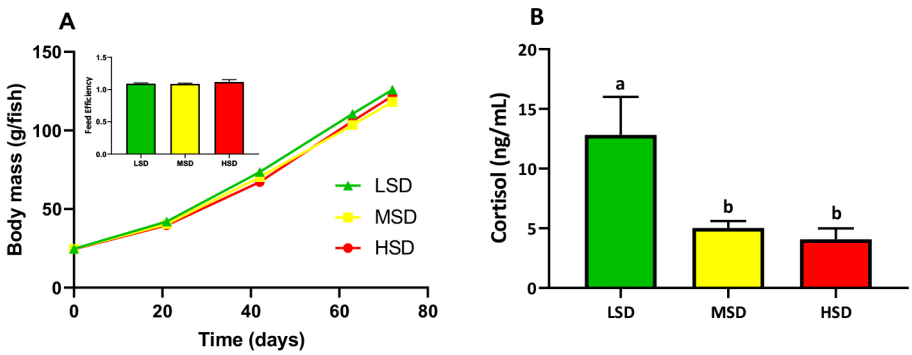

Fig. 1. Evolution of the wet body mass and feed efficiency (**A**), and cortisol levels (**B**) of *S. dumerili* juveniles cultured in three different stocking densities. The results are expressed as the average ± SEM (n = 12 fish). Different letters represent statistically significant differences at a p-value<0.05 resulting from the one-way ANOVA analysis.

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HOW TEMPERATURE AFFECTS INTERMEDIARY METABOLISM AND INTESTINAL HEALTH OF *Seriola dumerili* JUVENILES

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Introduction

Temperature is a key factor for achieving optimum fish growth and keep the welfare of the culture. Indeed, an incorrect temperature can affect ingestion, transit, digestion, assimilation and metabolism. Furthermore, this is magnified in a fast-growing fish such as the greater amberjack (*Seriola dumerili*). The culture of this species is becoming more and more important in the Mediterranean coasts mainly because of its fast development, its meat quality and its good acceptance in the market. For all these reasons, it is of utmost importance to establish a proper temperature, especially in RAS systems where this parameter can be modified quite easily, although its monitoring is especially important to avoid secondary artifacts that could compromise the metabolic and intestinal well-being of cultured fish. Taking all this into account, the aim of this study was to establish the consequences of water temperature on welfare, metabolic orchestration and intestinal integrity of greater amberjack juveniles accompanying the observed differences in growth performance (Yúfera et al., this conference).

Material and Methods

Greater amberjack juveniles (initial body mass 23.35 ± 5.07 g; mean ± SD) were randomly distributed in three independent RAS units set to 18, 22 and 26ºC of temperature, each one with three 900-L tanks. Juveniles were reared for 58 days under a photoperiod of 12h-light/12h-dark and fed until apparent satiation 3 times per day with a commercial diet (Skretting). At the end of the experiment, 5 fish per tank were sampled to check their body weight and to obtain plasma and liver samples. These samples were analysed for determination of metabolites and circulant cortisol. Intestinal integrity, permeability and electrogenic amino acid transport were assessed in using chambers. Differences were checked by ANOVA followed by Tukey's test.

Results and Discussion

In terms of growth performance, feed intake, final body weight and weight gain exhibited an increasing trend with the increase of temperature. Additionally, the feed conversion ratio was higher in the group maintained at 18ºC, so low temperatures produce a lower feed efficiency in this species (Yúfera et al., this conference).

Following biochemical results, the parameters measured in plasma shown a higher energy mobilization through glucose, as well as a higher presence of circulating proteins probably due to a higher protein retention at higher temperatures (Fernández-Montero et al., 2018). Furthermore, decreased circulating cortisol levels has been observed in the 18ºC group, probably due to the reduced metabolic rate associated with the low temperature, or even by a stimulatory effect of higher temperatures as a consequence of the anticipatory feeding activity. On the other hand, hepatic metabolites showed higher glycogen accumulation with increasing temperature, but lower triglyceride deposition in the 22 and 26°C group, indicating a temperature-dependent shift in energy substrate preference.

Intestinal health, evaluated by electrophysiological measurements, showed clear temperature-dependent variations. For example, an increase in mid gut transepithelial resistance (Rt, Fig. 2A) has been observed with increasing temperature. These results could also be associated with the higher transcellular transport in this section of the gut, as observed in the short-circuit current (Isc, Fig. 2B) for this group, as also confirmed the electrogenic amino acids transport in the mid intestine (ΔIsc, Fig. 2C). Both results could be indicative of increased gut integrity and selectivity, at least in the mid intestine, when fish are maintained at 26ºC.

Considering all these results, it could be stated that optimal culture temperatures for *S. dumerili* could be set between 22 and 26ºC, which agrees with the results obtained about growth performance and general health in previous studies with this species (Garcia-Gomez, 2000; Fernández-Montero et al., 2018).

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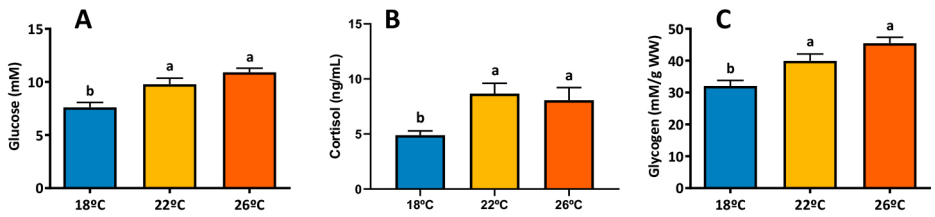

Fig. 1. Glucose (**A**) and cortisol (**B**) levels in plasma and glycogen deposits in liver (**C**) of *S. dumerili* juveniles cultured at three different temperatures (18ºC, 22ºC and 26ºC). The results are expressed as the average ± SEM (n = 15 fish). Different letters represent statistically significant differences at a p-value<0.05 resulting from the one-way ANOVA analysis.

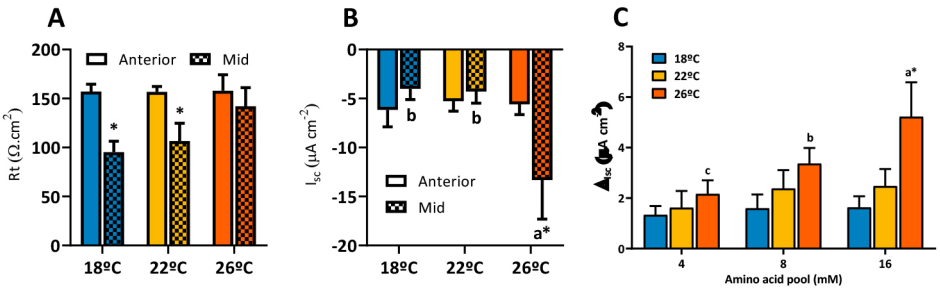

Fig. 2. Transepithelial resistance (**A**) and short-circuit current (**B**) measured in anterior and mid intestine, as well as electrogenic amino acids transport (**C**) in mid intestine of *S. dumerili* juveniles cultured at three different temperatures (18ºC, 22ºC and 26ºC). Further information as described in legend of Figure 1.

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A NEW VACCINE AGAISNT THE ZOONOTIC PATHOGEN *Vibrio vulnificus*: FIRST STEP

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Introduction

Vibrio vulnificus is an aquatic zoonotic pathogen whose distribution is expanding due to global warming. This pathogen causes a disease known as vibriosis in both aquatic animals and humans. The most severe form of vibriosis is a haemorrhagic septicaemia with a high probability of death. The species is subdivided into five potentially human-virulent lineages plus a polyphyletic pathovar with the ability to cause vibriosis in fish. Notably, this ability is associated to a series of genes encoding resistance to fish innate immunity. It has recently been shown that fish farms have played an important role in the evolution of this species as two of the five lineages emerged as human pathogenic variants in these sites after successive outbreaks of vibriosis in fish. Therefore, the control of this pathogen on farms is essential, not only to prevent animal and human infection but also to avoid the transference of this immunity system to other bacterial pathogens (1). This study is the first part of a larger project aiming at the development of a vaccine to protect any fish species not only from vibriosis caused by *V. vulnificus* but also from any other infection caused by a bacterium that had acquired this resistance system against fish immunity.

Materials and methods

Antigens. Selected genes (A1 and A2), related to fish immunity resistance, were cloned in pET28a (A1) and pET303a (A2), in frame with an N-terminal 6x-His tag. Recombinant protein expression was done in *Escherichia coli* BL21 (DE3) (A2) or *E. coli* BLR (DE3) (A1). Induced cells were suspended in lysis buffer, sonicated and centrifuged. Pellets were washed and sonicated several times with solubilization buffers (SB). Fractions containing the inclusion bodies were suspended in SB with 6M guanidine hydrochloride and solubilized by constant stirring overnight at 4ºC. Proteins were purified by nickel affinity chromatography (High Density Nickel, Agarose Bead Technologies) and quantified using the Pierce™ BCA Protein Assay kit (ThermoFisher Scientific).

Immunization. Four groups of 46 eels (*Anguilla anguilla*), selected as animal model, were intraperitoneally injected with 100 µl of purified proteins-based solutions according to Esteve-Gassent et al. (2). These formulations were prepared with A1, A2 or A1+A2 antigens (1:1 ratio) (0.75 µg protein/ml, in all cases), supplemented with Freund's incomplete adjuvant in a 1:1 ratio. In addition, a control group immunized with a 1:1000 dilution of the *V. vulnificus* bacterin enriched with extracellular components (2) was included. Plasma samples were collected before and 0, 3, 10, 14 and 21 days after immunization.

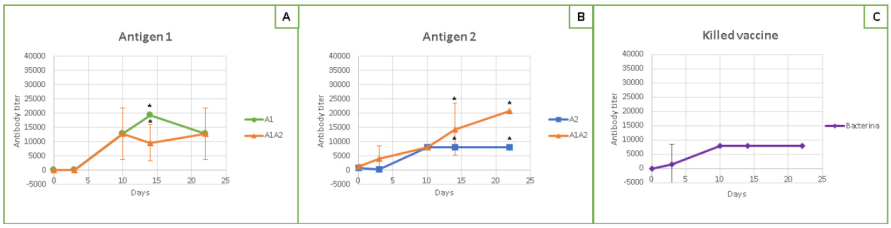

*Figure 1. Antibody response in plasma after eel immunization with purified antigens determined by ELISA. A) Groups immunized with A1 and A1+A2 against antigen A1. B) Groups immunized with A2 and A1+A2 against antigen A2. C) Group immunized with the bacterin against 1:100 diluted bacterin. *Significant differences in antibody production with respect to time 0.*

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Immune response. Specific antibody titers in plasma were determined by ELISA according to Esteve-Gassent et al. (2). Briefly, plates were coated with 10 ng of purified proteins or 1:100 diluted bacterin. Serial 1:2 dilutions of plasma samples were done. Reaction development was performed using rabbit anti-eel IgM antiserum (1:2000) and HRP conjugated goat anti-rabbit antiserum (1:2000) (Bio-Rad). Titer was established as the inverse of the dilution giving an A_{450} value twice greater than that of the blank (0.1% Bovine Serum Albumin in PBS).

Statistic. Two-factor ANOVA ($p < 0.05$), using R-Commader V.2.8 (R V.4.3.1).

Results

Proteins were successfully purified with a yield of 12.5 mg/l for A1 and 20.55 mg/l for A2. Immunization of eels led to the production of specific antibodies overtime (Fig 1). A significant increase in plasma antibody titers was observed 14 days after immunization in all groups, reaching the highest value 14 days (19200, in group A1) (Fig 1A) or 22 days (12800 to A1 and 20800 to A2, in group A1+A2) (Fig 1A & B) post-immunization. Immunization with formulation A2 elicited similar response (Fig 1B) to that of the bacterin group, in terms of plasmatic antibody titer (Fig 1C). However, in groups immunized with A1 and A1+A2 formulations, antibody levels were higher (Figs 1A & 1B).

Discussion and conclusion

There is a patented vaccine against *V. vulnificus* that showed high efficacy in fish farm trials but was never licensed due to its high production costs and low commercial value. The increase in the number of both outbreaks of vibriosis in fish farms and human infections related to global warming has substantially changed the picture. This work is part of an ambitious project to develop a multispecies vaccine that avoids not only the transmission of vibriosis but also the expansion of virulence genes encoding the innate immune resistance system of fish. In this work, we have selected the antigens, produced them recombinantly in *E. coli*, purified them from inclusion bodies and tested their efficacy in terms of antibody production. The specific response in eel was stronger after vaccination with purified proteins-based formulation than with the bacterin. The results obtained are encouraging and promise protection against the pathogen. The next step will be to test the protective efficacy of the protein-based formulations using different fish species and different pathogens.

Acknowledgments

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EFFECT OF DIFFERENT FEEDS ON GROWTH PARAMETERS DURING THE REARING OF CHUB (*Squalius cephalus*) ADVANCED FRY

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Introduction

Chub is an important gamefish in Hungary. There is a growing angling demand for this species (Borics et al. 2016). Several studies have already been carried out regarding the rearing of the chub larvae in a Recirculating Aquacultural System until the 30th day after hatching (Shiri Harzevili et al. 2003; Zarski et al. 2008; Crooks et al. 2013; Kupren et al. 2015). However, nowadays limited information is available on chub larvae rearing older than 30 days of age. Based on our previously experience, feeding with complete fish feed resulted high rate mortality and high rate of larval deformity compared to natural live food, for example *Artemia nauplii* (Bartucz 2020). One of the critical factors during rearing can be the lack of vitamin C (Berillis 2015). The study aimed to investigate the growth parameters on chub fries using different feeds.

Material and methods

The fries were reared for 28 days at a temperature of 25±0,5 °C and an oxygen level of 7,5±0,5 mg/L in Recirculating Aquacultural System (RAS). The lighting period was 12 h/day. Fish were stocked in with the capacity of 10 L tanks at a density of 10 individual/L. The effect of four different fish feed was examined in six repetition in each group. Fish were fed twice a day in a dosage of 2,5%/bodyweight.
Four different feeds were used:
- *Artemia nauplii* (A),
- *Artemia nauplii* with 1000 mg/kg Vitamin C (A+C),
- complete fish feed (Aller Infa EX GR 0,4 mm) (CF),
- complete fish feed (Aller Infa EX GR 0,4 mm) with 1000mg/kg Vitamin C (CF+C).

After 7 days of feed acclimatization period, wet body weight and standard length were recorded on the 0th, 14th and 28th days. Wet body weight and standard length were measured on 10 individuals/sampling occasion/tanks. Wet body weight was determined with a Mettler Toledo AB204-S analytical scale with an accuracy of ±1 mg. Standard length was measured with a Leica M205 FA type microscope and a Leica DFC 7000T camera mounted on it with an accuracy of ±1 mm. At the end of the experiment, the survival rate was determined.

Results

In wet body weight and standard length, no significant difference was observed as an effect of the addition of Vitamin C at any of the three measurements. On day 28, wet body weight and standard length were measured statistically verifiable higher in A (117±42 mg and 16±2 mm) and A+C (129±51 mg and 17±2 mm) groups than CF (107±54 mg and 15±2 mm) and CF+C (104±36 mg and 15±2 mm) groups. At the end of the experiment, similarly high survival rate was observed in all groups (A: 94,83%, A+C: 95,66%, CF: 94,33% and CF+C 94,83%).

Discussion and conclusion

We have successfully reared chub fry in a Recirculating Aquacultural System with artemia and a complete fish feed also. At the end of the experiment, between the two feed types, statistically verifiable higher wet body weight and standard length were recorded in the artemia groups, compared to the complete fish feed groups. The type of feed affected growth, but the addition of Vitamin C did not affect the offspring rearing period.

Acknowledgements

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EFFECTS OF FUNCTIONAL BREWERS' YEAST (*Saccharomyces cerevisiae*) ADDITIVES ON THE MUCOSAL HEALTH OF ATLANTIC SALMON PARR FED LOW MARINE CONTENT DIETS

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Introduction

Premium Atlantic salmon diets have contained up to 30% fishmeal (FM) and 20% fish oil (FO). Sustainability concerns over raw materials such as those derived from forage fish has led to continuous research and refinement of aquafeeds. Despite the growth of novel ingredients (such as insect meals, algal meals and single cell proteins) as FM/FO replacements, maintaining a low Fish-in : Fish-out ratio (FiFo) for salmon aquaculture largely depends on the more readily available plant derived raw materials. However, the combination of low levels of marine ingredients with high levels of plant derived raw materials can lead to poor gut health and welfare.

Functional feed additives such as probiotics, prebiotics, enzymes, and phytobiotic compounds have been incorporated in aquaculture diets for their ability to improve intestinal health and enhance disease resistance. Particularly, cell wall components of *Saccharomyces cerevisiae* (rich in β-1,3 and -1,6-glucans and mannan oligosaccharides) have been demonstrated to confer immunomodulatory effects in fish. These benefits are at least partially induced by improvements of intestinal health. However, despite these reported benefits, many knowledge gaps exist with the exact mode of action of yeast cell wall derivatives. Therefore, an experiment was conducted to investigate the efficacy of whole cell walls and highly purified β-glucans derived from brewer's yeast on the intestinal health of Atlantic salmon fed low marine content (15% FM, 8% FO) diets.

Methodology

A total of 120 parr (ca. 20g) were randomly assigned into six experimental units (20 fish per tank) and fed either 1] control (no yeast additives), 2] PβG (0.02% Leiber® Beta-S) or 3] WYCW (0.2% Biolex® MB40) treatments for 4 weeks. All treatment groups were fed to the same percentage of biomass (av. 1.5% per day). At the end of the experiment, distal intestine and skin tissues were collected from 5 fish per tank (N = 10 per treatment) for histology, electron microscopy and gene expression analyses according to protocols described elsewhere (Merrifield *et al*., 2009; Rawling *et al*., 2021).

Results and Discussion

At the end of the experiment, there were no significant differences in zootechnical performance (weight gain, SGR and FCR) between fish fed the different diets. Histological appraisal (Figure 2) revealed that fish fed the WYCW treatment had a 39% increase ($P = 0.0422$) in goblet cell abundance in the distal intestine and that the PβG treatment-fed fish had a 49% increase ($P = 0.0459$) in goblet cell abundance in the skin when compared to the control group.

In addition, electron microscopy analysis (Figure 3) of the distal intestine revealed significantly different microvilli morphometrics. Fish fed the PβG treatment had 20% longer ($P < 0.0001$) and 34% denser ($P = 0.0001$) microvilli compared to the control group. Fish fed the WYCW treatment had 25% denser microvilli arrangement ($P = 0.0056$) than the control group.

Targeted gene expression analysis of immunomodulatory and barrier function genes is on-going, however, the available data show that both functional feed additives demonstrated the potential to enhance the epithelial barriers studied.

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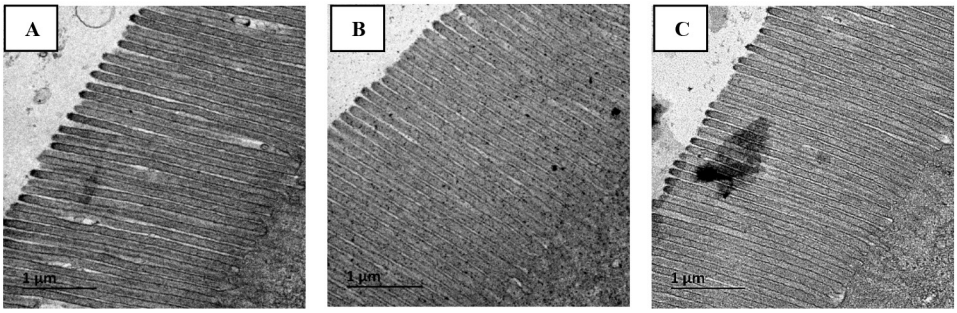

Figure 3: TEM micrographs showing microvilli length of the distal intestine of Atlantic salmon parr subjected to A] control B] PβG and C] WYCW dietary treatments.

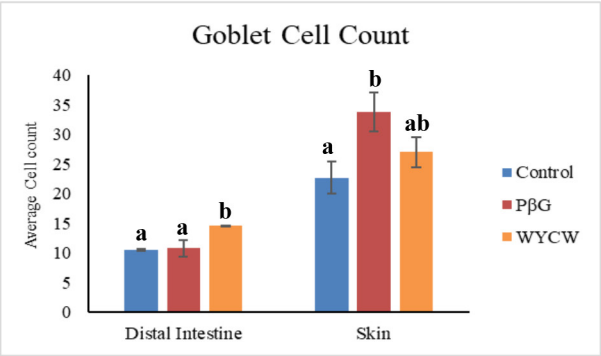

Figure 2: Goblet cells levels of the distal intestine and skin of Atlantic salmon parr after 4 weeks feeding on experimental diets. Different letters, within each tissue type, represent significantly different means between treatments ($P < 0.05$).

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CHARACTERIZATION OF LIPID PROFILE IN *Salicornia ramosissima* CULTIVATED IN IMTA SYSTEM: TOWARDS A MORE SUSTAINABLE AQUACULTURE

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Introduction

Over the last two decades, there has been a growing acknowledgment of the significance of fisheries and aquaculture in providing global food security and nutrition. Seafood is essential to over 1.2 billion individuals, with approximately 700 million relying on fish as their main protein source (FAO 2022). However, the expansion of aquaculture has raised concerns regarding the production of enormous amounts of organic waste and the associated environmental impacts. *Salicornia ramosissima* known as pickleweed or glasswort, a salt-tolerant plant, has been investigated for its potential to bioremediate marine and brackish aquaculture effluents. Also, this species shows great potential for diverse applications in pharmaceuticals, nutraceuticals, culinary, and aquaculture industries (Buhmann and Papenbrock 2013; Patel 2016). Furthermore, the use of *S. ramosissima* in fish effluent treatment systems that integrate IMTA (Integrated Multi-trophic Aquaculture) and RAS (Recirculating Aquaculture System) is still in its early phases of development, and further study is required to evaluate its full potential. Also, the biochemical profiles of *S. ramosissima* in every stage of its life cycle, on the other, are scarcely understood. Thus, this study aimed to characterize the amount of lipid, fatty acid profile, and lipid classes in *S. ramosissima*, considering its productivity, nutrient uptake, and life stages in utilizing fish effluents from RAS.

Materials and methods

The plant was cultivated in an integrated multi-trophic aquaculture (IMTA) system with floating plant rafts and in a fish-recirculating aquaculture system (RAS). The four-month experiment examined the plants' lipid, fatty acid content, and lipid classification considering their growth stages, biomass, and nutrient uptake.

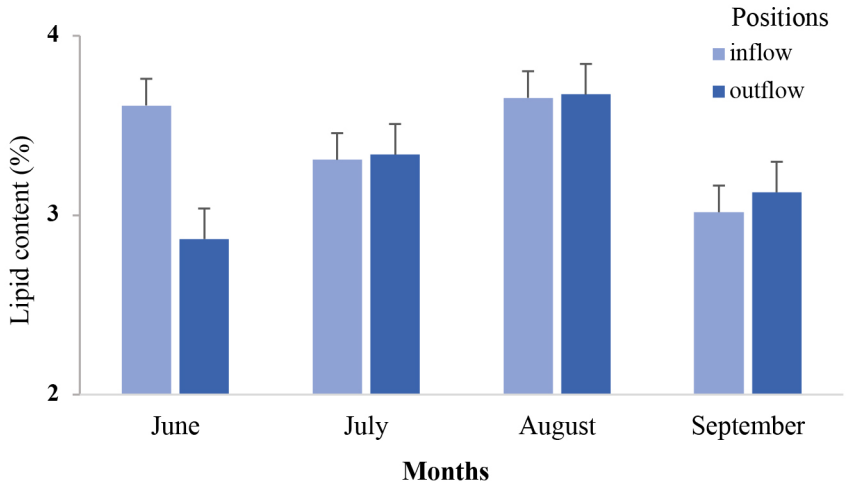

Figure 1. Total Lipid content (%) of S. ramosissima (edible part) throughout the 4 months of study at various positions on a raft system utilizing fish effluents from RAS for growing. Error bars are denoted by the standard errors.

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Results and discussions

S. ramosissima yield was significantly higher in the first month and decreased progressively until the end of the trial. It also revealed a high capacity to remove nutrients such as nitrite (64%), nitrate (82%), ammonia (89%), and phosphate (73%). Moreover, lipid percentages varied significantly throughout the life cycle with values of 3.4%, 3.5%, 3.8%, and 3.2% every month (Figure 1). The most abundant fatty acids (FA) were the polyunsaturated 18:3n3 (ALA) and 18:2n6 (LA), and the saturated 16:0 (PA). ALA and LA showed contrary tendencies, remaining constant until the final month, where ALA decreased significantly and LA increased. This coincided with flowering and seed production, highlighting LA's relevance in the senescence period. Regarding lipid classes, plants mainly showed high content of pigments (chlorophyll and β-carotene), glycolipids (mainly monogalactosyldiacylglycerols or MGDG and digalactosyldiacylglycerols or DGDG), phospholipids (mainly phosphatidylcholine or PC), flavonoids, and sterols. Most of these compounds are associated with antioxidant activity and exhibit health benefits. Thus, the study showed that the lipid profile of *S. ramosissima* is influenced by its life cycle and cultivation conditions. Also, these findings emphasize the significance of *S. ramosissima* in promoting eco-friendly and sustainable aquaculture practices while also providing valuable resources for food, feed, medicines, cosmetics, and biofuel.

Acknowledgments

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INSECT-BASED FISH FEED IN DECOUPLED AQUAPONIC SYSTEMS: EFFECT ON LETTUCE PRODUCTION AND USE OF RESOURCES

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Introduction

The utilization of insect meal-based fish feed as substitute to conventional fish meal-based feed is considered as a promising innovative alternative to boost circularity in aquaculture and aquaponics (Shaw et al. 2022a). However, basic research on its use in aquaponics and particularly in decoupled aquaponics is limited. So far, no reports on the effects of fish waste water, derived from a recirculating aquaculture system using Black-Soldier-Fly (BSF) meal-based diets, on the growth performance of lettuce were available. Therefore, this study aimed to compare the effect of reusing fish waste water (as a base for the hydroponic nutrient solution) from tilapia culture fed with a fish meal-based diet (FM) and a black soldier fly meal-based diet (BSF) on lettuce growth and water nutrient profile in decoupled aquaponic systems.

Material & methods

The experiment was conducted in a controlled climate chamber in nine separate hydroponics units. A conventional hydroponics nutrient solution (HP, control) was compared to two different aquaponic treatments (FM & BSF), and inorganic fertilizers were added to all groups to reach comparable target concentrations. All treatment groups were tested in triplicates. Lettuce fresh and dry weight, number of leaves, SPAD values, water consumption, and the usage of inorganic fertilizers were measured. Abiotic parameters of the nutrient solutions, environmental conditions, and micro- and macronutrients in the nutrient solutions were monitored in time series.

Results

Similar lettuce yield was seen in all treatments, with no significant effects on fresh and dry weight, the number of leaves, and SPAD values. Water use per plant was also similar between treatments, while the amount of total inorganic fertilizer required for preparing the nutrient solutions was 32% lower in FM and BSF compared to HP (Fig. 1). For the water nutrient profile analysis, higher sodium concentrations were found in the FM-based nutrient solutions compared to BSF and HP.

*Percentage difference**	FM	BSF
Nitrate – NO_3-N	- 29.21	- 25.85
Ammonia – NH_4-N	- 49.33	- 48.05
Potassium – K	- 8.40	- 10.67
Phosphorus - P	- 2.55	- 0.17
Calcium - Ca	- 53.30	- 53.75
Magnesium - Mg	- 50.62	- 52.58
Sulphur - S	- 93.66	- 91.77
Total inorganic fertiliser	**- 31.73**	**- 31.68**

Fig. 1. Average percentage difference between the two aquaponics and the control treatments for every macronutrient (NO_3-N, NH_4-N, K, P, Ca, Mg, and S) added in the form of inorganic fertilizer. The experiment tested lettuce production under three nutrient solution sources: conventional hydroponics solution (HP, control) and fish waste water solutions from tilapia culture fed with a fish meal-based diet (FM) and a black soldier fly meal-based diet (BSF).

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Discussion

The potential of using BSF-based feeds aquaponics for a combined production of fish and plants in a decoupled aquaponic approach was clearly shown in this study, as it was not negatively affecting lettuce growth. Nevertheless, also fish growth performance needs to be considered to evaluate the overall sustainability, since the use of BSF-meal-based diets might have a negative effect on the growth of Nile tilapia compared to a fish meal-based diet (Shaw et al. 2022b). In addition, BSF-based diets might be beneficial over FM-based diets in intensive, professional aquaponics applications due to the lower sodium concentration in the nutrient solution. This is especially important if the nutrient solution is recirculated in the hydroponics unit, as higher sodium concentrations might negatively influence plant growth (Hernández-Salinas et al.2022).

The results confirm that BSF as ingredient for fish diets is a promising alternative to FM in aquaponics without any adverse effects on lettuce growth, but more research is needed to evaluate the overall applicability.

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THE USE OF VIRTUAL REALITY AS A TOOL FOR A COMPREHENSIVE AND REALISTIC TRAINING IN AQUACULTURE

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Introduction

The use of Virtual Reality (VR) for the training of different aspects in aquaculture can be a valuable tool to simulate different working environments and situations e.g. with regard to animal welfare. Animal welfare in aquaculture does not attract enough international attention and there are no adequate international animal welfare standards for the breeding of fish, shrimp or mollusca. Therefore, the Aquaculture Welfare Standards Initiative (AWSI) formulated minimum requirements that are both comprehensible and implementable for producers of a wide variety of aquaculture animal species in a wide variety of countries. And in this specific context VR is used to offer comprehensive and realistic VR-training modules to address five core issues in aquaculture production (Fig. 1).

Material & methods

VR-training modules are developed based on scientific recommendations, basic guidelines for the different core issues as well as on feedback from practitioners. The first training modules for the species-specific stunning and slaughter of carp and trout as well as on general aspects of water quality are available in English and German.

Results

The first practical tests were very promising and clearly demonstrated the advantages of VR as an additional tool for training purposes in aquaculture. With this technology we were able to bring different aquaculture production systems such as recirculating aquaculture systems (RAS), flow through systems as well as ponds to the classroom and the users had the unique opportunity to get to know these systems without the need of travelling for several days. Additionally, critical scenarios, as the failure of different filtrations units or the stop of the main waterflow, can be simulated without putting the real systems and the produced fish at any risk.

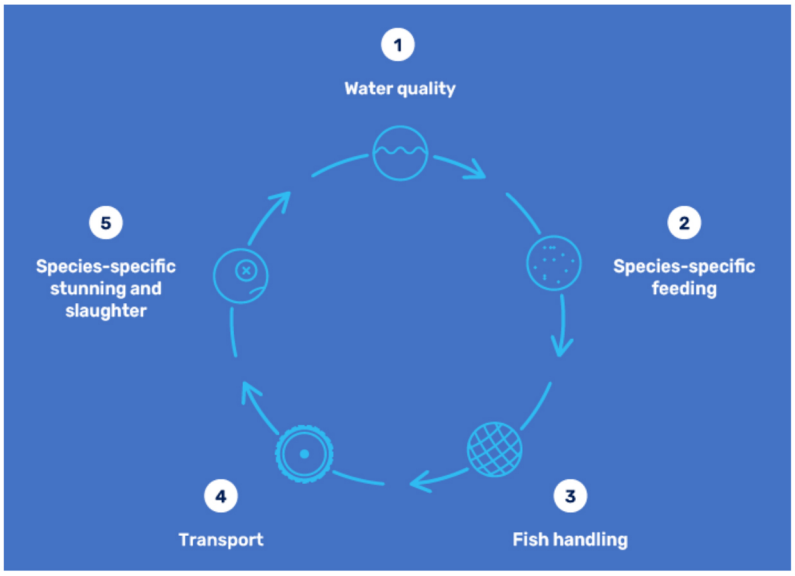

Fig. 1. The five aquaculture related topics which are particularly relevant for animal welfare. Specific VR-training modules are currently developed for these core issues.

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Discussion

There is a great potential for VR as a useful tool to improve the teaching and lifelong learning as well as to improve the awareness within the aquaculture sector. The topic animal welfare has been presented here with regard to the application of VR for training purposes but is of course not restricted to that. Many different use cases are possible, each one having their own challenges and beneficial effects. Next to the general development of further useful VR-training modules we would also like to scientifically study the effects of using VR as a training tool more in detail to further emphasize the potential benefits of this technology.

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DRIVERS OF MICROBIAL DYNAMICS IN REARING WATER OF A EUROPEAN PERCH RECIRCULATING AQUACULTURE SYSTEM

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Introduction

Microorganisms are omnipresent and key players in aquaculture, especially in recirculating aquaculture systems (RAS), where water is reused continuously. Monitoring and managing microorganisms in RAS rearing water is essential for optimal water quality, avoidance of microbial diseases, and sustainable fish production (1). Online sensors for multiple physicochemical parameters are standard in the industry; however, knowledge of microbial water quality has been hampered by the lack of tools available to fish farm managers.

Tracking and understanding microbial dynamics and the drivers of water microbiome disturbances requires fast-paced sampling and analysis, ideally in real-time, allowing for immediate reaction. Traditional methods have long time lags between water sampling and results and they are labor-intensive, time-consuming, and costly for implementation in a fish farm. onCyt Microbiology offers a real-time and fully automated microbial monitoring system based on flow cytometry that allows fast, accurate, and reproducible quantification and differentiation of total and intact microbial cells in aquaculture rearing water (2).

Materials and Methods

A fully automated flow cytometry monitoring system was used to monitor microbial concentration at multiple points across a European Perch RAS for 51 days. In the initial phase (biofilter start-up phase), water samples were drawn from the biofilter tank for 16 days every 30 minutes. In the second phase (fish farming phase), samples were drawn from up to 4 locations (2 fish tanks and their corresponding water inlets) for 35 consecutive days. Each water sample was automatically mixed with fluorescent stains, incubated, and then analyzed using flow cytometry. The resulting data sets (Approx. 4'000 data points) were batch-processed using onCyt proprietary software to determine total cell concentration (TCC) and intact cell concentration (ICC). Time series decomposition analysis was performed using R, version 4.21, and correlated with RAS operational and physicochemical sensor data.

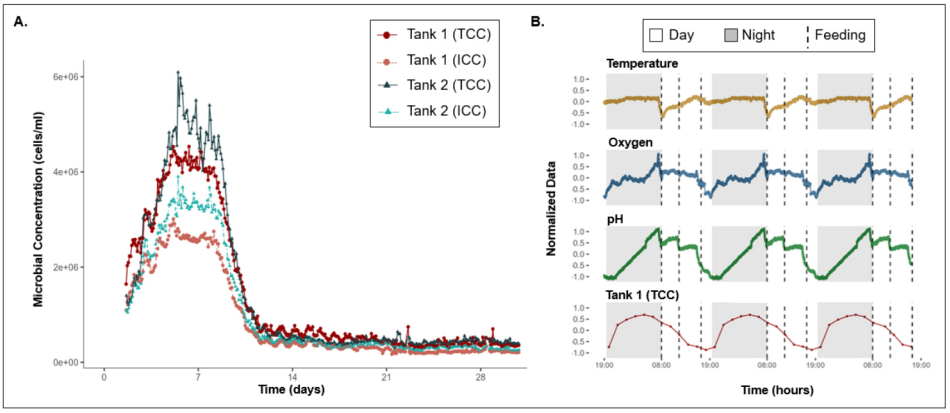

Fig. 1. (A). Microbial concentration in a Perch RAS measured by automated flow cytometry. Total and intact cell concentrations (TCC and ICC) were measured every 30 minutes for 35 consecutive days at two fish RAS tanks during the farming phase (data from inlets to the tanks is not shown). (B). Time series-decomposition of selected 3 days for TCC of tank 1, pH, and oxygen.

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Results

The onCyt automated flow cytometry system successfully delivered meaningful information on the microbial dynamics of the RAS. In the first 6 days, during the biofilter start-up phase, TCC and ICC remained relatively stable with an average of 1.5 x10^6 cells/ml and 1.1 x10^6 cells/ml, respectively. Over the following 2 days, a considerable increase (62%) in microbial concentration was observed, reaching a maximum TCC value of 4.1 x10^6 cells/ml, followed by a decrease and stabilization phase. Changes in microbial dynamics during this phase were attributed to operational changes, including the opening of the water recirculation and the start of water temperature regulation.

During the fish farming phase, measurements started one day after the perch entered the RAS. The first 10 days of this phase were highly dynamic and characterized by a massive increase in TCC and ICC at the three locations measured (two fish tanks and one water inlet), with tank 2 reaching up to 6.1 x10^6 cells/ml TCC (Fig. 1A). This expansion event was followed by a quick and sharp decrease in concentration to the order of 10^5 cells/ml (Fig. 1A). Thereafter, daily average microbial concentration remained relatively constant for the rest of the measurement period in all four locations, with water inlets showing similar trends but slightly lower concentration as the tanks they supply. Interestingly, a time series decomposition of selected 3 days revealed microbial trends linked to light and dark cycles, feeding times, and specific physicochemical parameters (oxygen and pH) (Fig. 1B).

Conclusions

Our findings provide strong evidence of the potential of advanced microbial monitoring for understanding the underlying mechanisms and operational consequences driving microbial dynamics in a RAS. The implementation of this tool in routine aquaculture operations can massively enhance microbial process management, improve operational efficiency, and reduce the risk of microbial infections.

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EVALUATION OF SELENIUM SOURCE ON GILTHEAD SEABREAM: A STUDY ON PERFORMANCE, QUALITY, AND STRESS RESPONSE

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Introduction

Selenium (Se) is a key essential trace mineral directly or indirectly involved in the maintenance of the body redox balance and antioxidant defense. Being a structural component of several selenoproteins, including glutathione peroxidase (GPx), Se helps to regulate lipid and protein oxidation processes, thereby enhancing the meat quality and its nutritional value. Traditionally, inorganic sources such as sodium selenite have been used to supplement Se in fish diets. However, in fish, research has shown that organic forms of Se have higher bioavailability and effectiveness in regulating oxidative stress and tissue retention rates than inorganic forms. The optimal levels of Se required to maximize fish performance may vary depending on the source, and further exploration in aquatic species is needed. The primary objective of this study was to compare the performance, product quality, and stress resistance of gilthead seabream (*Sparus aurata*) fed different sources of selenium (inorganic and two different organic sources) while meeting EFSA maximum allowed supplementation of organic Se as an additive of 0.2 mg kg $^{-1}$.

Materials and Methods

Three diets (CTRL, AVSe and ORGSe) were formulated to be isoproteic (48% DM), isolipidic (17% DM) and to vary in their Se source. Diets were supplemented with 0.2ppm Se as selenite (CTRL), Zn-L-SeMet (AVSe) or OH-Se-Met (ORGSe). All experimental diets were extruded and hand fed to apparent satiety, three times a day (9h, 12h, and 16h) for 137 days, to quadruplicate groups of gilthead seabream juveniles (50 g initial body weight). The fish were subjected to a 12-h light/12-h dark photoperiod regime and kept in 250L fiberglass tanks in a recirculating saltwater system (salinity 35‰, 22 ± 1 ºC). At the end of the growth trial, all fish were individually weighed and measured, and sampled for whole-body composition (n=4), total lipid and mineral composition of dorsal muscle (n=12), evaluation of hepatic oxidative stress (n=12), shelf-life evaluation over a period of 14 days (n=8), muscle evaluation of pH, WHC, color, texture and lipid peroxidation (LPO) at days 7 and 14 (n=8), and for sensory analysis (n=20). In order to determine fish metabolic and oxidative status, 12 additional fish per treatment were subjected to overcrowding (100 kg m^{-3}) for 5 min and air exposure for 1 min. After 1 hour of recovery, plasma and liver samples were collected from these fish.

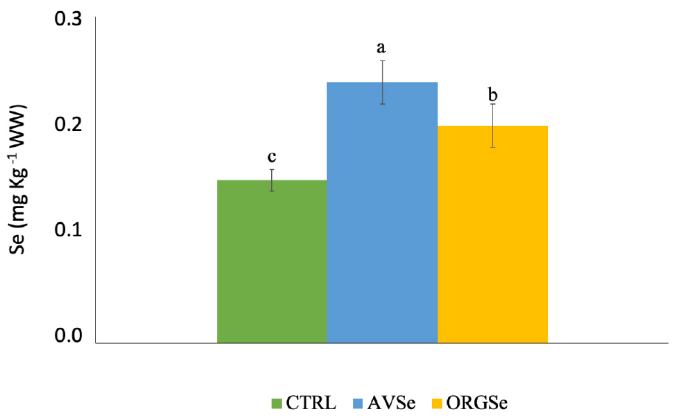

Figure 1. Se muscle content of gilthead seabream fed the experimental diets for 137 days.

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Results

All dietary treatments were equally accepted by fish regarding growth performance. However, nutrient utilization and fish whole-body composition were significantly affected by the diets. Fish fed AVSe had significantly higher whole body protein content compared to those fed the CTRL diet. These fish were the leanest but did not differ significantly from the CTRL. In terms of mineral utilization, increased Se gain and retention in fish fed AVSe and ORGSe diets, resulted in higher whole body Se content in these fish compared to the CTRL. It must be highlighted that only AVSe diet was able to significantly increase Se deposition in the muscle (**Figure 1**). The flesh quality parameters analyzed did not reveal major differences among the tested diets. The only exception was hardness, which was higher in fish fed ORGSe diet in comparison to CTRL. This difference could not be organoleptically confirmed by the sensory panel that gave a positive evaluation to all fish. Concerning stress tolerance, antioxidant enzymes' activity was either reduced (GR, GPx) or induced (GST) by the applied stress, although antioxidant enzymatic mechanisms did not differ significantly among dietary treatments. The source of Se supplemented to the diets has affected non-enzymatic antioxidant mechanisms. In fish exposed to the acute stress challenge, oxidized glutathione (GSSG) and oxidative stress index (OSI) were significantly higher with AVSe than with CTRL diet. Despite the increased levels of GSSG and OSI, no signs of oxidative damage were observed in the liver, as evidenced by similar LPO levels amongst dietary treatments and stress conditions.

Discussion and Conclusion

All the tested diets were equally effective in promoting the growth of fish and ensuring high feed efficiency. There were no significant differences in the overall consumer acceptance between the different muscle samples from the various diets. Stress exposure was shown to decrease GR and GPX activities which led to an increased level of oxidized glutathione (GSSG), particularly fish fed AVSe. This suggests a quicker response by these fish in challenging situations, by direct reaction of GSH with H_2O_2. The cause behind the lack of activation of the antioxidant enzyme response could not be completely addressed in the present study and should be considered and evaluated in future studies. The present study suggests that supplementation with organic Se, particularly from AVSe leads to leaner gilthead seabream fillets fortified in Se. The use of organic sources of Se, in particular of Zn-L-SeMet, could also have a positive environmental impact by improving Se gain and retention and therefore reducing Se emissions into the water bodies.

Acknowledgments

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IMMUNOMODULATORY EFFECTS OF LAMINARIN FROM *Laminaria hyperborea* ON ATLANTIC SALMON LEUKOCYTES– FIRST STEPS TOWARDS NOVEL FUNCTIONAL FEED APPLICATION FOR SALMON

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Introduction

Fish health and welfare are great concerns to the aquaculture industry [1]. In this regard, the search for sustainable feed compounds with bioactive properties, which contribute to maintaining fish health, is highly relevant. Seaweeds are important natural resources for the food and feed industry worldwide and volumes of cultivated seaweeds are increasing over the years [2]. Seaweeds contain bioactive compounds such as laminarin and fucoidan which have shown antioxidant, immunostimulant and anti-bacterial properties in several mammalian and fish cells [3]. Few studies have evaluated effects of laminarin in salmon cells, and so far, no studies have been done to evaluate laminarin as a bioactive compound in diets for salmon with the aim to improve/enhance immune responses.

To assess whether laminarin has immune-modulating activity on salmon cells, and to reduce the use of experimental animals, a "stepwise approach" was used. Step 1 involved an *in vitro* screening of differentially processed laminarin on the viability and on immune responses of salmon cells. Step 2 will involve an *in vivo* salmon trial evaluating the effects of different dietary inclusion levels of laminarin on growth performance and immune-related parameters. Together, this work will contribute to setting a basis for the inclusion of laminarin in salmon feed, with an overall aim to support and improve fish health and welfare.

Methods

Laminaria hyperborea biomass was processed by water extraction (50 ºC, 4 h). A precipitate consisting of enriched laminarin was formed after storage of extract at 4 ºC for several days. After extensive washing in 4 ºC ion-free water and in 50 % ethanol, three fractions were made by acid hydrolysis for further testing: L1, hydrolyzed 1 h, L2, hydrolyzed 3 h, and L3, control fraction without hydrolysis. To evaluate their effects in Atlantic salmon-isolated leukocytes from spleen and head kidney (HK), viability and bioactivity assays were performed using the three laminarin fractions. For the viability assay, 100, 250 and 500 µg mL^{-1} were used, and the cell viability was measured after 6, 24, 48 and 72 h. For the bioactivity assay, spleen and HK cells were incubated with laminarin fractions at 250 µg mL^{-1} for 6 and 24 h, and a panel of immune-related biomarkers were measured by qPCR.

Results and discussion

None of the fractions exhibited a toxic effect on spleen and head kidney (HK) cells; L1 even showed a positive effect on cell viability in cells from both organs at 250 µg mL^{-1}. Regarding bioactive properties on salmon leukocytes, L1 elicited an up-regulation of *tnfa*, *il1b*, *inos*, *ifng, cath-2*, *tgfb* and *sod* in both organs, which is similar to a mammalian macrophage type 1 profile (M1), evidencing a short-term inflammatory response that decreased 24 h after the exposure to laminarin. Moreover, in HK, L3 induced an up-regulation of *tgfb*, while no significant gene expression modulation was observed with L2. On the contrary, in spleen L2 and L3 showed an up-regulation of *ifng*, *il1b, inos* and *tgfb*, suggesting a differential response between these two organs.

Conclusions

Results show that laminarin from *L. hyperborea* has immuno-modulating capacity on salmon leukocytes. Therefore, laminarin could be a promising functional component for aquafeeds, especially when exposed to a low processing level such as in fraction L1. Further work is being carried out to elucidate whether laminarin may exert further immune effects on other salmon cells. Based on the promising results obtained in Step 1, Step 2, which will focus on including laminarin in salmon diets, is now being planned, with a future view to its application in novel functional feeds for the salmon aquaculture industry.

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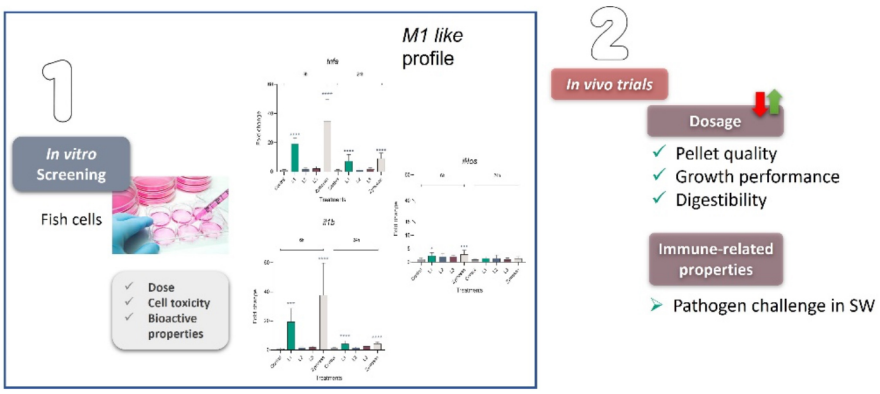

Figure 1. Graphical summary of the stepwise approach used to evaluate the potential use of laminarin as a bioactive compound in salmon feed.

Acknowledgements: This work was supported by The Norwegian Seaweed Biorefinery Platform (The Research Council of Norway; grant number 294946) and Foods of Norway, a Center for Research-based Innovation (The Research Council of Norway; grant number 237841/030).

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SPIRULINA *Arthrospira platensis* AND PROCESSING INDUSTRY FISHERY BY-PRODUCTS AS ALTERNATIVE PROTEIN SOURCES TO MARINE ORIGIN MEALS IN WEANING DIETS OF MUGIL *Liza aurata*

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The present study evaluated the use of *Arthrospira platensis* cyanobacterium and fish by-products as alternative protein sources in *Liza aurata* larvae of 31 dph (days post-hatching) with an initial weight and length of 1.92±0.12 mg and 10.47±1.01 mm, respectively. Five microdiets were tested: Control diet, with 100% protein from squid meal; Arth10, Arth20 and Arth40 diets with 10%, 20% and 40% *A. platensis* as partial substitution of squid meal; and Circular diet, with 100% squid meal substitution by fishery by-product. This research revealed that *Spirulina* can be included in the microdiet of mullets up to 40% maximum selected replacement, reporting high growth rates in size and weight, as well as high survival rates and resistance to stress. The inclusion of aquafeed by-products, despite reflecting significantly lower growth, it presented similar survival rates and proximal composition in *L. aurata* larvae. The skeletal anomalies analysis showed that mullet larvae do not present high percentages of severe deformities, except for the presence of stones in the urinary ducts. These results open a path towards sustainability for *L. aurata* production and the use of fisheries by-product resources, giving rise to a circular economy necessary for the aquaculture sector.

Introduction

The continuous expansion of the aquaculture sector as well as the high demand of the population and the decreasing production of fishmeal and fish oils are speeding up the search processes for new raw materials and/or alternative protein and lipid ingredients to supply, effectively, proper feeding of cultured aquatic organisms. For this, the sector must focus on growing to offer products both in greater quantity and quality following the principle of sustainability of the environment and natural resources (FAO, 2022). Numerous scientific studies are directing their investigations towards the search for the highest percentage of fishmeal replacement by different routes and in a multitude of potential species for aquaculture industry (Yarnold *et al.,* 2019). However, research on mugilids, candidate species for diversification, is scarce.

For this reason and taking into account that the exorbitant increase in prices will affect the composition of diets in the future and, therefore, the cultivation of species with high requirements, the species of *Liza aurata* has been selected. Omnivorous organism with a low trophic level that presents a high potential in terms of the use of alternative ingredients and by-products of low added value, as well as the potential that its cultivation presents in different environments and even extreme conditions, and the opportunity for introduction into the regional aquaculture production in a differentiated way from the rest (Crosetti and Blaber, 2016; Rosas *et al.,* 2019a).

Therefore, the main objective of the work is to evaluate the effect and potential use of these ingredients as alternative protein sources to squid meals used in microdiets for weaning of *Liza aurata* larvae.

Material and methods

The trial was carried out in the Parque Cientifico Tecnológico de Taliarte (PCTM) of the ECOAQUA University Institute of the University of Las Palmas de Gran Canaria.

Liza aurata larvae of 31 dpe (N=400) were randomly seeded in 15 200-L cylindrical tanks (5 treatments in triplicate). A co-feeding protocol was carried out with metanauplii of *Artemia* sp. and 5 different experimental microdiets according to the percentage of inclusion and replacement of squid meal by the cyanobacteria *Arthrospira platensis* in 10% (Arth10 Diet), 20% (Arth20 Diet) and 40% (Arth40 Diet) and by marine origin by-products not intended for human consumption (Sandach III) in 100% (Circular Diet). To evaluate the effect of the inclusion of the alternative ingredients, the larvae

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were measured (total length; mm) and weighed (wet weight; mg) to estimate parameters related to growth at 31, 40 and 52 dpe. Likewise, the proximal composition and fatty acids of the auxiliary cultures used during larval rearing, enrichers, experimental microdiets and larvae were analyzed following standardized chemical procedures (AOAC, 2005). In addition, larval quality was evaluated by performing a test of activity and resistance to an increase in salinity (38 to 70 ppt) and an analysis of identification and quantification of severe skeletal anomalies along the vertebral axis described by Boglione *et al.,* (2014).

Results and discusión

The larvae fed with Arth10, Arth20 and Arth40 spirulina-based microdiets showed growth performance similar to the Control diet and significantly higher than the Circular diet. However, all treatments present high survival rates, greater than 90%, and similar values in the proximal composition as those found by Rosas *et al*. (2019a) in *Mugil Liza*. Larvae fed high levels of squid meal substitution exhibited a similar stress response as those fed the Control diet. On the other hand, the increasing inclusion of alternative ingredients to squid meal reflects a tendency to decrease the percentage of severe skeletal anomalies and, therefore, to increase the total percentage of high-quality larvae.

These results demonstrate the robustness of the larvae and the feasibility of producing juveniles with more sustainable diets, in addition to contributing to the promotion and growth of complementary industries, as well as the future development of the sector based on the sustainability of the environment and resources.

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CORRELATION BETWEEN THE SUSTAINABLE DEVELOPMENT GOALS (SDGs) AND SUSTAINABILITY INDICATORS FOR THE EVALUATION OF AQUACULTURE SYSTEMS IN BRAZIL

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Introduction

The Sustainable Development Goals (SDGs) are seventeen interlinked objectives designed to serve as a guide for peace and prosperity for people and the planet, now and into the future (UN, 2015). Sustainability Indicators are variables defined to reflect the main features of sustainability in a simplified way (Valenti et al., 2018).

This work aims to achieve legal integration between the Sustainable Development Goals (SDGs) and available aquaculture sustainability indicators based on the legal principles of the Brazilian Constitution.

Materials and methods

An analysis of the Brazilian Federal Constitution was carried out from the perspective of constitutional principles related to environmental law to establish the correlation between these principles, the SDGs, and sustainability indicators of aquaculture.

It was defined 63 desirable states for sustainable aquaculture (DS) divided into 4 dimensions of sustainability. The SDGs were then associated with each of the desirable state characteristics. A hundred and eight six sustainability indicators were selected from the scientific literature and technical documents. They were associated with DS and SDGs.

The indicators must consider one or more of the SDGs to harmonize with the idea of sustainability in its most diverse aspects. The use of quantitative indicators is essential so that sustainability can be measured more accurately and that the intermediate stages of the process towards more sustainable systems and, consequently, the contribution of aquaculture to achieving the SDGs can be evaluated.

Results and Discussion

The analyses showed that aquaculture sustainability indicators make it possible to meet one or more of the SDGs of the United Nations 2030 Agenda (UN, 2015). These manage to relate to sustainability in its most diverse areas, as we can see in the figure below.

The use of quantitative indicators is fundamental so that sustainability can be measured more accurately and that the intermediate stages of the process towards more sustainable systems can be evaluated and, consequently, the contribution of aquaculture to achieving the SDGs. Even indicators based on qualitative variables should be transformed into a semi-quantitative scale to assess the degree of sustainability and the evolution after interventions by the public and private sector actions. (Valenti et al. 2018). This would allow public policies to be better evaluated and redirected to meet their purposes.

In addition to allowing the evaluation of the contributions of aquaculture to the achievement of the SDG targets, aquaculture sustainability indicators can serve as a tool to assist in the analysis of other sustainability measures such as the Corporate Sustainability Index or the ESG System – Environment, Social, and Governance.

Conclusions

This study showed that the Brazilian Federal constitution supports the four dimensions of sustainability; therefore, the concepts can be applied to national aquaculture and other economic activities.

The study also showed that the Desired States (DS) to achieve sustainable aquaculture are closely linked to the Sustainable Development Goals (SDGs). This means that achieving sustainability also contributes to achieving the SDGs.

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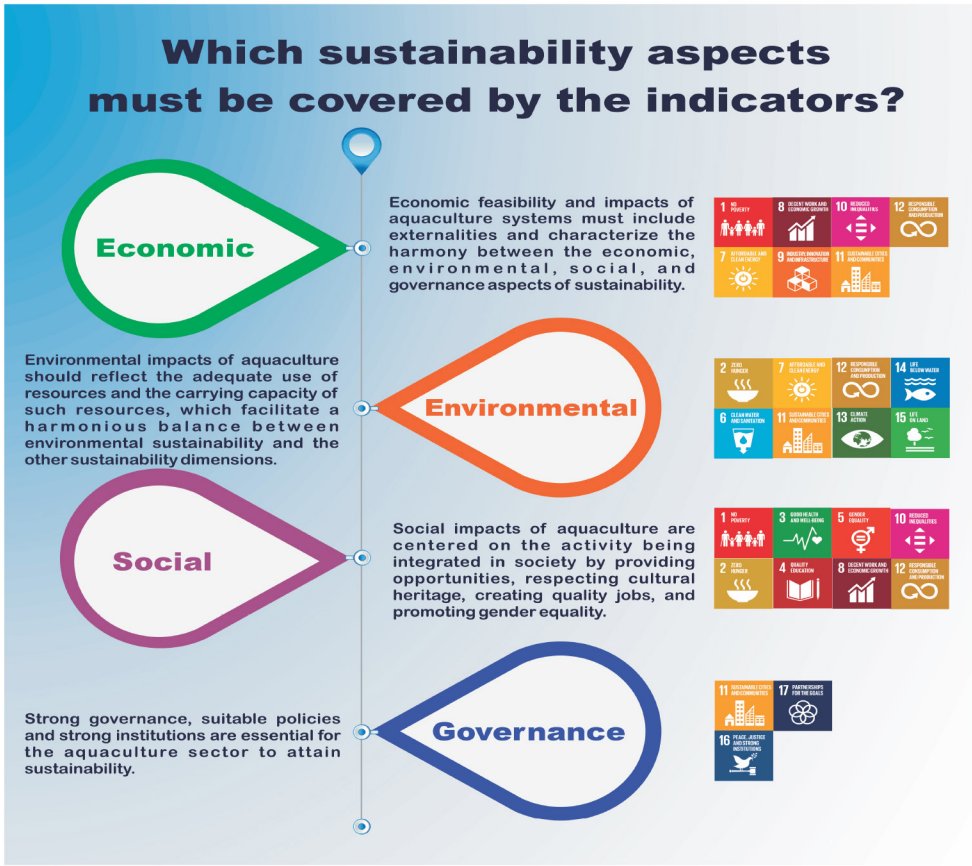

The sustainability indicators in aquaculture selected in this study can represent the desirable states, measuring their variation and evolution towards sustainable systems in the environmental, social, and economic dimensions. Therefore, they can also be used to assess the evolution of aquaculture as a driver of sustainable development for various purposes contained in the SDGs,

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FEED PALATABILITY ENHANCER IMPROVES FEED INTAKE IN EUROPEAN SEABASS *Dicentrarchus labrax*, ESPECIALLY UNDER STRESS

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Introduction

Palatability of feed, associated to its chemical and sensory properties, determines its acceptability by fish and total feed intake. Some fishes are more demanding in this respect than others, but common stressors in captivity and farming conditions are known to severely accentuate feed intake problems in most species, especially when diet palatability is sub-optimal. A decrease in feed intake is normally the first sign of stress which, when repeated often or prolonged, leads to performance loss and disease susceptibility. Hence, maintaining the fishes' motivation to eat during stressful events or production phases is key. A potential strategy to achieve this is to supplement feeds with chemosensory active substances that are quickly sensed by the fish and enhance attractive smell and taste cues of the feed, triggering fish appetite. In order to test a new palatability enhancer (PE) developed for European seabass, *Dicentrarchus labrax*, a trial was performed under both undisturbed and disturbed (induced stress) rearing conditions.

Materials and methods

The trial was performed in 16 indoor 250L tanks, equipped with a self-feeding system (activated by string sensors pulled by the fish) connected to a computer that registers and integrates the number of demands continuously (24/7). Feed demands are converted into grams, as feeders can be programed to deliver a set amount of feed, which is further corrected by weighing the amount added into each feeder and subtracting the feed remaining. Seabass juveniles were acclimated to the tanks (9 fish/tank, 8 tanks/treatment) and fed either a control feed (CTR: 42% CP, 18% CF; containing 12.5% fish meal, 4.8% fish oil and all remaining ingredients of plant origin) or the same basal formulation further supplemented (by vacuum coating with oil) with 0.1% of the PE. Fish average initial body weight (BW) was 146 ± 3.1 g (mean ± SEM) in the CTR and 154 ± 3.7 g in the PE group. The trial was divided in 2 phases: an undisturbed period lasting 35 days and a stressed period of 14 days, during which tanks were cleaned every day during the first 4 days and in alternate days afterwards, generating mechanical (brushing) and crowding (during 2 mins) stress. The trial was conducted from November to December 2022 under natural conditions of decreasing water temperature and photoperiod. Average water temperature during the undisturbed and stressed periods was 21.2ºC and 16.4ºC, respectively. The effect of treatment and stress were analyzed with a repeated measures ANOVA by the Proc MIXED (SAS), with tank as the experimental unit repeated through time (days), and differences between groups were assessed using the Tukey-Kramer test.

Results and discussion

During the 35 days undisturbed period only a non-significand trend (P=0.10) was observed, with the PE-fed fish showing 18% higher average daily feed intake (Fig. 1).

When stress was induced, a substantial decrease in feed intake was immediately observed in both treatments. Over the 14 days of the stress period, voluntary feed intake of the PE group gradually increased, although it never reached the level measured in the equivalent preceding undisturbed period (Fig. 2). It should be noted, however, that there was also a decrease in water temperature during this time, which might have contributed to the results. On the other hand, feed intake remained low during the whole stressed period in the CTR treatment. Interestingly, feed-demand peaks occurred in the CTR group in two of the days when no stress was imposed (it should be noted that on day 10 practically all feeding demands were recorded before 12h30, which is when cleaning of the tanks started), while feeding demands were more regular in the PE treatment.

Comparison of the average feed intake during an equivalent period of time (14 days) in the undisturbed and stressed phases (Fig. 3) revealed that stress induced a significant (P<0.05) decrease in both treatments, but the reduction was substantially lower in the PE than in the CTR treatment (43% *versus* 60%). Furthermore, feed intake in PE-fed fish was significantly (P<0.05) or nearly significant (P=0.057) higher than in the CTR in both the undisturbed and stressed periods, respectively.

In conclusion, present results suggest that the tested PE has the potential to increase feed intake in seabass fed diets containing low-moderate levels of marine ingredients (12.5% fish meal and 4.8% fish oil in this trial). Furthermore, and more significantly, results clearly indicate that the PE is effective in ameliorating the reduction in feed intake caused by stress.

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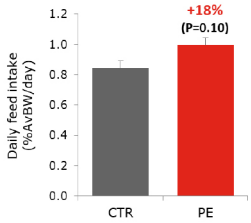

Fig. 1. Average voluntary daily feed intake (%BW/day) during the whole (35 days duration) undisturbed period.

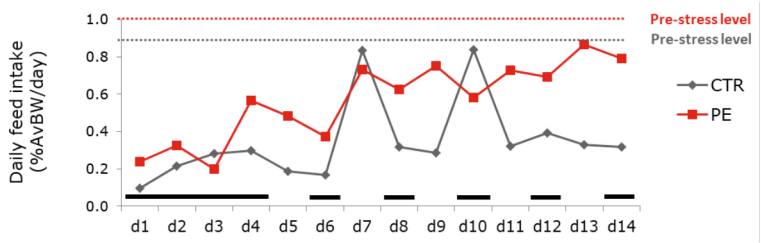

Fig. 2. Average voluntary daily feed intake (%BW/day) during the stressed period. Red and grey bars indicate the average feed intake in the 14 days preceding the stress in the PE and CTR treatments, respectively. Black bars at the bottom mark the days in which tank cleaning was performed.

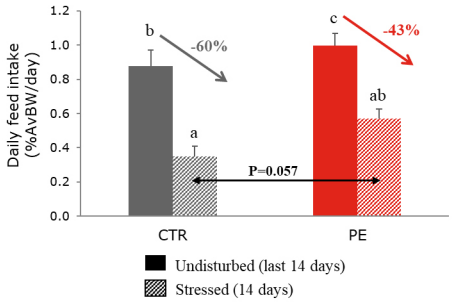

Fig. 3. Average voluntary feed intake percent reduction comparing the 14 days preceding (undisturbed) and the 14 days of the stressed period, in the CTR (grey) and PE (red) treatments. Columns containing different letters are significantly different ($P<0.05$), and a near significant difference between the CTR and PE treatments in the stressed period is indicated by an arrow.

NUTRITIONAL PROGRAMMING USING FUCOIDAN FROM SUGAR KELP AS A FEED ADDITIVE TO IMPROVE TRAINED IMMUNITY IN ATLANTIC SALMON AGAINST *Tenacibaculum maritimum*

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Introduction

Bacterial skin pathogens (e.g., *Moritella viscosa* and *Tenacibaculum* spp) are affecting fish health and welfare in farmed salmon during seawater stage (Sommerset et al., 2022). Both bacteria can be the primary cause of an infection, or establish a "consortium of pathogens" together with others such as *Aliivibrio wodanis* (Karlsen et al., 2014), which increases fish mortality. To control this situation, fish-specific pathogen vaccines are used in salmon farming. However, the effectiveness of some of these immunological strategies still do not meet industry expectations, especially those associated with long-term protection (Figueroa et al., 2022). In addition, there is a lack of vaccines against emerging bacteria. Interestingly, Trained Immunity-based Vaccines (TIbVs) have been reported in higher vertebrates during recent years (Sánchez-Ramón et al., 2018). This approach focuses on the innate response and how the vaccines can provide immunological protection against challenges that are not related to the vaccine antigens (Sánchez-Ramón et al., 2018). Considering this, TIbVs could be relevant to explore in fish, since there are currently no specific vaccines available against *Tenacibaculum* spp.

In the Resilient Salmon project (RCN 294821), in coordination with Foods of Norway, a Centre for Research-based Innovation at the Norwegian University of Life Sciences (NMBU), we are exploring nutritional programming with novel aquafeeds containing fucoidan isolated from sugar kelp (*Saccharina latissima*) to modulate trained immunity-related responses against *Tenacibaculum maritimum* in vaccinated Atlantic salmon (*Salmo salar*).

Materials and methods

Atlantic salmon pre-smolts were maintained at the NMBU Center for Sustainable Aquaculture. Before the feeding trial, fish were vaccinated with ALPHA JECT micro 6 vaccine (PHARMAQ) which contained antigens from *Aeromonas salmonicida*, *Listonella anguillarum* O1 and O2α, *Vibrio salmonicida*, *M. viscosa* and infectious pancreatic necrosis virus. Later, fish were randomly distributed into six 300 L tanks (45 fish per tank) supplied with recirculated fresh water. Each tank was assigned one of three diets (CD: commercial-like diet; FD: CD + 0.2% fucoidan extract from sugar kelp; MG: CD + 0.2% MacroGard®) for four weeks.

After this period, 35 fish per tank were transferred to seawater at the Norwegian Institute for Water Research (Solbergstrand, Norway) and fed the CD for 4 weeks before being intraperitoneally injected with inactivated *T. maritimum*. Samples (e.g., distal intestine: DI and head kidney: HK) were taken from six fish per tank at different time points: at the end of freshwater stage, after four weeks in seawater, one day post-stimulation and seven days post-stimulation. Total RNA was extracted from the tissue samples, and qPCR was performed to determine the gene expression of specific immune-related biomarkers.

Results and Discussion

In the different dietary groups, an immunological profile that could be associated with trained immunity (up-regulation of type-1 immune biomarkers such as *il-1b*, *il-8* and *tnf-a* at the end of the first stimulus, a decrease in their expression during rest time, and an enhanced response after the secondary stimulus) was detected in HK and DI of vaccinated Atlantic salmon. In higher vertebrates, there are reports showing similar results (Sánchez-Ramón et al., 2018), which have demonstrated the ability of vaccines to modulate the innate immunity against bacterial challenges that are not related to the vaccine antigens. This is interesting to explore in fish, since that there are no specific vaccines against *T. maritimum*, and because this bacterium is causing a higher number of natural outbreaks in salmon farms in recent years (Sommerset et al., 2022). Furthermore, the inclusion of fucoidan in novel aquafeeds as a bioactive additive showed up-regulation (one day post-stimulation with *T. maritimum*) of genes related to effector molecules such as antimicrobial peptides (e.g., *hepcidin*), which are crucial in the innate humoral response. Antimicrobial peptides are part of the first line of defence against a wide spectrum of pathogens and may contribute to a decrease in the use of antibiotics (Valero et al., 2020).

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Conclusion

Vaccines could induce the modulation of biomarkers associated with trained immunity against *T. maritimum* in HK and DI of Atlantic salmon. Moreover, nutritional programming using fucoidan may enhance the innate humoral response (e.g., antimicrobial peptides) related to TIbVs in immunological organs. This data supports the proposal to use fucoidan as a feed additive in novel aquafeeds for Atlantic salmon to improve fish health and welfare and to strengthen the sustainability of the aquaculture industry.

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AQUAFEEDS SUPPLEMENTATION WITH MULTIBIOTIC COMPOUNDS EXTRACTED FROM MACROALGAE FOR THE PHYSIOLOGICAL EVALUATION AND WATER QUALITY AFTER A MID-HYPOXIA CHALLENGE

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Introduction

The climate change has given the world an alarm to start making improvements in the way we exploit natural resources, specifically for animal and human diets. The actual problem found in aquaculture is directly associated with this natural resources shortage and its high demand, making substitutions in the bases of fish diets, from fish meals to vegetable ones. Although these changes show compatibility in carnivorous species, it may produce physiological alterations which can be modulated by nutraceutical compounds provided by macro and microalgae (Perera et al., 2020). In turn, the increase in global temperature as well as high stocking densities have triggered problems associated with oxygen demand, decreasing its concentration and availability in the environment. Therefore, this may be associated with internal changes leading to physiological stress (Martos-Sitcha et al., 2020). In addition, a strong relationship has been seen between the stressful effect and the intestinal microbiota, which can be attenuated through its maintenance and strengthening, where the addition of symbiotic nutraceuticals provides beneficial properties to the individual (Egerton et al., 2018). Moreover, it has shown the importance of gut enzymes and bacteria relationship, related to an appropriate digestibility along with better protein and energetic utilisation, in relation to the ammonia, nitrates and nitrites released to the aquatic environment (Hlordzi et al., 2020).

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 *Prebiodo 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, allowing to obtain feed efficiency (FE) and weight evolution for each group. After the feeding period, a hypoxia challenge was performed on the 3 experimental groups, being subjected to moderate hypoxia (35-40% O_2 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).

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).

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Table 1. *Results of Feed Efficiency (A), Mesenteric Index (B) and Intestine Length Index (C). Results expressed as mean ± 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
A	**FE (%)**	0.71 ± 0.02[a]	0.76 ± 0.02[ab]	0.78 ± 0.01[b]	0.041
B	**MSI (%)**	1.08 ± 0.09[a]	0.82 ± 0.09[ab]	0.58 ± 0.06[b]	<0.001
C	**ILI (%)**	97.89 ± 4.00[a]	81.05 ± 4.38[b]	83.89 ± 4.80[b]	0.014

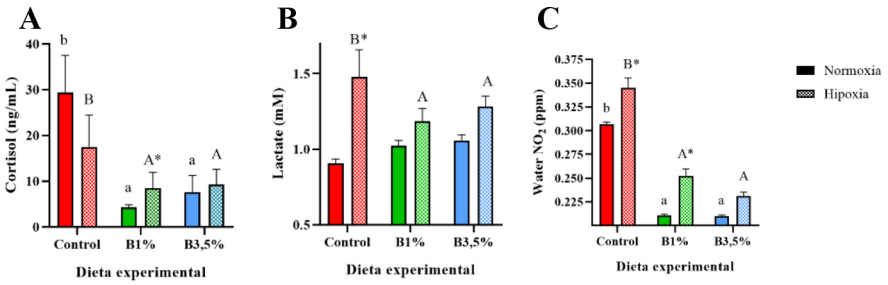

Figure 1. *Plasma cortisol (A), lactate (B) and nitrite in water (C). Results expressed as mean ± SEM, and analysed by two-way ANOVA analysis. Different letters represent statistically significant differences between groups (p-value<0.05).*

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ARGININE AND LYSINE ABSORPTION IN RAINBOW TROUT: UNDERSTAND THEIR ABSORPTION AND FATE IN CELLS TO COPE WITH AQUACULTURE STAKES

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Introduction

The use of plant proteins (PP) in fish feeds has considerably increased, at the expense of fishmeal (FM) inclusion, over the last decades to better cope with aquaculture sustainability. Nonetheless, after decades of research, 100% plant-based diets (PBD) still disturb fish physiology owing to multifactorial reasons among which an imbalance of PP amino acids (AA) profiles with some essential AA (EAA), mainly Methionine (Met), Lysine (Lys) and less frequently Arginine (Arg), being underrepresented in PP compared to FM. To overcome this issue, those EAA are usually added in free form to the PBD to fulfil requirements of fish determined previously using FM-based diets as standards. Although supplementation in these EAA improves fish growth, it does not rescue the full growing potential of fish fed FM-based diets. This could be explained, at least partially, by the fact that free AA are differentially absorbed compared to protein-bound AA, leading to differences in their bioavailabilities[1]. Interestingly, we noticed in two independent studies that some intestinal AA transporters (AAT), crucial for Arg, Lys and potentially Met absorption, and described in mammals to work by exchanging Arg and Lys with neutral AA[2], were overexpressed in fish fed PBD[3,4]. Thus, we wondered if the difference in bioavailabilities of protein-bound neutral AA versus free Arg, Lys and Met added to PBD could compromise their absorption, leading to dysregulations of these AAT and subsequent negative outcomes on fish physiology and growth. Thus, our study aimed to challenge this hypothesis by i) identifying and characterising the whole sub-family of cationic AAT (in charge of Arg and Lys uptake) in response to cationic AA starvation, ii) studying the consequences of cationic AA starvation on two major AA-sensing dependent pathways called GCN2 (General Control Nonderepressible 2) and mTOR (mechanistic Target Of Rapamycin) pathways and iii) proposing, through fundamental knowledge gathered using rainbow trout (RT) cell lines as models to study AA absorption, a new formulation strategy that promotes the absorption of cationic AA supplemented in their free forms to 100% PBD *in vivo* to improve the zootechnical parameters of fish fed such PBD.

Materials and methods

Cell culture experiments were performed at 18°C using RTH-149 and RTgutGC cell lines. Cells lines were subjected to various nutritional challenge (*e.g.* AA starvation or specific cationic amino acid (CAA) starvations…) prior to determine cellular absorption of AA in cell lines, using UPLC-FL as well as the activation of AA-dependent signalling pathways, such as GCN2 and mTOR, involved in growth and stress through RT-qPCR and western blot analyses. For the presented *in vivo* results, RT were fed *at libitum* for 21 weeks with a full PBD supplemented with free Arg and Lys (RK) or the same PBD also supplemented with free Glycine (RKG). Plasma were sampled from the caudal vein 5 hours after the last meal (during the post-prandial amino academia peak) and AA levels were analysed by UPLC-FL to assess the effect of Gly supplementation on absorption of Arg and Lys.

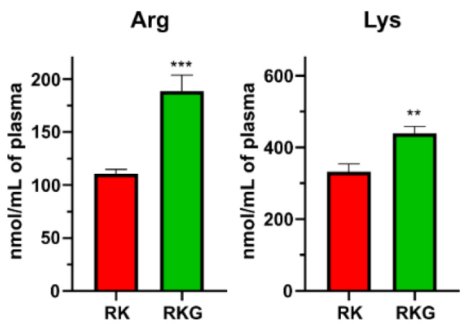

Figure. Free glycine supplementation to plant-based diet improves free arginine and lysine absorption

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Results

Using *in silico* analysis we identified 42 orthologous genes of the 16 human cationic amino acid transporters (CAAT) in the rainbow trout (RT) genome. At least 28 of them are expressed in trout tissues. Both RTH-149 and RTgutGC cell lines express the same 16 CAAT which are also expressed in their tissues of origin, liver and intestine respectively. Of the 16 CAAT expressed in the cell lines, 6 of them were systematically shown to be-upregulated upon nutritional challenges in AA- and CAA-dependent manners, very likely through the activation of the GCN2 pathway. Interestingly, one of them, called y^+LAT2 which is an important CAA exchanger (notably in the intestine) is one of the most upregulated by Arg and Lys starvation in cells while it was also shown to be upregulated in previous *in vivo* studies where fish were fed a 100% PBD supplemented with free CAA[3,4]. Therefore we supposed that the y^+LAT2 upregulation observed in fish fed PBD could be due to an activation of the GCN2 pathway caused by a compromised ability of fish to absorb the supplemented free CAAs and therefore leading to decrease fish growth performances. Knowing from our *in vitro* assays that part of the cellular absorption of CAAs relies on the presence of non-essential neutral AA, among which glycine certainly exchanged by y^+LAT2, we therefore build a working hypothesis according which the known difference of bioavailability of free AA *versus* protein-bound AA, could considerably dampens the absorption of supplemented CAAs in PBD. Consequently, we challenged this hypothesis by comparing zootechnical parameters of fish fed a PBD supplemented with Arg and Lys (RK) to those fed with the same diet also supplemented with Gly (RKG). Following a 21 weeks feed trial, we first could observed that such Gly supplementation improves significantly the feeding efficiency (as already observed in broilers). Very strikingly, we could also noticed that such Gly supplementation allows a better absorption of CAA as demonstrated by the significant increase in post-prandial AA levels of Arg and Lys (see figure) while, with the exception of Gly and Serine, no other AA levels was observed to be significantly increased. These results demonstrate that free Gly supplementation increases free Arg and Lys absorption that in turns could improve feed efficiency.

Conclusion

In this study, we first identified the CAAT expressed in RT and showed with the use of RT cell lines that some of them are upregulated through activation of the GCN2 pathway during Arg and Lys starvation. One of them, y^+LAT2, is also upregulated in fish fed with PBD supplemented with free Arg and Lys. Because y^+LAT2 is 1) a strict exchanger of CAA against neutral AA and 2) an essential intestinal CAA transporter in mammals, we supposed that bioavailability difference between protein-bound neutral AA and free CAA affect absorption of the latter. Thus, we demonstrated with combined *in vitro* and *in vivo* approaches, that most of this working hypothesis was correct by showing that free CAA absorption can be improved by supplementing PBD with free neutral AA like Gly. Altogether, this study shed a new light on the importance considering AAT functions and activities in the biology of fish of agronomic interest and open new exciting avenues to develop news feed formulation that better meet the sustainable developmental goals of the aquaculture industry.

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OXYGEN CONSUMPTION AND LOCOMOTORY BEHAVIOUR DURING A SWIM FITNESS TEST OF EUROPEAN SEABASS (*Dicentrarchus labrax*): RELATION WITH ORIGIN AND EARLY LIFE EXERCISE TRAINING

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Introduction

Swimming capacity plays a crucial role in the fitness of fish, crucial for their survival and reproductive success. Origin and early life experiences may have important consequences for swimming capacity later in life (Zambonino-Infante *et al.,* 2017; Vandeputte *et al.,* 2019). In this study, we investigated the influence of origin and early life exercise training on the swimming economy and locomotory behaviour during later life in the European seabass (*Dicentrarchus labrax*).

Materials and methods

Experimental fish, originating from the Atlantic, Eastern Mediterranean, and Western Mediterranean, had been subjected to an early life exercise training consisting of swimming against an increased flow of 0.3 $m.s^{-1}$ from 92 to 162 dph at the facilities of Ifremer (Palavas-les-Flots, France) by M-L Bégout and colleagues. The controls had been reared at regular flow conditions of 0 - 0.1 $m.s^{-1}$ during this period. After training and PIT tagging, fish were reared in common garden until an average weight of about 20 g was reached. Fish were then transported to Wageningen University and Research experimental facilities (CARUS, Wageningen, The Netherlands). The fish (N= 36, with 3 origins x 2 training conditions is N= 6 fish per group) were grown until approximately 60 g in weight, when the swimming experiments were executed using a 30-L Loligo swim tunnel (Loligo systems, Viborg, Denmark) (Fig.1).

The flow in the swim-tunnel was set at five different speeds during the experiment, starting at the lowest speed of 0.1 $m.s^{-1}$ and then increasing stepwise with 0.1 ms^{-1} per hour up to a maximum speed of 0.5 $m.s^{-1}$. Oxygen consumption and locomotory behaviour were assessed at each interval using a galvanic oxygen probe and a Basler 2040-90um NIR USB3 high-speed camera, respectively (see also Arechavala-Lopez *et al.,* 2021). From the experiment we obtained the following data thus far: oxygen consumption rates at each of the swimming speeds (MO_2 in $mg.kg^{-1}.h^{-1}$), optimal swimming speed (U_{opt} in $m.s^{-1}$), Cost of Transport at the optimal speed (COT in $mg.kg^{-1}.km^{-1}$) and critical swimming speed (U_{crit} in $m.s^{-1}$). Locomotory behaviour parameters tail beat amplitude and frequency, and head width amplitude and frequency, are still being analysed.

Results and discussion

Our results provide valuable insights into the swimming economy of juvenile seabass. Group averages of MO_2 ranged from 206 to 231 $mg.kg^{-1}.h^{-1}$ when swimming at 0.1 $m.s^{-1}$; 174 to 218 $mg.kg^{-1}.h^{-1}$ at 0.2 $m.s^{-1}$; 190 to 232 $mg.kg^{-1}.h^{-1}$ at 0.3 $m.s^{-1}$ and 260 to 459 $mg.kg^{-1}.h^{-1}$ at 0.4 $m.s^{-1}$. Fish then started to fatigue at U_{crit} values of 0.38 up to 0.43 $m.s^{-1}$ or 2.01 up to 2.48 $BL.s^{-1}$. Average U_{opt} was calculated at 53-67% of U_{crit} as 0.30-0.33 $m.s^{-1}$ or 1.57-1.73 $BL.s^{-1}$. At U_{opt}, COT values were ~200 $mg.kg^{-1}.km^{-1}$. U_{opt} values were significantly lower than the 0.69 $m.s^{-1}$ reported for similar sized seabass swimming in Blazka-type swim-tunnels (Graziano *et al.,* 2018) which may well be due to the longer swimming compartment in those tunnels allowing for burst-and-glide swimming behaviour. COT values at U_{opt} were similar.

Surprisingly, our results revealed no significant differences between fish from the three different origins, nor between trained fish and their controls. These findings suggest that origin and early life training do not have any relation with the swimming performance during later life. As we did observe that Atlantic fish were generally more active than fish from the Mediterranean sites, we expect to find differences in locomotory parameters between fish from different origin.

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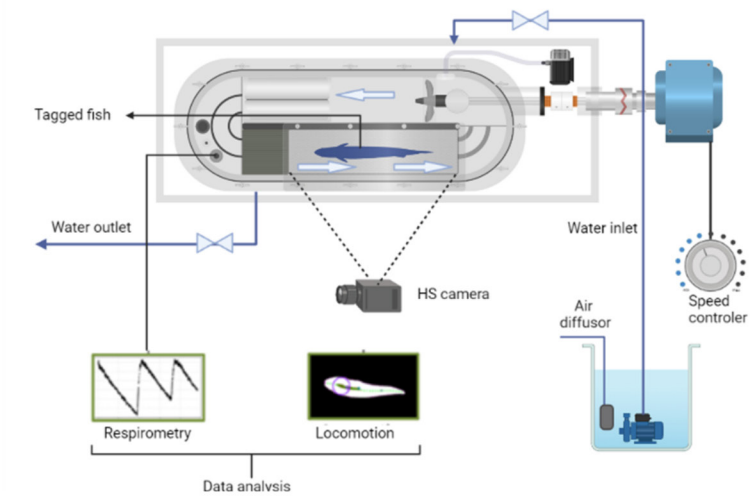

Figure 1. Schematic drawing of a Loligo swim-tunnel with the experimental setting for *Dicentrarchus labrax* swimming performance and locomotion at different speeds.

Understanding the swimming economy of seabass has important implications for both scientific research and practical applications in aquaculture (McKenzie *et al.,* 2020). By gaining a deeper understanding of the swimming economy, we can determine climate change impacts and optimize breeding programs, and develop more effective strategies for the cultivation and management of this economically significant fish species.

Acknowledgements

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BIOENERGETIC MODELLING: A TOOL FOR IMPROVING AQUACULTURE SUSTAINABILITY

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Introduction

To ensure sustainable production and maximize the efficiency of aquaculture, it is essential to implement strategies that maximize growth while minimizing the invested resources and the environmental impacts.

Our work aims to report one example of how bioenergetic modelling, and specifically the DEB theory, can link fish physiology with profits/impacts of the industry. Dynamic Energy Budget (DEB) theory (Kooijman, 2010) supports a fully mechanistic bioenergetic model that enables to predict individual growth under different scenarios of food and temperature. Interestingly for our matter, DEB theory assesses the energy and mass balance of an individual fish (or group of fish) at any moment of the life span, explicitly including, for example, the food and oxygen consumed and the waste produced at any time. All this information can be upscaled from the individual fish level to the cage or the farm level (Chary *et al.,* 2022). Here, as a proof of concept, we aim to explore through computer simulation experiments the landscape of yield that emerges from several fish displaying different but realistic combinations of values for three key DEB parameters that have direct relation with the food assimilation and the energy mobilization processes: (1) digestion efficiency (kappa_X), (2) maximum assimilation rate (p_Am) and (3) energy conductance (v). Then, to enhance comprehension of the implications of different values of DEB parameters, we explored the emerging correlations between the three DEB parameters and 4 response variables: (1) final wet weight, (2) total food ingested, and two widely used efficiency indicators in aquaculture: (3) Specific Feeding Rate (SFR) and (4) Specific Growth Rate (SGR).

Methods

Sparus aurata (gilt-head seabream), a fish commonly cultivated in the Mediterranean Sea, was used as model species. Feeding (gr of ingested food) and growth (gr, gain in wet weight) were estimated using a DEB model for 200 simulated fish that only differ in the three DEB parameters above. The specific values of these parameters for a given fish were randomly sampled form a multinormal distribution. The means, variance and covariance of p_Am and v came from the actual values estimated for 69 gilt-head seabream male individuals (Moro-Martínez *et al.,* 2023) reared at an aquaculture company (Aquicultura Balear, ABSA, S.A.U.). For kappa_X, we did not have yet empirical data because the challenge of quantifying the food ingested by each fish. In the meantime, the parameters for the distribution of kappa_X was approached as follows: the mean was taken from the add-my-pet database (Add-my-Pet, 2023) and a coefficient of variation of 12.5% was assumed for between-individual variability. We assume also no correlation between neither pAm nor v with kappa_X. Feeding and wet weight of each simulated fish was then used for estimating SFR and SGR.

Results and discussion

The simulation experiment supports two main findings. First, high interindividual variability in the feeding and growth rates was reported, which suggest that distinct individual feeding and growth strategies can coexist in aquaculture systems.

Second, concerning the emerging correlations between the three DEB parameters and 4 response variables, the main patters found were: (1) Final wet weight is strongly, positively correlated with pAm and weakly, negatively correlated with v; (2) Total food consumed is weakly, positively correlated with pAm, and weakly, negatively correlated with kappa_X; (3) SFR is strongly, negatively correlated with kappa_X; and (4) SGR is weakly, positively correlated with pAm, and weakly, negatively correlated with v. The mechanistic explanations for all these emerging patterns will be discussed in detail.

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Overall, this preliminary simulation is aimed to be interpreted as a proof-of-concept, to demonstrate the potential of understanding how bioenergetic processes at the fish level scale up to aquaculture indicators. Finally, we aim to motivate discussion on the utility of DEB theory for bridging bioenergetics at the individual level to yield indicators at the aquaculture level, and eventually to be used as a tool to optimize resources in aquaculture and, consequently, make it more sustainable.

Acknowledgment

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DANCE WITH SAMBA: AN INNOVATIVE APPROACH TO STUDY FISH GUT MICROBIOME USING BAYESIAN NETWORKS

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Introduction

Gut microbiota creates a profound connection with the host, taking part in numerous essential aspects of its physiology (Naya-Català et al., 2022). Moreover, microbial populations build a mazy network of interconnections between species, characterized by cooperation or competition that can affect the whole microbiome (Yajima et al., 2023). It is well documented that several factors can affect the microbial composition, but the interpretation of the probabilistic factors that underlie these interactions are still unrevealed. Indeed, the current approaches in fish microbiota research are mainly focused on the comparison of bacterial abundances between experimental groups or meta-analyses matching different studies. In this context, to make a step forward in the understanding of the fish microbiome dynamics, we implemented SAMBA (Structure-Learning of Aquaculture Microbiomes using a Bayesian Approach), an open-source, web-based platform with a graphical user interface implemented with Shiny (Soriano et al., 2022). This tool uses probabilistic Bayesian Networks (BN) to evaluate the conditional dependencies within a set of experimental variables and taxa. The aim of this study is to show how the implementation of SAMBA works as a model of causal predictions in the gut microbiota of aquaculture species.

Materials and methods

Experimental data for training SAMBA were taken from Spanish national (ThinkInAzul) and H2020 EU projects (AquaIMPACT, AQUAEXCEL[2020], AQUAEXCEL3.0, and EATFISH). The experiments were carried out on gilthead sea bream (*Sparus aurata*) under specific experimental conditions such as changes in genetic background, diet composition, or feed additives supplementation, among others. To define how and which biotic and abiotic factors modulate the fish microbiota, and which causal relationships exist within microbial taxa, we set different goals in line with the potential of SAMBA. In a first approach, we combined the taxa abundances within one specific experiment with BN, identifying the positive and negative relationships among the most representative bacteria. In a second approach, we filtered, from the data of already published experiments, those taxa present in both trials that took part of the core microbiota in at least one experiment. The counts of the remaining taxa were introduced in SAMBA, and we built two separated models, aiming to detect common microbes' causal relationships occurring in multiple experiences.

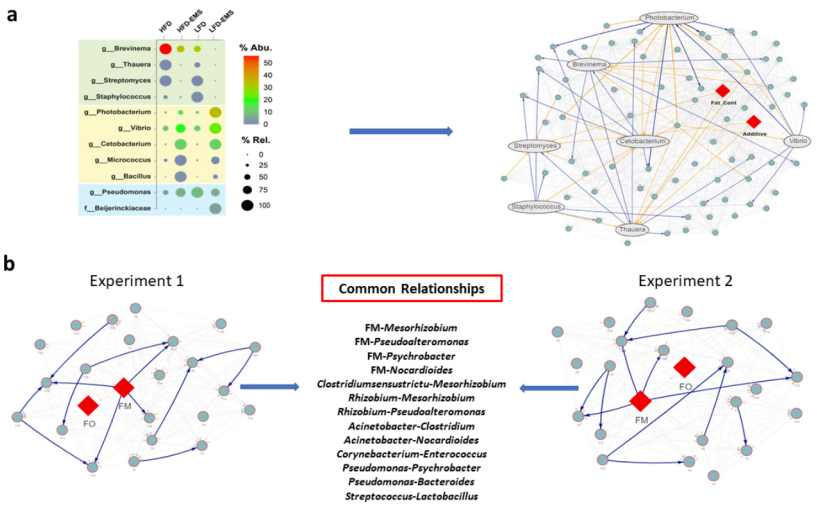

Figure 1. a Graphical representation of the relationships of the most abundant OTUs within the experiments (blue arrows= directional relationships from the OTUs, orange arrows= directional relationships to the OTUs, red diamonds=Experimental variables). **b** Graphical representation of the common relationships, shared between two different experiments (blue arrows=shared relationships, red diamonds=Experimental variables, dots=OTUs).

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Results and discussion

Both the intra-experiment and the inter-experiment approaches allowed us to obtain the hierarchical disposition of the experimental variables and the taxa within the data populations (Figure 1). Therefore, SAMBA tool can constitute a real step forward microbiomic studies, as it can take advantages from the taxa abundances to deeper understand the role and influence of each OTUs in the gut of livestock species. The directed acyclic graph (DAG) created in the intra-experiment approach (Figure 1a) disclosed the taxa primarily influenced by the experimental variables and the effect of these over other members of the microbial population. Interestingly, our results showed that the taxa affecting other taxa (i.e., parent taxa) are not always the most abundant, which are the ones usually reported in current 16S analyses. Regarding the inter-experiment approach, we obtained a total of 13 relationships present in both models (Figure 1b). From these results, it is feasible to draw a common structure, identifying which interconnections, shared by the two different experiments, and belonging to the core microbiota taxa, remain unaltered or are strictly linked with the variables that characterize the experiments. In addition, with the SAMBA implementation of the pipeline to infer metagenomes using PICRUSt2 (available with Metacyc and KEGG protocols), it is also possible to correlate the functional metabolic profiles of those taxa to better define their role in the specific relationships and in the total framework of the core microbiota (data not shown).

Concluding remarks

The implementation of SAMBA arises as an innovative approach, not yet exploited for aquaculture data (Ruiz-Pérez et al., 2021), to offer researchers relevant information beyond taxa abundances comparison when working with 16S metagenomics datasets. Although the tool is still being trained, when a sufficient amount of shared variables (i.e. mutual taxa, core microbiota) will be available, SAMBA will predict matching information of several experiments, discerning common associations between them that can be related with the experimental variables. In fact, future experimental designs are expected to be adapted to feed SAMBA. Moreover, as this tool is a constantly evolving project, SAMBA will soon include the integration of machine learning algorithms and new interfaces for different omics data, to be able to process complex and integrated results in an easy-to-use package.

Funding

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HISTOPATHOLOGICAL CHANGES IN THE COMPACT MYOCARDIUM OF ATLANTIC SALMON (*Salmo salar*) DYING AFTER THERMAL DELOUSING TREATMENT

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Introduction

The critical phase with regards to mortality of farmed Atlantic salmon during the sea stage used to be the shift from freshwater to seawater. However, in recent years substantial mortality is now also observed later in the sea stage. This late-stage loss is often connected to stressful events, such as delousing where mechanical and thermal salmon lice treatment appear to result in particularly high mortalities.[1–3] While the reason for this increase in stress-related mortality is largely unknown, accumulating evidence points towards diseases of the heart and blood vessels (*e.g.*, arteriosclerosis), which are becoming more common in farmed salmonids as farming practices intensify.[4–7] These conditions are thought to make the fish's heart less robust and able to handle stress, but few studies have directly examined the links between cardiovascular pathophysiological deviations and sudden stress-related mortality in farmed salmonids.Thus, the present study was designed to comprehensively evaluate salmon mortality in connection with thermal delousing treatment in the Faroe Islands. Here we present preliminary histological findings in the hearts of farmed Atlantic salmon that died in conjunction with treatment. As a comparison, hearts were also sampled from seemingly healthy salmon one week prior to the treatment at the same farming site.

Material and methods

Sampling was undertaken at a commercial farm in the Faroe Islands in late March 2022. The sampling was done approximately 10 months after sea transfer.

Prior to thermal delousing treatment, apparently healthy fish (N=20) were netted from the sea cage and euthanized with an overdose of anaesthetics (Tricaine methanesulfonate). The hearts were immediately dissected out and fixed in 4% formalin. Similarly, fish that died after delousing treatment (N=20) were pumped up from the sea cage and their hearts were sampled and fixed in the same way. After fixation the bulbus arteriosus and atrium were removed and the heart ventricle was longitudinally cut in half and routinely processed for histological examination. Sections (4 µm) were cut and stained with haematoxylin and eosin (HE) before being digitized and examined.

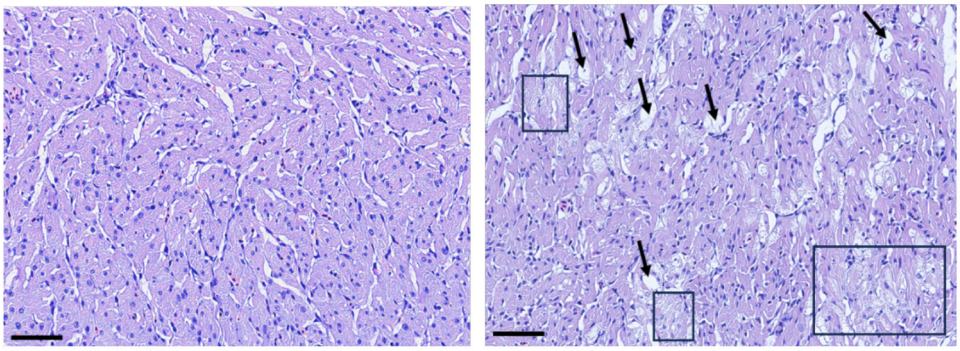

Fig. 1. Left: Normal histology of cardiomyocytes in the ventricular compactum from a salmon sampled prior to delousing (bar= 60 µm). Right: Degenerating cardiomyocytes in the ventricular compactum from a salmon that died after thermal delousing treatment. It shows multifocal areas with swollen, lighter stained cardiomyocytes, with granular (squares) or vacuolated (arrows) cytoplasm (bar= 60 µm).

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Results and discussion

In 14 out of the 20 ventricles sampled from fish that died in conjunction with the thermal delousing, there were changes indicating ischaemia (reduced oxygen supply) in the compact myocardium as previously described by Poppe et al (2021).[8] Microscopic evaluation revealed multifocal areas in the compact myocardium with swollen, lighter stained cardiomyocytes (heart muscle cells), with granular or vacuolated cytoplasm (Fig.1). These histopathological changes are interpreted as cardiomyocyte degeneration due to ischaemia. None of the 20 ventricles sampled prior to delousing showed any histological changes consistent with ischaemia.

The ventricle of the salmonid heart consists of an outer compact myocardium, supplied by the coronary circulation with oxygen-rich blood, while the inner spongy myocardium receives oxygen from the oxygen-poor venous blood returning from the body. Thus, the ischaemic lesions in the compact myocardium observed here indicate that the coronary arterial blood flow is insufficient, and may have caused heart failure and possibly explaining why the fish died during the stressful and likely highly metabolically demanding delousing treatment.

A possible cause of the compact myocardial ischaemia is coronary arteriosclerosis, a condition in which coronary blood flow is partially or completely restricted. Coronary arteriosclerosis is becoming more frequent and severe in farmed Atlantic salmon.[9,10]

Another condition that can reduce the coronary blood supply to the myocardium, resulting in ischaemic lesions, is coronary artery spasm. Several factors can trigger coronary artery spams, including stress and chronic inflammation.[11] 39 out of the 40 ventricles examined from both apparently healthy and dead fish exhibited mild to moderate inflammation in both spongious and compact myocardium that may be compatible with viral myocarditis, most likely heart and skeletal muscle inflammation (HSMI).

Conclusion

Ischaemic lesions were observed in the compact myocardium in the majority of the hearts sampled from fish that died in connection with delousing. No lesions consistent with ischaemia were observed in the hearts from fish sampled prior to treatment. We therefore argue that cardiac failure due to ischaemia was the most plausible cause of mortality.

The underlying cause(s) for the ischaemic lesions observed here are unknow and needs to be investigated further. To do this, we will now examine and quantify the severity of coronary arteriosclerosis in the collected hearts and relate that to the incidence of myocardial ischemic lesions reported here, as well as the overall mortality risk during thermal delousing treatment.

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POTENTIAL OF GENOMIC SELECTION FOR GROWTH AND QUALITY TRAITS IN MIXED-FAMILY BREEDING DESIGNS FOR THE PACIFIC OYSTER *Crassostrea gigas*

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Introduction

Oyster farming is a major aquaculture industry worldwide, with the Pacific oyster being the most widely cultivated species. Controlled reproduction in hatcheries enables the production of genetically improved seed using polyploidy or selective breeding. Selective breeding programs have been applied successfully in different oyster species, including the Pacific oyster, using mass selection and pedigree-based approaches. Recent advances in genomics have led to the development of high-density SNP arrays for several bivalve species, including the Pacific oyster. Genomic selection can accelerate genetic gain by reducing the generation interval and increasing selection accuracy. However, the success of genomic selection relies on the availability of accurate and reliable phenotype and genotype data, as well as appropriate statistical models. The aim of our study was to assess the potential of genomic selection for growth and quality traits in two independent mixed-family breeding designs at commercial scale in *C. gigas* selected lines.

Material and Methods

The study used oysters from two French breeding companies, each from a population that underwent six to eight generations of mass selection, mostly for resistance to OsHV-1, growth, and morphology. The first population resulted from seven full-factorial crosses of ten males and ten females each, generating 700 full-sib families, while the second population resulted from six full-factorial crosses of ten males and eight females each, generating 480 full-sib families. The oysters were phenotyped at 36 and 31 months old for various traits, and their genetic variability was monitored with genetic markers. All parents and offspring were genotyped on the bi-species Axiom Affymetrix 57K oyster array, Axiom_Oyster02, comprising 40,625 markers for *C. gigas*, and quality control analyses were carried out to ensure the quality of the data.

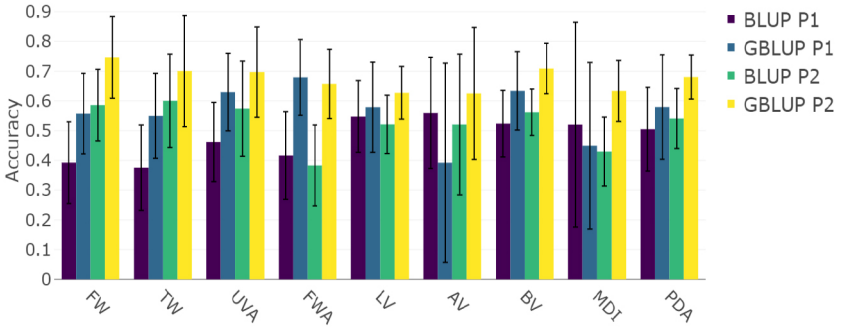

Figure 1. Selection accuracy and standard deviations using BLUP or GBLUP in two studied population for growth and colour traits. FW: Wet Flesh Weight, TW: Total Weight, UVA: Upper Valve Area, FWA: Flesh Weight Adjusted by TW, LV: Mean Upper Valve L value, AV: Mean Upper Valve a* value, BV: Mean Upper Valve b* value, MDI: Mean Darkness Intensity, PDA: Percent of Dark Area*

(Continued on next page)

Results & Discussion

LD (linkage disequilibrium) strongly decreased with distance between pairs of SNPs for both populations, and that P1 had higher LD than P2 throughout the genome. Parentage assignment rates were high in both populations, with P2 having a lower assignment rate due to missing genotypes for four parents. Effective population size (Ne) was estimated at 107 for P1 and 76 for P2. Heritability was estimated for each trait and population and ranged from 0.08 ± 0.04 to 0.56 ± 0.08 for a pedigree-based model and from 0.04 ± 0.02 to 0.69 ± 0.04 for a genomic-based model. Growth-related traits were generally highly genetically and positively correlated with each other, but weakly correlated with colour traits. Accuracy of prediction was generally higher with the genomic model (GBLUP) than with the classical BLUP model, with a maximum gain of accuracy (from 0.38 to 0.66) for flesh weight adjusted by total weight in P2. Accuracy of breeding values was slightly higher for colour traits for P2, with higher heritability estimates.

Conclusion

The study found that genomic selection and mixed-family designs can improve growth and color traits in Pacific oysters. Both breeding programs evaluated showed substantial genetic variation and good genetic diversity. However, better genomic tools are needed, and interactions between genotype and environment should be evaluated to optimize breeding programs for hatcheries.

Acknowledgement

The data presented here were obtained in the Quality-Huitre project which received funding from the European Maritime and Fisheries Fund (EMFF) research grant number PFEA470018FA1000011.

INDUCTION OF *VIBRIO* BIOFILM FORMATION BY BENZALKONIUM CHLORIDE

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Introduction

Vibrio spp. are Gram-negative, rod-shaped bacteria that are widely distributed in freshwater, estuarine, and marine environments. They have the potential to cause infections in animals and are frequently isolated from aquaculture farms, resulting in mss mortalities and significant economic losses. *Vibrio* bacterial pathogens may persist in aquaculture ponds in a biofilm form, a complex microbial structure, attached to a surface and integrated into an adhesive extracellular matrix. This matrix provides protection against environmental stressors, including cleaning and disinfection procedures used in aquaculture industry. Therefore, it is crucial for professionals to use effective disinfectants to eliminate these biofilms and prevent the transfer of *Vibrio* spp. cells from surfaces to food.

Objective

The aim of the present study was to assess the effectiveness of benzalkonium chloride, a commonly used disinfectant in aquaculture farms, against biofilms of *Vibrio* species that are frequently encountered in aquaculture and pose significant economic challenges.

Methods

Two strains of *V. parahaemolyticus*, two strains of *V. alginolyticus*, and one strain each of *V. harveyi* and *V. cholerae* were studied. Biofilms were grown in a 96-well microtiter plate at 23°C for 24 hours to simulate conditions encountered in aquaculture farms. Treatments with disinfectants or water (as control) were applied either before biofilm formation or on pre-formed 24-hour biofilms. The total biomass of the biofilms (matrix and bacterial population) was evaluated by crystal violet staining, the metabolic activity of cells within the biofilms was measured by 2,3,5-triphenyl tetrazolium chloride, a metabolic dye used as a mitochondrial redox potential indicator staining. The viability status of these bacterial populations was then evaluated by flow cytometry and epifluorescence microscopy coupled with a live/dead staining.

Results

Surprisingly, in four out of six strains of *Vibrio* studied, crystal violet quantification showed that benzalkonium chloride induced overproduction of *Vibrio* biofilm biomass. Metabolic activity was not always correlated with the overproduction of biofilm. Flow cytometry and microscopy data indicated that injured cells were responsible for this overproduction of biofilm.

Significance

This study demonstrates for the first time the ineffectiveness of benzalkonium chloride in removing biofilms formed by *Vibrio* species that pose significant economic problems in aquaculture. Worse still, this study shows that benzalkonium chloride induces overproduction of *Vibrio* biofilms, which represents a major risk in the resistance, dissemination and persistence of this pathogenic organism.

PREVENTATIVE CARE: CO-EXPOSURE WITH FUNGAL AND HERBAL STIMULANTS IN RAINBOW TROUT (*Oncorhynchus mykiss*) TO OPTIMISE BENEFICIAL IMMUNE EFFECTS

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Introduction

Disease outbreaks are a major source of economic losses to the aquaculture industry. Preventive biological control strategies, using compounds that also meet food safety standards for subsequent human consumption, are urgently needed to improve animal health and welfare while reducing impacts from antibiotic use on human and environmental health. The addition of functional ingredients or immunostimulants in fish diets is a promising option with the ability to enhance the protective function of both humoral and cellular components of the immune system, increase host resilience and host resistance to pathogens and disease. The use of generally recognized as safe (GRAS) medicinal mushrooms already authorised for human consumption by the U.S. Food and Drug Administration (FDA), such as fungi belonging to Basidiomycetes, have significant potential as immunostimulants for farmed fish, as it was already proved in human or terrestrial animals. In addition, capsaicin, the principal pungent component in hot peppers, has received considerable interest for its implication into numerous biological activities with potential pharmacological application. Several studies have thus shown a correlation between the addition of capsaicin into the diets and increased nutrient uptake. In finfish, capsaicin has been implicated in having a modulating effect on the gastrointestinal mucosal barrier, enhancing the uptake of immunomodulatory compounds such as vaccine particles. While specific interactions within the microbiota remain unpredictable, it is clear that modifying the microbiome with the goal of optimising metabolite bioavailability is a promising approach to improve therapeutic efficacy.

Objective

In this study, we evaluated a holistic and cross-disciplinary pipeline to assess the immunostimulatory properties of two fungi: *Trametes versicolor* and *Ganoderma lucidum*; one herbal supplement, capsaicin in the form of Espelette pepper (*Capsicum annuum*), and a combination of these fungal and herbal additives on rainbow trout(*Oncorhynchus mykiss*).

Methods

After dietary exposure during a 7-week trial, fish were collected to investigate the effect of the feed additives on multiple parameters: growth, cellular and humoral immune parameters (blood samples), molecular immune parameters (spleen samples), and intestinal microbiota characteristics (intestinal samples). Fish cellular immune parameters (number of leucocytes and phagocytosis capacity) were then studied using flow cytometry, alongside with humoral immune parameter (alternative pathway of complement activities) using hemolytic assays, as well as gene expression patterns of specific immune organs (target genes *il-1*β, *ifn-*γ, *tlr2*, and *tnf-*α) using reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR). In parallel, the response of the fish intestinal microbial community to these additives was conducted via metabarcording and predictive functional analyses. Ultimately, this study demonstrates the necessity for congruity between molecular, cellular, microbial, and humoral methods as a prerequisite to establish true changes in fish physiological state following the inclusion of novel feed ingredients.

(Continued on next page)

Results

Uptake of herbal and fungal compounds influenced the expression of immune related genes, without generating an inflammatory response. Significant differences were detected in the spleen-*tlr2* gene expression. Supplementation with herbal additives correlated with structural changes in the fish intestinal microbiota and enhanced overall intestinal microbial diversity. Results demonstrated that the different treatments had no adverse effect on growth performance and survival, suggesting the safety of the different feed additives at the tested concentrations and their potential for application in aquaculture. While the mechanisms and multifactorial interactions driving the observed changes remain unclear, this study provides a holistic and cross-disciplinary investigation not only in regard to nutrition and safety, but also when considering potential mechanisms of action from a combined immune and gut microbiota perspective.

Significance

Novel fungal and herbal feed ingredients are on the crossroad between fish, human, and environmental health, with the potential to improve sustainability without compromising health outcomes. There is a pressing need in existing literature to apply a more holistic and integrative approach as part of the *One Health* concept to assess the impact of such functional additives. The use of compounds in aquaculture that are already authorized for human consumption - hence without environmental or public health concerns - is therefore a promising approach. This study provides a detailed cross-disciplinary investigation not only of the safety of these compounds for use in the target organism, but also provides insight into actual effectiveness in relation to combined immune and microbiological parameters.

MICROALGAL BIOFILMS AS A SOURCE OF UNEXPLORED BIOACTIVE COMPOUNDS: ANTI-ADHESIVE EFFECT AGAINST BACTERIAL PATHOGEN

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Introduction

In food industry and aquaculture, successful bacterial pathogen colonisation and persistence begin with their adhesion to a surface, followed by the spatial development of mature biofilm of public health concern. A biofilm is a complex assemblage of cells embedded in a matrix of Extracellular Polymeric Substances (EPS), conferring bacteria increasing tolerance against antibiotics and disinfectants. Compromising bacterial adhesion with natural inhibitors is a promising alternative to conventional anti-fouling treatments typically based on chemical biocides or antibiotics that contribute to the growing burden of antimicrobial resistance.

An attractive alternative lies within the exploitation of marine microalgae and derivatives, which harbor a wide range of functionalities and bioactive components, such as proteins, lipids, pigments, or exopolysaccharides. Although polysaccharides from algae, bacteria and fungi have shown promising anti-biofilm activities against widespread pathogens, no studies have investigated microalgal EPS for applications in food-contact surfaces. Yet, their high biodegradability and expected non-toxicity, combined with the opportunity of large-scale production thanks to innovative biofilm-based microalgae cultivation technologies, make them ideal candidates for the development of natural and sustainable anti-adhesive surfaces, with promising applications in food industries. Indeed biofilm-based cultures have emerged lately to overcome the main drawbacks of conventional suspended microalgae cultures: low productivity and high operating costs. Especially, the two microalgae *Cylindrotheca closterium* and *Tetraselmis suecica*, are known to be able to develop photosynthetic biofilms and such a self-organized community might be a source of unexplored bioactive agents to tackle biofouling and bacterial biofilms of health concern.

Objective

The objective of this study was to investigate for the first time the bacterial anti-adhesive/antifouling potential of EPS fractions extracted from microalgae biofilms cultivated in innovative large-scale pilots, for application in food-contact materials.

Methods

Microalgal EPSs were extracted from biofilm cultures of *Cylindrotheca closterium* and *Tetraselmis suecica* resulting in the obtention of three fractions: fraction C from *C. closterium* and fractions Ta and Tb (cell-bound and insoluble EPSs respectively) from *T. suecica*. Early adhesion inhibition was tested using eight food-borne bacterial pathogens belonging to *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica* subsp. *enterica*, and *Listeria monocytogenes* and quantified through Confocal Laser Scanning Microscopy (CLSM). In order, to better understand the mechanisms behind the modification of bacterial adhesion, the chemical composition of the different EPS fractions was characterized using analytical methods, including Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy and High-Performance Liquid Chromatography (HPLC).

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Results

The results showed that, among the three EPS fractions, the fraction Ta (*T. suecica*) significantly reduced adhesion of strains of *E. coli*, *S. aureus*, and *L. monocytogenes*. Overall EPSs coating on polystyrene surfaces of the different fractions increased the hydrophilic character of the support. Differences in bacterial adhesion could be explained by several dissimilarities in the structural and physicochemical EPSs composition, according to HPLC and ATR-FTIR analysis. Interestingly, while fractions Ta and Tb were extracted from the same microalgal culture, distinct adhesion patterns were observed, highlighting the importance of the extraction process. Overall, the findings showed that EPS extracted from microalgal photosynthetic biofilms can exhibit anti-adhesive effects against a wide range of food-borne pathogens and could help developing sustainable and non-toxic anti-adhesive surfaces for the food industry.

Significance

Biofilm and bio-fouling formation in food industry and aquaculture are major public health concerns, which represent reservoir of bacterial pathogens that could be armful for animals or human. In order to reduce the growing burden of antimicrobial resistance, the development of novel anti-fouling compounds is of paramount importance. As such, microalgal EPSs which are non-toxic and biodegradable represent a promising alternative for the development of sustainable anti-adhesive surfaces. Besides, such compounds may have inherent wide applications, ranging from marine, agricultural and industrial equipment and aquaculture sea-cages, to biomedical devices such as biosensors and implants.

MODE OF APPLICATION OF A FUNCTIONAL FEED DETERMINES THE IMMUNE RESPONSE OF ATLANTIC SALMON TOWARDS ACUTE OXIDATIVE STRESS

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Introduction

It is widely recognized that stress has profound effects on the immune system of fish. Although actions are undertaken to minimize stress and adverse effects during aquaculture production mitigation strategies are necessary when stress cannot be avoided. A variety of stressors impact health and performance of Atlantic salmon produced in intensive land-based recirculating aquaculture systems (RAS). Among them oxidative stress occurs during the regular disinfection of RAS facilities and is induced by strong oxidants such as peracetic acid (PAA) [1]. Besides its beneficial effects PAA alters the stress and immune response of Atlantic salmon [2,3]. Dietary mitigation strategies may prove useful under these conditions in mitigating the effects of such oxidative acute stress. In this study we explored whether differently applied microalgae enriched functional feeds influence the immune and stress response of Atlantic salmon towards an acute oxidative stress (PAA treatment).

Material & Methods

Atlantic salmon smolts (20 per tank, initial weight ~ 126 g) were reared in triplicates in a recirculating aquaculture system. The fish were fed either a control diet (CD), a diet containing 2 % (CV2) or 14 % (CV14) of *Chlorella vulgaris* on a daily basis, or the diet containing 14 % *Chlorella vulgaris* once weekly (CV14w). Following eight weeks of feeding, all groups were subjected to an acute oxidative stress induced by treatment with peracetic acid (WOFA-steril, Kesla, Germany) at 2.5µl PAA / L water. We assessed growth performance plasma stress indicators as well as gene expression of stress- and immune-related genes in the head kidney and gill.

Results & discussion

Conclusion

References

MULTI-FUNCTIONAL GENOMIC ANALYSES IDENTIFY *IFI27L2A* AS THE GENE MODULATING VIRAL NERVOUS NECROSIS RESISTANCE IN EUROPEAN SEABASS

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Introduction

Viral nervous necrosis (VNN) disease caused by the nervous necrosis virus (NNV) inflicts significant losses to seabass producers through mortalities and impaired growth of the infected fish (Barsøe *et al.* 2021). Genetics studies have demonstrated the potential for improving resistance to the disease through selective breeding in different seabass populations (Palaiokostas *et al.* 2018; Griot *et al.* 2021). Additionally, recent studies have identified genomic regions or markers with significant effect on VNN resistance in different European seabass populations (Palaiokostas *et al.* 2018; Griot *et al.* 2021; Vela-Avitúa *et al.* 2022). However, precise genetic basis of VNN resistance in European seabass remains unknown. Therefore, in the current study we employed multi-omics tools to identify and functionally characterize genomic components underpinning resistance to VNN in farmed European seabass.

Materials and Methods

Approximately 1,500 European seabass from a full factorial cross of 25 dams and 25 sires were challenged with NNV. Mortality of the challenged fish was then recorded twice a day for a period of 29 days and used as the primary phenotype for resistance to the virus (binary survival). Time of death (in days) of the challenged fish was also recorded. A total of 1,066 fish (including the 50 parents and 1016 NNV challenged fish) were genotyped on the MedFish SNP array for 30K SNPs. The 50 parents and 40 offspring were full genome sequenced, and used as references for full genome imputation of all the offspring. Additionally, 322 offspring from the same parents were recruited in a transcriptome challenge experiment where 110 and 212 fish were mock- and NNV-challenged respectively. Tissue samples for RNA sequencing were collected from brain and head kidney. Whole genome GWAS was performed for both binary survival and days to death using GCTA software. Additionally, correlation analyses between gene expression and VNN resistance breeding values were performed. We subsequently performed expression QTL analyses for the main candidate genes identified in the QTL region.

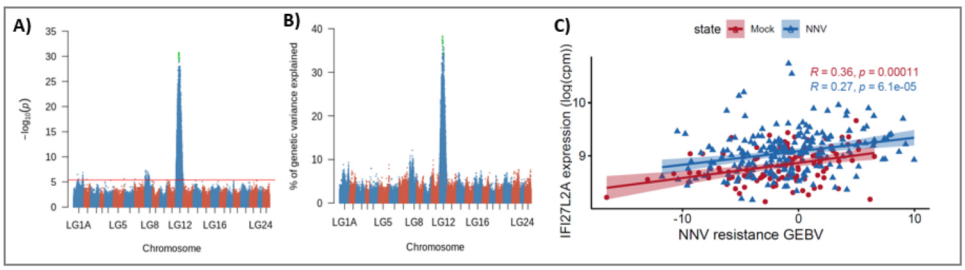

Figure 1: A) Manhattan plot summarizing whole genome GWAS results by P-value; B) Manhattan plot summarizing whole genome GWAS results by additive genetic variance explained; C) Plot showing the correlation between *IFI27L2A* expression and NNV resistance GEBV in the head kidney.

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Results

Our results showed moderate heritability estimates for VNN resistance of 0.400 ± 0.058 and 0.451 ± 0.058, for binary survival and days to death respectively, indicating the potential of selective breeding to reduce the impact of the disease. Of the 8.5M variants, 48,787 were associated with VNN resistance, with a large majority (90%) located on LG12 (scaffold CAJNNU010000003.1) as shown in Figure 1A. The most significant variant explained 37% of the total additive genetic variation in VNN resistance (Figure 1B) and showed a clear additive effect with individuals homozygous for the resistant allele showing 81% survival upon infection. This demonstrates the potential of utilizing marker assisted selection to enhance selective breeding for resistance against VNN. The eight most significant variants were located in the *IFI27L2A* gene, and our results revealed a significant correlation between *IFI27L2A* expression and VNN resistance (Figure 1C). Interestingly, variants with the strongest effect on VNN resistance also showed a clear association with the expression levels of *IFI27L2A*.

Conclusions

Our results provide a more refined insight into the genomic basis of resistance to VNN, which could be utilized for enhancing selective breeding or to design genome editing approaches to produce fish that are more resistant to the disease.

Funding

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OCCURRENCE OF MYCOTOXINS IN COMMON RAW INGREDIENTS USED IN AQUA FEED JAN-JUNE 2023

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Introduction

Mycotoxins are secondary fungal metabolites which are frequently found in various plant-based ingredients and can negatively affect health and performance of aquatic species. The increasing inclusion of plant protein in aqua feed, leads to the introduction of mycotoxins in aqua diets. Mycotoxin occurrence in raw ingredients as well as finished feed is monitored within the DSM Mycotoxin Survey, the longest running global mycotoxin survey available. Global results between January-June 2023 of prevalence and concentrations of the six well known mycotoxins aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), T-2 toxin (T-2), fumonisins (FUM) and ochratoxin A (OTA), in commonly used ingredients are presented.

Material and methods

Samples were analyzed with ELISA, HPLC and LC-MS/MS (liquid chromatography coupled to tandem mass spectrometry) based methods. For Asia, mainly the multi-mycotoxin method Spectrum Top®50 developed by Romer Labs®, was used. This method allows the simultaneous detection of >50 different toxins and metabolites, including masked and "emerging" (unregulated) mycotoxins.

Results and discussion

Table 1 shows the number of samples analyzed, % of samples tested positive for each mycotoxin, average concentration of all positive samples (ppb), median concentration of all positive samples (ppb) as well as the maximum (ppb) detected.

Table 1: Global mycotoxin occurrence in soybean, wheat, corn, corn gluten meal and corn DDGS collected between Jan-June 2023. All concentrations in ppb.

		Soybean + meal	Wheat grain + bran	Corn	Corn gluten meal	Corn DDGS
	Number of samples	1461	705	2874	71	140
Afla	Contamination (%)	35%	13%	21%	41%	18%
	Average of positives	3	3	37	23	11
	Median of positives	2	3	4	18	5
	Maximum	14	8	9 846	163	156
ZEN	Contamination (%)	59%	42%	40%	97%	86%
	Average of positives	47	61	136	1 008	342
	Median of positives	41	38	55	259	211
	Maximum (	241	715	4 310	1 1693	5 357
DON	Contamination (%)	9%	54%	53%	89%	93%
	Average of positives	248	734	958	1 680	2 821
	Median of positives	133	386	569	849	2 199
	Maximum	1 700	10 556	20 440	21 092	16 884
T-2	Contamination (%)	27%	25%	16%	21%	16%
	Average of positives	36	24	54	64	46
	Median of positives	24	18	28	20	40
	Maximum	659	275	665	547	128
FUM	Contamination (%)	14%	28%	70%	97%	98%
	Average of positives	915	468	2 400	6 953	2 004
	Median of positives	536	290	1 060	2 443	1 152
	Maximum	4 672	6 009	142 773	43 136	37 949
OTA	Contamination (%)	4%	8%	9%	24%	11%
	Average of positives	15	13	14	5	8
	Median of positives	5	2	3	3	2
	Maximum	63	162	386	18	44

Afla occurs most frequently in soybean, but at moderate levels. Wheat/-bran shows high abundance of DON. Corn is frequently contaminated with *Fusarium* toxins (ZEN, DON, FUM), but shows also an extreme Afla maximum. Corn gluten meal and corn DDGS are risky ingredients due to high contamination. Poster will also provide details on rice bran, cottonseed - and sunflower cake, rapeseed meal as well as cassava.

Conclusion

Prevalence and concentrations of the six well known mycotoxins underline the importance of a proper mycotoxin risk management including regular testing of ingredients as well as testing of aqua finished feed and inclusion of a mycotoxin deactivator.

RESPONSE TO SELECTION FOR CYTOGENETIC QUALITY IN *Mytilus edulis* AND *M. galloprovincialis*

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Introduction

Mussel is one of the most cultured and high-value species in France. French mussel culture entirely depends on the wild spat collection. In the last decade, Abnormal Mass Mortality (AMM) has been observed in mussel farms along the Atlantic coasts, and the causes are not clearly established. Recent studies suggest that genetic factors are involved in mortality outbreaks, and poor cytogenetic quality is one among them (Degremont et al., 2019; Benabdelmouna et al., 2018). Abnormal mass mortality was shown to be strongly correlated to genomic abnormalities, in particular with a poor cytogenetic quality which is defined as a lower proportion of diploid to non-diploid cells in their haemolymph (Benabdelmouna and Ledu, 2016). The purpose of our study is to estimate the response to selection/realized heritability of cytogenetic quality using a divergent selection in the two main mussel species cultivated in France and to explore the relationship between the cytogenetic quality and the resistance to mortality using a cohabitation protocol with wild mussels sampled in site regularly impacted by AMM.

Materials and methods

Wild adult mussels were collected for *M. edulis* in Agnas and for *M. galloprovincialis* in Biarritz in January 2022. For each species, 420 mussels were phenotyped for cytogenetic quality after collecting haemolymph using flow cytometer analyses (FCM). For each species, a divergent selection was applied using an intensity of selection of 1.40 (i.e. 20% of the population selected for each group) producing a low and a high cytogenetic quality groups, respectively LCQ and HCQ, as well as a control group. In May 2022, five replicate spawns per group were produced for each species using two to five females and three to six males per spawning. Spawning, larval rearing and grow-out culture were carried out in our experimental hatchery at ASIM, Ifremer, La Tremblade, France. The cytogenetic quality of each replicate spawn will occur in summer 2023. Survival evaluation started on March 2023, using a cohabitation protocol between groups and wild mussels sampled in a mussel farm impacted by AMM. Thirty mussels per replicate spawns are tested in triplicate in a flow-through tanks, and mortality is recorded weekly until the summer 2023.

Results

The mean proportions of non-diploid cells were significantly higher for *M. edulis* (12%) than *M. galloprovincialis* (9%) (p <0.0001). The previous studies on non-diploid cells percentage of French mussels stocks varies between 2.44% to 21.37% (Sample size = 74-117) before mortality events (Benabdelmouna and Ledu, 2016). The mean proportions of non-diploid cells of LCQ, control and HCQ mussels were 28%, 12%, and 3% for *M. edulis*, and 26%, 9%, and 2% for *M. galloprovincialis*. HCQ has been found significant between the species (p <0.0001). However, LCQ have not showed any significant difference (p = 0.36) between species. The mean of non-diploid percentage of selected parents is shown in figure 1, and those of their progenies will be known during the summer, as well as their survival examined in the cohabitation experiment will be identified in summer. The mean mortality of the mussel species after 45 days was showed less than 1%.

Expected outcomes

It will be the first report on the possibility of selection for cytogenetic quality in shellfish. The previous results suggest that mortality level is significantly negatively correlated with the cytogenetic quality of the mussel. Selection for cytogenetic quality would not only improve the survival against the specific pathogen, but it should also improve the overall health status/fitness of the animal. If the cytogenetic character is heritable, it will give an important insight into the selection approach on the cytogenetic quality of the shellfish and will lead to new selection criteria for disease/survival/welfare traits of the shellfish.

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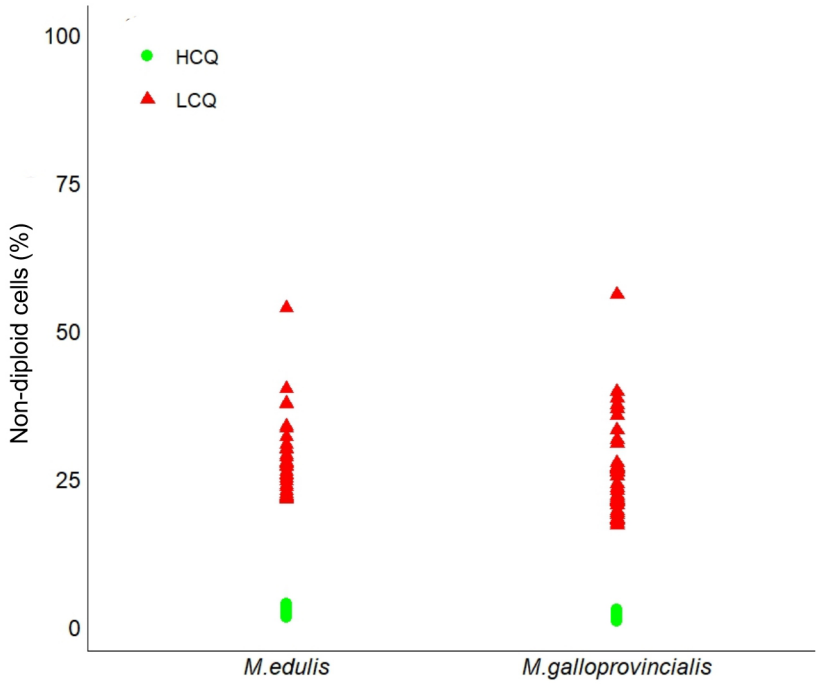

Figure 1: Percentage of non-diploid cells for selected parents used to produce the high and low cytogenetic quality groups, respectively HCQ and LCQ, in *Mytilus edulis* and *M. galloprovincialis*.

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GENERATION OF CALCIUM-SENSING RECEPTOR (CASR) KNOCK-OUT MEDAKA *Oryzias latipes* AND ITS PHENOTYPIC ANALYSIS

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Introduction

There is a growing use of plant ingredients in aquafeeds due to limited availability of marine protein sources (fishmeal). However, plant proteins included at high levels reduce fish growth, which can be partially explained by their lower digestibility (Krogdahl et al., 2022; Murashita et al., 2022). It is, therefore, important to understand the physiological mechanisms involved in the regulation of digestion, and how these are affected by nutrients and dietary ingredients. In mammals, the complex regulatory interaction between nutrient sensing and digestion involves the Calcium-sensing receptor (CaSR). However, the physiological roles of Casr in fish are still unclear. To explore the role of Casr in the regulatory mechanisms of fish digestion, this study described the tissue distribution of the *casr* gene, generated a *casr* knock-out (KO) model and analyzed its phenotype with focus on digestive enzyme secretion and growth, using medaka *Oryzias latipes*.

Materials and methods

Tissue distribution of *casr* gene in medaka**:** A Cab inbred strain of medaka was used in this study. The tissue distribution of medaka *casr* gene was analyzed by real-time quantitative RT-PCR (qPCR) in sixteen different tissues (whole brain, pituitary, eye, tongue, gill, skin, muscle, hart, gallbladder, liver, spleen, kidney, gonad, foregut, midgut and hindgut) (n = 8 fish).

Generation of *casr* KO medaka: *casr* gene was targeted and the gene-specific sgRNA was synthesized. A mixture of Cas9 protein and the synthesized sgRNA was injected into fertilized eggs before the first cleavage. The sgRNA activity (induction rate of somatic mutation) in the F0 embryo and its germ line transmission rate in the F1 fish (offspring of F0 founder and wild type (wt)) was estimated by High Resolution Melting analysis. The obtained F1 mutants were genotyped and heterozygous mutants (+/-) were selected. The selected F1 heterozygous mutants were incrossed to generate null F2 mutants (-/-). The population of null mutants was expanded by incrossing of the seleted null mutants.

Phenotypic analysis of *casr* KO medaka: To compare digestive enzymes secretion in wt and *casr* KO fish, the same amount of commercial diet (2.5% body weight) was fed, and the intestinal content was collected. Activities of digestive enzymes (trypsin, chymotrypsin and lipase) of the collected intestinal contents were analyzed (n = 8 fish). This can be regarded as the levels of secretion from the pancreas. To compare the growth rates between wt and *casr* KO mutant, fish were fed with two isoenergetic (20.5 kJ/g) diets with different protein/lipid ratios, a high protein diet (HP; protein/lipid, 60%/11%) and low protein diet (LP; protein/lipid, 53%/15%) for seven weeks (from 3 to 10 weeks, triplicate tanks). Every week, the fish body weight, total length and Fulton's condition factor were recorded until 10-week post hatch.

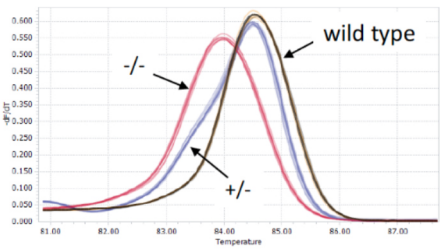

Fig. 1. Detection of *casr* mutant by High Resolution Melting analysis.

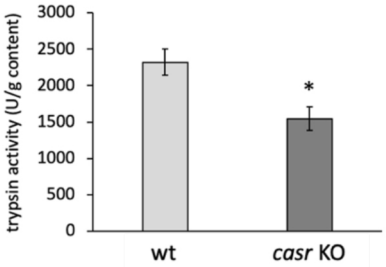

Fig. 2. Digestive enzyme (trypsin) secretion. Trypsin enzyme activitiy of the intestinal content was analyzed to estimate its secretion levels. Asterisk (*) shows significant difference between the strains (t-test, p < 0.05).

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Results and Discussion

Tissue distribution of *casr* gene in medaka: *casr* gene was highly expressed in the kidney and in all intestinal segments, suggesting the functions related to Ca^{2+}excretion and nutritient sensing, respectively, exist in medaka as seen in mammals (Hannan et al., 2018).

Generation of *casr* KO medaka: The induction rate of somatic mutations in the F0 embryo (n = 24) and its germ line transmission rate in the F1 fish (n = 46) were both 100%. The mutation in genotyped/isolated F1 mutant of *casr* gene showed a frameshift with 19-bp deletion, and the mutant allele yields a truncated protein due to an additional region of altered translation, in which a stop codon is generated before the functionally important domain of Casr. The homozygous mutant was successfully generated by incrossing the selected heterozygous mutants and the population was expanded (Fig. 1).

Phenotypic analysis of *casr* KO medaka: Lower secretion levels of proteases (trypsin (Fig. 2) and chymotrypsin) were observed in the *casr* KO fish compared to the wt fish whereas no diffrences were found in the lipase secretion between the strains. Regarding the medaka growth performance, although no differences were found between the *casr* KO and wt strains for the fish fed the LP diet, *casr* KO fish fed the HP diet showed markedly lower growth performance compared to the other diet/strain groups.

The findings of this study indicate that fish with *casr* KO demonstrate a lower capacity for protein digestion, potentially due to a decrease in the intestinal nutrient (protein) sensing ability.

Acknowledgments

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RESPONSIBLE CATFISH IS NEXT ON THE MENU! THE SCOPE EXTENSION PROJECT OF THE AQUACULTURE STEWARDSHIP COUNCIL (ASC)

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The Aquaculture Stewardship Council (ASC) is the world's leading certification scheme for farmed seafood. In 2022, the total global production of ASC certified seafood amounted to more than 1.9 million tonnes, which corresponds to 1.55% of the global aquaculture production, while certified fish accounted for 1.4 million tonnes, meaning 2.4% of the total finfish global production. Our certification programme uses market-based approaches that incentivise famers to achieve strict standards of environmental and social performance. Compliance with the requirements of ASC Standards can help to reduce farm's environmental footprint, conserve natural resources and contribute to worker's well being.

Currently, the ASC covers 17 species groups under 11 standards, plus a joint ASC-MSC standard for seaweed. They all provide industry leading, robust, environmental and social requirements, but because they have been developed at different times, their requirements vary on some of the impacts that are common across aquaculture. Therefore, ASC is currently aligning all 11 standards into one ASC Farm Standard. This will cover all impact areas and all currently certifiable species in one rigorous standard. Additionally, it will also allow the ASC to more efficiently expand its reach (e.g. adding production systems or new species) and will facilitate the swifter update to specific metrics. The ASC Farm Standard will include a core set of indicators and will monitor legal, social and environmental impacts consistently across species and regions and set species-specific limits where necessary (e.g., mortality levels, feed conversion, etc.). New species will be added to the ASC portfolio by applying common management and system specific indicators form the ASC Farm Standard. Additional species-specific indicators will be evaluated for addition when the current indicators do not align cover major impacts of the new species. As a matter of fact, there is a huge potential for aquaculture volumes that are not within scope of the current 11 standards. This potential is explored by the ASC Species Scope Extension Project.

The next group of interest for the Scope Extension Project is catfish, including the following species: *Clarias gariepinus; Ictalurus punctatus; Hybrid of Heterobranchus Longifilis X Clarias Gariepinus*. The production of *Clarias gariepinus* has been exponentially increasing since the 1990s and peaked in 2015 with more than 250K tonnes of live weight produced in aquaculture (Food and Agriculture Organization of the United Nations, 2023). The genus *Clarias spp.* accounts for 2.3% of the total fish production in 2020, according to the FAO and it is a fish species with a remarkable potential for the development of freshwater aquaculture. They are air beathing species, meaning that they are highly adaptable, widely distributed geographically and tolerant to adverse conditions (Lisachov et al., 2023). *Ictalurus punctatus* is also a species of high commercial interest worldwide. It is a key aquaculture species especially in North America and Asia (Wen et al., 2023). It has a high economic importance in China due to its adaptability and resistance (Yu et al., 2023). Finally, catfish hybrids such as the "heteroclarias"(*Heterobranchus Longifilis X Clarias Gariepinus)* have been widely used to select ideal parental features for breeding fast growing, efficient and healthy fish (Sobczak et al., 2022). Catfish and its hybrids are therefore gaining importance worldwide.
All these species seem to have competitive profit margins for large-scale production which is leading farmers to opt for more intensive aquaculture practices with associated social and environmental risk as well as risks for the fish welfare (Wise et al., 2023). These risks will be covered by the implementation of the ASC Farm Standard with species-specific indicators. Thus, the ASC sees an urgent need to include these species the portfolio to actively address major concerns and issues.

Our goal is to involve relevant stakeholders in the content development, especially in regard to metric setting. To set credible metrics, data from literature and real farm data will be used. Expanding ASC's scope to include the potential for catfish certification expands accessibility to ASC standards and increased the environmental and social benefits awarded through certification. Farmers also benefit from this scope extension as ASC certification allows them to drive additional revenue and income though access to global market demand, demonstrate commitment to responsible farming practices, workers, communities and to have access to education in effective and efficient farming. This is the next step to bring responsible catfish on our consumers' tables!

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REPRODUCTIVE BEHAVIOR AND PARENTAL CONTRIBUTION OF MEAGRE (*Argyrosomus regius*) IN AQUACULTURE CONDITIONS, IN RELATION TO SOUND PRODUCTION DURING SPAWNING

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Introduction

The meagre (*Argyrosomus regius*) is an emerging species for the Mediterranean aquaculture industry and its reproductive dysfunctions have been resolved using gonadotropin releasing hormone agonist (GnRHa)-based spawning induction protocols. However, to eventually be able to obtain fertilized eggs spontaneously, more information on the environmental requirements of the species and its breeding behavior is needed. Although sound production has been described so far in little more than 1100 fish species, recent studies demonstrate that this communication modality is shared by nearly two-thirds of actinopterygian species. The meagre is a member of the Sciaenidae family -called "drums" or "croakers"- and has a wide vocal repertoire, emitting sounds made from a single pulse up to more than 100 pulses. The calling rate and sound temporal features have been suggested as indicative of reproductive events. The aim of this study was to describe the reproductive behavior and parental contribution of meagre in aquaculture conditions, after spawning induction with (GnRHa), and document sound production during spawning and investigate how sounds may relate with specific behaviors in aquaculture facilities.

Materials and Methods

Two meagre broodstocks (n=2 females and 3 males, each) were utilized for two consecutive years (2021 and 2022). At the expected spawning period, fish were induced to spawn with a GnRHa injection (15 μg kg^{-1} for females) or implant (50 μg kg^{-1} for males). Each breeder was externally tagged for individual identification, and their behavior was monitored using underwater cameras. Egg production and quality was evaluated in terms of fecundity, fertilization success, 24-h embryo survival, hatching and 5-d larval survival. A sub-sample of the eggs from each spawn was analyzed for parental contribution. In 2022 only, two HTI- 96-Min hydrophones were also connected to the audio input of the cameras. Using Adobe Premiere, audio files were extracted from the videos before and after spawning induction (5–13 May, 2022). All audio files were analyzed by audio and visual assessment (Raven Pro 64 1.4). Sounds produced by the fish were manually labelled on the basis of the number of pulses as knocks (1-3 pulses), or short (4-7 pulses), intermediate (8-29 pulses) and long grunts (≥30 pulses). When a sound could be associated with one of the identified stereotype behaviors -such as "male(s) trailing a female", "darting", "male-to-male agonistic behavior" or "spawning" the occurrence was annotated from the video observations.

Results

Spawning induction led to three consecutive daily spawns, 2 days after GnRHa treatment, with maximum fecundity on day 3. Spawning usually took place with all three males following a female (Fig. 1), and after numerous male-to-male agonistic interactions. Mean daily fecundity (n=4 years x broodstock) was 159,743±23,315 eggs kg^{-1}, and mean fertilization success and 24-h embryo survival were 83-92% and 93-100%, respectively.

Of the eggs produced in both years, 77±3% were spawned by one of the females in each tank, while 77±9% of the eggs from each tank were fertilized by one of the three males in each broodstock. In broodstock 1 (Fig. 2), the larger male fertilized 68±9% of the eggs in both years and in broodstock 2, the most aggressive male fertilized 86±8% of the eggs.

After GnRHa treatment, the number of sounds produced increased significantly during spawning days, especially during the evening hours when spawning took place. Long grunts, in particular, were emitted only during spawning days. Knocks, on the other hand, were associated with all identified behaviors and were the only sound type associated with male-to-male agonistic behavior. Grunts, on the other hand, were associated with "male(s) trailing a female" during spawning days.

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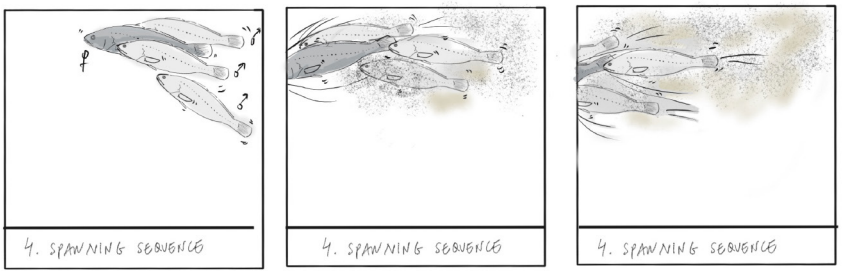

Fig. 1. Spawning sequence with one female (darker) being trailed by all males in the tank.

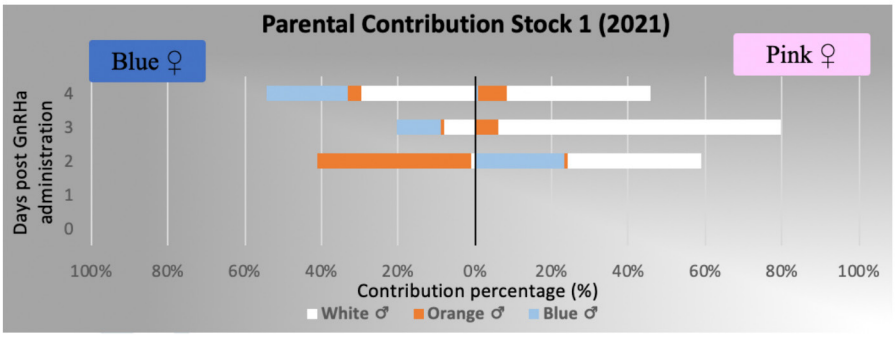

Fig. 2. Female (left and right side of the Y-axis) and male (different grey shades of each horizontal bar) parentage contribution in one of the meagre broodstocks in 2021.

Discussion

The study demonstrated that hierarchies developed within meagre broodstocks, and even after exogenous GnRHa stimulation of all individuals in the broodstock, spawning success was not equal among breeders. In both years, the same male and female dominated the produced progeny. It is not clear at this stage what factor contributes to dominance of the two sexes, especially in females, but in males it may be size or aggressiveness. The video and sound production results agree with previous studies that recorded long grunts during spawning nights, as well as with studies conducted in the wild, which reported long choruses of knocks. Knocks and long grunts are, therefore, suggested as "carriers of information" during spawning in meagre. In the future, we will examine the potential of sound "play-back" as an environmental inducer of spawning in cultured meagre and a disruptor of male hierarchies.

The work was funded by the project NewTechAqua (EU H2020, GA 862658).

THE USE OF CHEMICAL AND BIOLOGICAL SETTLEMENT CUES IN ENHANCING THE LARVAL SETTLEMENT OF ABALONE *Haliotis midae*: IMPLICATIONS FOR HATCHERIES AND OCEAN RANCHING

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Introduction

The seeding of abalone larvae has been considered as a viable alternative for stock enhancement programs due to high production costs of raising juvenile abalone for seeding (Preece et al., 1997). However, poor larval settlement and metamorphosis in the hatchery is one of the significant constraints with seed production on abalone farms, resulting in low post-larval survival (Gapasin and Polohan, 2004; Li et al., 2006). Improving settlement and metamorphosis of abalone larvae is critical to ensure that abalone larvae settle within the seeding site for ocean ranching or to increase production in hatcheries. This study investigated the effect of biological (planktonic *Nitzschia* sp.) and chemical (potassium chloride) cues in inducing settlement and metamorphosis of *Haliotis midae* larvae. Specifically, the effects of dilution of such cues upon release of the larvae into the ocean is immitated by removing the settlement cues after a certain time period.

Materials and Methods

Three potassium chloride concentrations (10, 15, and 20 mM) and a control (with no KCl) were used to determine the optimal concentration for potassium ions to induce larval settlement for *Haliotis midae*. The optimal concentration of KCl was used to determine the effect of longer exposure period (12- and 24-hours) on the larval settlement and metamorphosis with the treatments also include the combination of KCl optimal concentration and biological cues (*Nitzschia*). Larval settlement and metamorphosisi was also determined after a brief exposure to chemical and biological cues and a combination of both using settlement sheets that were coated with diatoms.

Results

For larvae exposed to different KCl concentrations (10–20 mM), settlement was highest at 10 mM in the first 20 hours. Larval settlement exposed to KCl-12 hours and KCl and *Nitzschia* combined-24 hours was higher in the first 20 hours than larvae exposed to KCl-24 hours and the controls (Figure 1). However, in both experiments, larval settlement in the controls increased over time and surpassed other treatments after 20 hours. Finally, the settlement was very low on uncoated sheets, compared to diatom-coated sheets, regardless of exposure to different combinations of KCl and *Nitzschia*.

Discussion

Due to the dramatic decrease in the mean settlement post-exposure, these results should be interpreted with caution when drawing biological conclusions for field studies. We, therefore hypothesize that exposure of *Haliotis midae* larvae to 10 mM KCl and *Nitzschia* will not enhance settlement in the ocean, as the inducers are primarily effective when KCl concentration levels remain at 10 Mm. The level of K^+ in the ocean are typically below 10 mM (Todd et al.,1991) and it does not improve settlement without adding extra KCl in the seawater. However, long-term exposure to both KCl and *Nitzschia over* 24 hours could be used in hatcheries to improve the settlement of *Haliotis midae* larvae.

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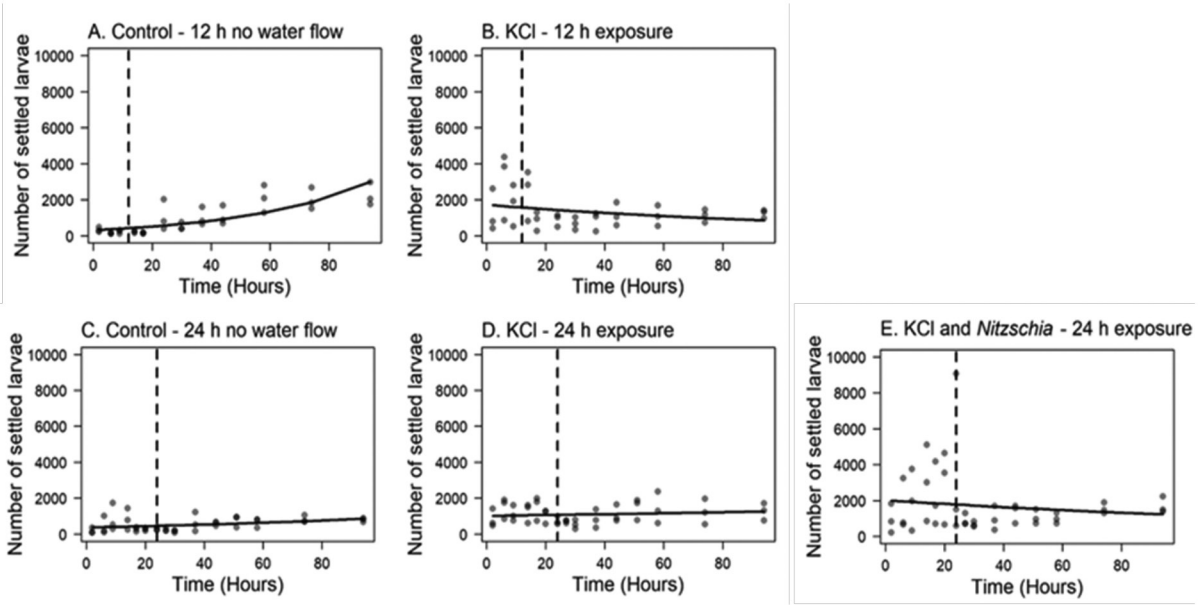

Figure 1: Number of settled abalone (*Haliotis midae*) larvae during and post-exposure for 12 hr to 10 mM KCl, 24 hr to KCl, and exposure to a combination of 10 mM KCl and *Nitzschia* for 24 hr with control treatment for each exposure time. The vertical dashed line indicates exposure period for each treatment and no water flows for each control treatment.

AQUACULTURE ADAPTATIONS TO CLIMATE CHANGE: INVESTIGATING THE EFFECTS OF SHADING MATERIALS ON THE REARING ENVIRONMENT

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Climate change with its heatwaves and water shortages has an impact on aquaculture. Aquaculture practice has a strong need to find ways on how to adapt to these changing conditions. One option to increase the resilience of the sector towards heatwaves is the application of shading material.

In order to investigate the effects of different shading materials, a 31-days summer experiment (28.06-28.07.2022) was conducted using twelve fish-free round tanks (Depth: 100cm, diameter: 158cm). Each unit had a total volume of 1.96m³ and was embedded in the ground at a depth of approx. 80cm. Tanks were randomly equipped with four different shading nets (0 (control), 40, 60 and 85% grade) in triplicates. The nets were installed 115cm above the top of the terrain. At the beginning of the experiment, the tanks were initially filled with water from Lake Sacrow to a level of 80cm. No exchange of water was conducted. Henceforth, water temperature (°C), oxygen concentration (mg/L), turbidity (FNU) and water level (cm) were monitored on daily basis by manual measurements. Furthermore, data loggers for air and water temperatures as well as light intensity (lx) were installed in and above the tanks. In order to determine the growth of periphyton (in g dry matter/m²), plastic stripes were placed in the tanks. In addition, a weather station was operated at the experimental site (Lat.: 52.449182, Lon.: 13.098415).

The shading nets had a significant effect on air and water temperatures. Mean air temperatures were reduced by 3.1 to 6.7 °C and water temperatures by 2.3 to 4.0 °C. Here, a significant decrease in water temperatures could already be reported in tanks with the installation of 40% shading material. Also, the increase of the water temperature (ΔT) in the tanks between 10 a.m. and 2 p.m. was reduced when applying shading material. Hereby, an increase of 2.8°C was observed in the control, 1.6°C at 40% shading, 1.1°C at 60% shading and 0.6°C at 85% shading. Thus, shading nets can effectively reduce water temperatures and provide homogenization of rearing conditions. Illumination levels above and within the tanks were reduced according to the material properties of the nets, which also went along with a homogenization of the rearing environment.

Only the 85% shading grade significantly increased the water level. But, in addition to the reduction of evaporation, an increased collection of precipitation was observed when applying this particular material. This can have implications in practical applications, as this rainwater accumulation could affect the statics of shading units.

Shading had no significant effect on oxygen concentration and turbidity in the tanks, but it did influence periphyton growth. Compared to the periphyton biomass in the control (6.2g DM/m²), shading significantly increased the growth of periphyton (up to 15.9g DM/m²). Associated effects and arising consequences for tank cleanness, fish and product quality aspects need to be evaluated in future research.

In view of the results, the installation of shade material appears to be a simple, effective and relatively inexpensive measure for aquaculture practice that supports adapting to heatwaves and water shortages. Results will also be discussed with perspective on the use of floating photovoltaic in aquaculture.

DEVELOPMENT OF A LARGE-SCALE SPERM CRYOPRESERVATION METHOD AND TESTING ITS APPLICABILITY IN PROPAGATION OF CHUB (*Squalius cephalus*)

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Introduction

Chub is a rheophilic species, preferring rapid rivers with gravelly bottoms. It is distributed in Europe and Asia Minor (Caffrey et al. 2008). Based on monitoring studies natural populations of chub have decreasing tendency due to overexploitation, loss of spawning habitat, water pollution, interspecific hybridization, remarkable angling demand and climate change (Cejko & Krejszef 2016). The improvement of applicable propagation technology, in which sperm cryopreservation plays a key role, is important for maintaining and stabilising the populations and for gene conservation. Cryopreserved sperm collected from farmed or natural populations is part of maintaining biological and genetic diversity. It can also contribute to increasing the natural abundance of fish species with high angling interest (Bernáth et al. 2021). The study aimed to compare the effects of two hormonal agents (Ovopel and carp pituitary extract) on chub sperm production, its cryopreservation and post-thaw storage time, moreover, the development of a novel large-scale cryopreservation procedure by testing various freezing methods.

Materials and methods

Experiment 1.

In the first experiment, 6-6 males were hormonally stimulated with two hormone preparations, carp pituitary extract and Ovopel. Sperm samples were frozen at a dilution ratio of 1:9 (sperm:diluent + protectant) in 0.5 mL straw in a Styrofoam box. Sperm motility assessment was carried out using Computer-Assisted Sperm Analysis (CASA) system. Sperm quality was determined by pMOT (progressive motility, %), VCL (curvilinear velocity, µm/s) and LIN (linearity, %). Motility parameters were recorded for fresh, cryopreserved and thawed chilled stored samples (at 0, 3, 6 h post-thawing).

Experiment 2.

In the second experiment, (*N*=5) sperm samples were cryopreserved in a Styrofoam box (dilution ratio: 1:9) using 5 mL straws and 4 mL cryotubes. Motility parameters of both fresh and thawed samples were recorded by CASA.

Experiment 3.

In our first propagation test, sperm samples (*N*=5) were pooled and filled into 4 mL cryotubes (dilution ratio: 1:9). The samples were frozen in a Styrofoam box and a Controlled-rate freezer (CRF). The kinetic parameters of fresh and frozen samples were recorded by CASA. In the experiment, 1-1 g of egg batches were fertilized with 10 μl of fresh and 100 μl of cryopreserved sperm. The control and cryopreserved groups were incubated separately at 19±0.8 °C using spawning nests. The hatching rate (hatched larvae per total egg number * 100) was determined for both groups (~100 larvae or eggs) at the moment of hatching (3 days post fertilization).

Experiment 4.

The second fertilization trial was carried out based on the results of the previous experiments (Ovopel, dilution ratio 1:1, 4 ml cryotube, Styrofoam box). Fertilization of 1-1 g egg batches was performed with fresh (10 μl) and frozen (20 μl) sperm. Kinetic parameters were recorded using CASA system in fresh samples, immediately before application (control, ~1 h post-collection) and after thawing. The hatching rate was determined for both fresh and frozen groups.

Results

Experiment 1.

No significant difference was measured between the carp pituitary extract and Ovopel treated groups for fresh and cryopreserved sperm samples (0, 3, 6 h post-thaw). Reduced pMOT values were also observed after 3 hours of chilled post-thaw storage in comparison to 0 h.

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Experiment 2.
There was no significant difference in the motility parameters tested between the groups frozen in a Styrofoam box using 5 ml straws and using 4 ml cryotubes.

Experiment 3.
No significant difference was observed between the efficacy of the two cryopreservation methods either in terms of kinetic parameters or in terms of hatching rates (Styrofoam box (35±7%) and CRF (25±9%).

Experiment 4.
In the second fertilization trial, in pMOT and VCL parameters, a significant decrease was observed in the cryopreserved sperm in comparison to the fresh and control groups. Moreover, similar high (with no significant difference) hatching rates were observed in the control (72±19%) and cryopreserved (4 ml cryotube and Styrofoam box, 61±5%) groups.

Discussion and conclusion
Although there was no significant difference between the treatments. The chub sperm showed high sensitivity during 6 hours of chilled storage, the pMOT values were already significantly reduced after 3 hours. Before its implementation in common hatchery practices, it needs to be considered. Both the 5 mL straw and the 4 mL cryotube have been proven to be effective in freezing large-scale chub sperm. Both sperm frozen in a Styrofoam box and in a CRF seemed to be effective in propagation. For the first time, our study presents a novel and applicable method for the large-scale cryopreservation of chub sperm.

Acknowledgements
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EFFECT OF DIFFERENT REARING ENVIRONMENTS ON THE WATER QUALITY AND ON THE PRODUCTION TRAITS OF HYBRID STRIPED BASS *(Morone saxatilis x Morone chrysops)* IN HUNGARY

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Introduction

In past decades, aquaculture production in the European Union has stagnated and even a slight decrease can be observed. In 2020, 89 % of aquaculture production was used directly for human consumption while further growth of aquaculture industry is forecasted with increasing fish consumption and declining natural populations (FAO, 2022). However, the further growth of production can be slowed down by the lack of usable land, the increasingly scarce water resources, and the ecological carrying capacity of the surrounding areas. An intensification trend can be observed in the world aquaculture production, especially in Asia. These systems typically have high nutrient retention. Therefore, continuous monitoring of water quality plays a very important role in modern aquaculture production. The deterioration of water quality is caused by the inputs (fertilizer, complete feeds) used to increase production (Boyd, 2018). These substances result in greater production, on the other hand, it can lead to phytoplankton blooms and a decrease in dissolved oxygen (Boyd and Tucker, 1998). The aim of the study was to determine the effect of different rearing systems on water quality and on the growth of hybrid striped bass *(Morone saxatilis x Morone chrysops)*.

Materials and Methods

The 13-week experiment was carried out at the Hungarian University of Agriculture and Life Sciences (MATE) Research Centre for Fisheries and Aquaculture (HAKI), Szarvas, Hungary. 1-year-old hybrid striped bass individuals with an average initial weight of 128.5±8.1 g were randomly distributed in two replicates to small ponds (treatment SP, surface 324 m^2) and large ponds (treatment LP, surface 700 m^2), in two replicates to cages in the small ponds (treatment SPC, 3x3x2 m, 18 m^3) and in the large ponds (treatment LPC, 3x6x2 m, 36 m^3). Common carp (*Cyprinus carpio*) were also stocked in the ponds containing the cages. Fish were fed with commercial floating feed for African catfish (*Clarias gariepinus*) (Aller Aqua Group, Denmark). The fish were fed 1-3 % of the biomass. Water samples were collected twice per week from the outflow of each pond. At the beginning of the experiment, 32 randomly chosen specimens were measured for length (± 1cm) and weight (± 1g) individually. During the experiment, sample harvest was conducted every two weeks to monitor biomass growth.

Results and conclusions

Data of growth parameters and production traits determined at the end of the experiment are summarized in Table 1. After thirteen weeks of rearing significant differences were found between the groups in terms of growth (final weight, conditional factor, SGR and DGR). The final body weight was significantly affected by both the technology and the size of the ponds and the cages. The SGR and DGR were affected significantly by the technology. The parameters of water quality are summarized in Table 2. The technology had a significant effect on all parameters examined. NH_4-N and TN values were also affected by the size of the rearing environment. In term of water quality and production traits the most favourable result was achieved by monocultural rearing.

Aknowledgement

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Table 1. Effect of different rearing systems on the production traits of hybrid striped bass

Production trait	Rearing systems				*p*-value	
	SPC	SP	LPC	LP	technology	size
Initial weight (g)	129.2±7.4	127.1±7.1	126.4±8.4	132.1±9.1	0.434	0.629
Final weight (g)	282.9±10.9[a]	301.9±14.8[b]	287.3±12.7[a]	313.1±17.2[c]	< .001	0.016
Condition factor final	1.6±0.1[a]	1.4±0.0[b]	1.5±0.1[a]	1.5±0.0[b]	< .001	0.668
SGR (% day^{-1})	0.9±0.0[ab]	1.0±0.0[bc]	0.9±0.0[b]	1.0±0.0[bc]	0.010	0.170
DGR (g day^{-1})	1.7±0.0[ab]	1.9±0.0[b]	1.8±0.0[b]	2.0±0.1[bc]	0.007	0.188
FCR (g g^{-1})	1.6±0.1	1.4±0.0	1.5±0.1	1.5±0.0	0.102	0.560
Survival (%)	99.3±0.4	99.6±0.6	99.8±0.3	99.3±0.8	0.764	0.764

Table 2. Effect of different rearing systems on the water quality

	Rearing systems				*p*-value	
	SPC	SP	LPC	LP	technology	size
NH_4-N (mg/l)	0.14±0.06[a]	0.11±0.05[b]	0.11±0.05[b]	0.10±0.05[b]	0.005	0.002
TN (mg/l)	1.31±0.55[a]	0.75±0.37[c]	1.04±0.34[b]	0.69±0.34[c]	<.001	0.004
TP (mg/l)	0.18±0.05[a]	0.13±0.07[b]	0.17±0.04[a]	0.12±0.05[b]	<.001	0.936
TSS (mg/l)	80.8±25.7[a]	5.7±5.0[b]	74.8±21.8[a]	5.5±6.2[b]	<.001	0.203
Chl-a (mg/l)	32.6±17.1[a]	12.5±15.9[b]	38.3±21.7[a]	13.9±18.6[b]	<.001	0.171
DO (mg/l)	6.4±1.0[a]	7.3±1.0[b]	6.7±1.0[a]	7.1±0.8[b]	<.001	0.669

THE USE OF CARBON AND NITROGEN STABLE ISOTOPES TO ELUCIDATE TROPHIC TRANSFERS BETWEEN SEABREAM, SHRIMP, CLAM AND OYSTER REARED IN AN INTEGRATED MULTITROPHIC AQUACULTURE PONDS

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Introduction

In a context of rapid development of aquaculture, ecosystem responsible practices are a deep challenge. Aquaculture feeds have been traditionally based on fish meal (FM) and fish oil (FO) derived from fisheries of small pelagic species. Efficient alternatives to FM and FO are crucial to preserve wild fish populations. Another concern of aquaculture is the release of high amounts of wastewater into the environment. The excess of particulate and dissolved nutrients, i.e., uneaten feed and metabolic wastes of fish, induce the eutrophication of surrounding ecosystem. The generic concept of Integrated MultiTrophic Aquaculture (IMTA) covers a large set of practices but is based on the production of fed species (*e.g.*, finfish fed sustainable commercial diets) with extractive species, which utilize the inorganic (*e.g.*, seaweeds) and organic (*e.g.*, suspension-feeders) excess nutrients from fed aquaculture for their growth (Chopin, 2013). IMTA have the principal advantages to decrease the dependence on external inputs, increase the system efficiency, diversify farm-products and decrease waste effluents. An important challenge, to increase the development of IMTA practices, is the understanding of trophic relationship between species co-habiting in the system. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) natural stable isotopes have proven to be a useful tool to characterize trophic interactions in aquatic ecosystem (Fry and Sherr, 1984). The use of stable isotopes is based on the assumption that isotope signatures of consumers reflect those of assimilated dietary sources. The $\delta^{13}C$ signatures of consumer tissues are usually close to those of their diets, making possible an elucidation of the origin of food sources. In contrast, $\delta^{15}N$ signatures are enriched by 3.5‰ between a consumer and its prey and thus are typically used to estimate the trophic position of a consumer.

In this study, seabream (*Sparus aurata*) was raised in one pond and feed with commercial pellets exclusively containing terrestrial plant ingredients. Unmarked batches of mussels were given to seabream as supplementary food to underbalanced deleterious effects of the total replacement of FM and FO in their diet. In three other ponds, filter-feeders (oyster and clam) and detritivores crustacean (shrimp) have been raised. The four ponds were only connected by water flow. The aim of our study was to determine the food sources used by seabream, shrimp, clam, and oyster raised in this IMTA system. The proportion of commercial diet and mussels used by seabream were quantified. We also evaluated the ability of shrimp, clam, and oyster to feed on fish detritus. The present study is part of SIMTAP (Self-sufficient Integrated Multi-Trophic AquaPonic systems) a PRIMA project aiming to improving food production sustainability and brackish water use and recycling.

Material and methods

From June 2020 to September 2020, experiment has been conducted in 4 ponds on the Atlantic French Coast at the "Lycée de la Mer et du Littoral, Bourcefranc le Chapus" (fig.1.). Seabream (*Sparus aurata*) were fed 5 a week with commercial pellets and once a week with mussels at a rate of 3.7 ± 1.3% of live weight day^{-1} and adjusted according to water temperature. Commercial feed and mussels were distributed to seabream with a ratio of 0.86:0.14. The oyster (*Crassostrea gigas*), shrimp (*Penaeus japonicus*) and clam (*Ruditapes philippinarum*) were not artificially fed. At the end of the experiment, 10 individuals from each species were collected in each ponds. Sediment organic matter (SOM) and suspended organic matter (SPOM) were also sampled in each pond. Sampled were dried, weighed and packed into pressed tin capsule for simultaneous $\delta^{13}C$ and $\delta^{15}N$ analysis, using an isotope ratio mass spectrometer interfaced with an elemental analyzer. The contribution of food sources to diet were estimated using Bayesian stable isotope mixing models (simmr package of R software, Parnell et al. 2013).

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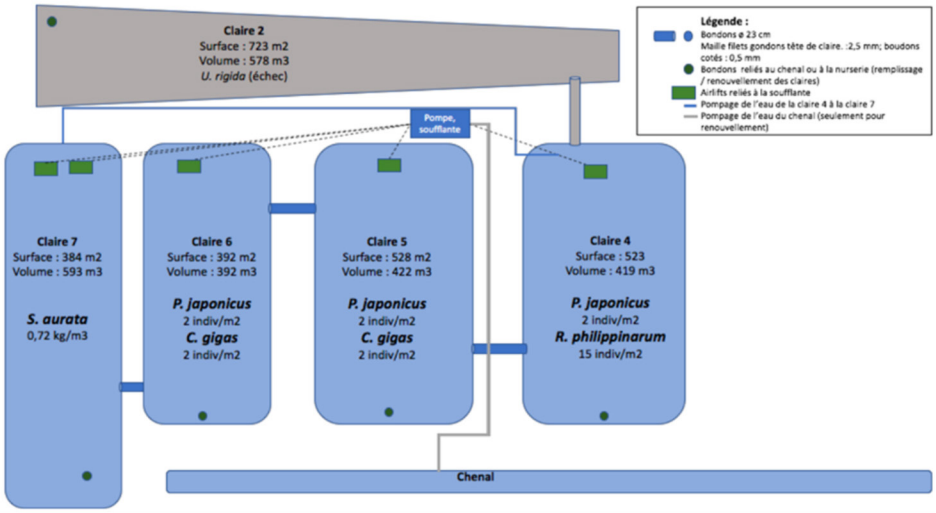

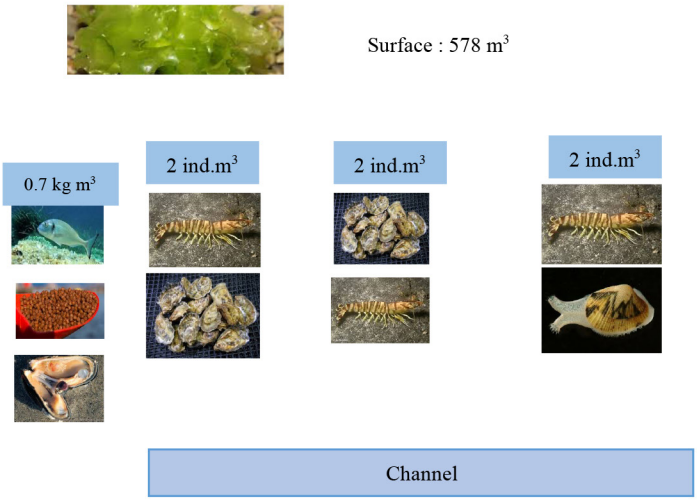

Results and discussion

Seabream, oyster, clam, and shrimp had significantly different $\delta^{13}C$ and $\delta^{15}N$ values ($p < 0.05$). Seabream were enriched by 5.5‰ and 5.49 ‰ in ^{13}C and ^{15}N respectively compared to commercial feed. Mixing model indicated that commercial feed contributes for 72.7% while mussels contribute for 27.3% to seabream diet. Proportion of mussel used by seabream was higher than those distributed (14%). Such difference is probably due to a better appetence and/or digestibility of mussel than plant-based diet. Oysters were enriched by 0.35 ‰ and 3.03 ‰ in ^{15}N and ^{13}C compared to SPOM that is consistent with a consumption of SPOM. Oyster did not directly ingest fish aquaculture waste. However, oyster helped to regulate the level of SPOM (i.e., bloom of phytoplankton and zooplankton) that is boosted by the release of nitrogen and phosphorus by fish. Mixing model indicated that clam consumed 67.3% of SPOM and 32.7% of SOM. Clams were in competition with oyster for SPOM but helped to reduce the level of organic matter in sediment. Shrimp had the higher $\delta^{13}C$ and $\delta^{15}N$ values suggesting that they not directly fed on organic matter from sediment but had a carnivorous diet feeding on naturally present organisms in sediment. The results of this study confirm that seabream, oyster, clam, and shrimp have complementary diets and can be raised together in IMTA ponds.

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ATLANTIC SALMON'S CORE INTERFERON RESPONSE GENES REGULATION IN REACTION TO POLY I:C

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Examining the genome-wide regulatory response of Atlantic salmon to viral infection is central to understanding the control of antiviral immunity. This study investigated the epigenomic and transcriptomic response to stimulation with the viral mimic poly I:C in Atlantic salmon. We employed ATAC-seq and ChIP-seq in combination with RNA-seq analysis to comprehensively examine changes in chromatin accessibility, histone modifications and gene expression. We identified a core set of 197 interferon-stimulated genes (ISGs) that were epigenetically modulated and highly up regulated in response to poly I:C. Fifty-four of these genes were also ISGs in rainbow trout, zebrafish, and humans, highlighting their evolutionary conservation. Our analysis revealed key transcription factors involved in the interferon response, including IRF9, STAT1, and STAT2. Regulatory elements showing increased activity to poly I:C were enriched in conserved vertebrate binding sites for STAT6, PRDM1, IRF6, JDP2, NR2E1, and BCL6, suggesting their central roles in the antiviral immune response. Focused analysis of Interferon Stimulating Response Elements (ISRE) indicated that genomic regions containing ISREs were modulated in response to poly I:C stimulation. Finally, we investigated differences in response to poly I:C stimulation among key ISGs retained as salmonid-specific paralogues, including MX, RSAD2, IRF9, DHX58, STAT1, IRF7 and CD9. This revealed paralogue-specific enrichment of ISRE motifs in promoter regions. Overall, this study provides novel insights into the regulatory mechanisms underlying the antiviral response in Atlantic salmon and underscores the significance of the epigenetic landscape in transcriptional regulation.

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TEMPORAL VARIATION OF DIGESTIVE ENZYME ACTIVITY IN FLATHEAD GREY MULLET *Mugil cephalus* FED INCREASING LEVEL OF BACTERIAL SCP

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Introduction

Grey mullet (*Mugil cephalus*) is a promising omnivorous species for the sustainable diversification of aquaculture due to its low dietary nutrients requirements and the broad market for its roe (bottarga). Bacterial single cell ingredients produced by fermenting industry by-products represent a sustainable alternative of fishmeal and soybean meal (Glencross et al., 2020). The application of bacterial single cell protein (SCP) ingredient was assessed on growth, digestive enzyme activity and on the gene expression of PepT-1 at three different time T0 (0h before feeding), T1 (0/6h after feeding), T2 (6/12h after feeding). To the best of our knowledge, this is the first characterisation of digestive enzymes at different times in grey mullet under captive conditions and different diets.

Materials and Methods

Three isonitrogenous, isolipidic and isoenergetic experimental diets were formulated to contain increasing inclusion levels of bacterial SCP (SCP0, 0%; SCP10, 10%; SCP20, 20%). Diets were tested on triplicate fish groups of 45 individuals (initial weight: 67.9 g) over a period of 113 days. During the trial, feed was provided to apparent satiation during a 6 hours meal once a day, temperature was kept at 26.8 ± 2.5 °C and oxygen above 7.5 mg L^{-1}. At the end of the trial final body weight (FBW), specific growth rate (SGR), feed intake (FI) and feed conversion rate (FCR) were calculated. Digestive enzyme activity (total alkaline protease, trypsin, chymotrypsin, leucine aminopeptidase, α-amylase, bile salt-activated lipase), anterior and posterior intestine of brush border (aminopeptidase and alkaline phosphatase) and gene expression of PepT-1 were performed in three different times T0 (0h before feeding), T1 (0/6h after feeding), T2 (6/12h after feeding). Differences among treatments were considered significant at $P < 0.05$.

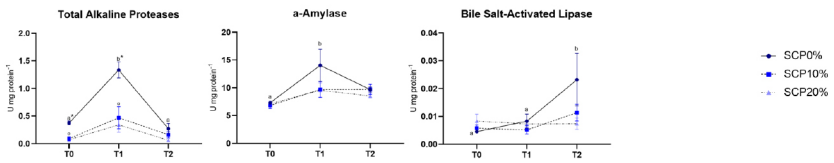

Figure 1. SCP0, SCP10, SCP20 diets at different times for total alkaline proteases, α-amylase, bile salt activated lipase. Differences among each treatment and its timing (T0, T1, T2) were considered significant at P< 0.05 and are marked with letters. Different symbols (*°) stand for significant differences at P< 0.05 between times for the same diet.

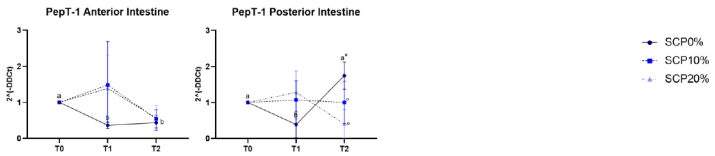

Figure 2. SCP0, SCP10, SCP20 diets at different times for gene expression. Differences among each treatment and its timing (T0, T1, T2) were considered significant at P< 0.05 and are marked with letters. Different symbols (*°) stand for significant differences at P< 0.05 between times for the same diet.

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Results

At the end of the trial, fish fed SCP0 showed a significantly higher body weight and SGR and a significantly lower FI and FCR compared to SCP10 and SCP20 ($P < 0.05$). Temporal variation of digestive enzymes activity in different diet is reported in Fig.1. In fish fed SCP0, total alkaline proteases activities, trypsin and chymotrypsin increased significantly at T1 in comparison to T0, followed by a decreasing pattern at T2. No significant temporal variation for total alkaline protease, trypsin and chymotrypsin was detected in SCP10 and SCP20. On the other hand, in T0 and T1 the SCP0 diet was statistically different from SCP10 and SCP20. No significant differences were found in T2. In leucine aminopeptidase fish fed SCP0 showed an increase significantly in T2 compered to T0 and T1 which gave the same results. The same trend has been found for other diets. Fish fed with SCP10 and SCP20 in T2 exhibited significant differences from T1 and T0 that followed the same values. Comparison between time with diet, it turned out that in T1 the diet values SCP0 were different from SCP10 and SCP20 values. No differences in T0 and T2. In fish fed SCP0 diet, α-amylase was significantly higher in T1 than T0. No differences were found in the comparison between time with diet. In bile salt-activated lipase, fish fed SCP0 diet in T2 showed a significantly high increase compared with fish fed SCP0 in the other two times. No differences in the comparison between time with diet. The results measured in the intestinal brush border (anterior and posterior intestine) were very similar to each other. Significantly higher differences of aminopeptidase were encountered in fish fed SCP0 diet in the anterior intestine at T1 than fish fed SCP0 at T0 and T2. No significant temporal variation for aminopeptidase was detected in SCP10 and SCP20. No differences in comparison of time with diet. No significant temporal variation for alkaline phosphatase were found. Instead there has been observed a significant difference in the time-diet interaction. In the posterior intestine, alkaline phosphatase displayed an increase in fish fed SCP10 diet at T1 from SCP0 diet at T1. In the results of gene expression of PepT-1 as shown in Fig. 2, there was a significant reduction in the anterior intestine in fish fed SCP0 diet at T0 than T1 and T2. No significant temporal variation was detected in SCP10 and SCP20. No differences in the comparison time with diet. There was a significant reduction in fish fed SCP0 diet in posterior intestine at T1 than T0 and T2. In the comparison time with diet, it turned out that in T2 in posterior intestine the diet values for SCP0 were higher from the SCP10 and SCP20 values.

Discussion and Conclusion

The lower performance of *C. glutamicum* SCP as a protein source for grey mullet is probably due to a particular organisation of the digestive system because there was incomplete protein utilisation of SCP due to poor lysis of cell-wall components and also associated with the captive conditions (failure in the development of a functional gizzard). Analyses of enzyme activity showed the important key role in the physiology of the grey mullet digestive system. Proteases, in particular, played a key role but were negatively affected by diet. The temporal variation of grey mullet is similar to that found in the literature for other Mediterranean species of commercial interest. These are the first results concerning the enzymatic activity of the grey mullet. This highlights the extreme importance of analyses of the physiology of the species and the most adaptable diet.

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FEEDING IS THE MAIN DRIVER OF GUT MICROBIOTA ACTIVITY DYNAMICS IN GREATER AMBERJACK JUVENILES *Seriola dumerili*

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Introduction

Gut microbial communities are highly dynamic and respond to factors such as diet and host metabolism. However, there is limited knowledge on gut microbiota changes during the daily cycle. In aquaculture, the characterization of gut microbiome dynamics is necessary to identify and understand the relationship it have on host physiology and fitness. To investigate how interactions between feeding and water temperature affected the diel dynamics and activity of the microbiota, we performed two experiments with water temperature and feeding regime as experimental variables, respectively. The qualitative and quantitative composition of the active bacterial community in feces was analyzed along a day cycle in greater amberjack (*Seriola dumerili*) juveniles.

Materials and methods

In the first experiment, with water temperature as experimental variable, fish were reared in three independent RAS systems set to 18, 22 and 26°C, under a light/dark photoperiod and fed *ad libitum* three times daily with a commercial diet. In the second experiment, the fish had two different feeding regimes, continuous feeding or intermittent feeding (three meals per day), both under continuous illumination. Water temperature was maintained at 22ºC. Sampling was performed along a 24h cycle. For quantitative abundance and activity, we quantified copy numbers of total 16S rDNA genes and rRNA per gram of feces by quantitative PCR (qPCR), and used these data as a proxy of absolute bacterial density and protein synthesis, respectively. RNA:DNA ratio was used as an approximation for the specific activity of bacteria. The qualitative composition of the active bacterial community was analyzed by rRNA-based amplicon sequencing.

Results

Results from the first experiment identified feeding as the main driver modulating the diel variations in bacterial community activity. A peak in bacterial activity (based on RNA:DNA ratio) was observed at 6 h after first meal for the three temperatures (Fig. 1).

The composition of the active bacteria community in fish feces varied in relation to postprandial time. At the order level, feeding promoted a decrease in the relative abundance of active Spirochaetes and an increase in Vibrionales. Although the relative abundance of active Mycoplasmoidales was stable throughout the day in all treatments, the absolute abundance of active bacteria peaked at first postprandial sampling (6 h) for this order, being the order mainly responsible for the increase in absolute bacterial activity. At the lowest water temperature, the diel variations were slower.

Results from the second experiment confirmed the role of feeding as a synchronizer of the diel dynamics of the activity of the bacterial communities in the fish gut. Fish fed continuously did not have significant daily variations either in quantitative or qualitative composition of the active bacterial community. By contrast, and in agreement with the data shown above, intermittently fed-fish revealed a peak in specific bacterial activity after the first daily meal and postprandial changes in the relative abundance of active bacteria along the day. At the order level, the composition of the active bacterial community in continuously fed fish was dominated by Vibrionales during the whole day cycle, with minor fluctuations in its relative abundance (Fig. 2).

PERMANOVA based on the Sørensen similarity indicated that communities' composition from the two feeding regimes, based on the ASV table, were different depending on sampling time. Active bacterial communities were significant different at the beginning of the day, before the first meal was offered to the intermittently fed-fish (0 h, $p<0.05$) and close to being different ($p<0.08$) at the end of the day cycle (8 and 12 h after the last meal for the intermittent fed-fish).

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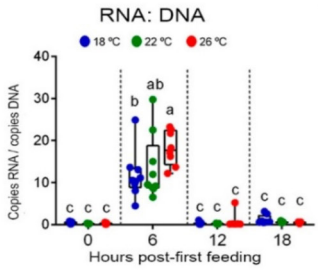

Fig. 1. Bacterial specific activity (16S rRNA:16S rDNA ratio) in feces of greater amberjack reared at 18, 22 and 26ºC at different sampling points (n = 9 per temperature). Different letters mean statistical differences between sampling points (one-way ANOVA, $p<0.05$).

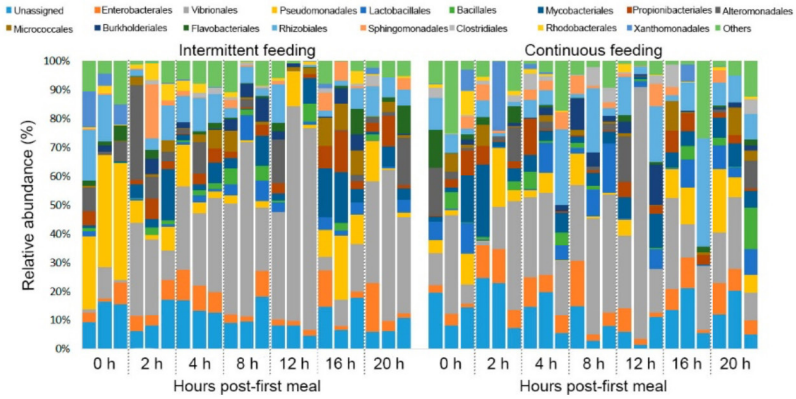

Fig. 2. Composition of the active bacterial community at the order level expressed as relative abundance in *S. dumerili* juveniles fed on an intermittent (three meals per day) or continuous feeding regime sampled along a 24h cycle. The plot represents the most abundant orders (> 1%).

Discussion

This is the first study assessing the quantitative and qualitative effects of water temperature and daily feeding rhythm on the gut microbiota dynamics in a poikilothermic animal. 16S rRNA:rDNA ratio revealed a postprandial increase in bacterial-specific activity, pointing to feeding as the main driver of the bacterial activity dynamics. The most noticeable change in the composition of the active bacteria community was a postprandial increase in the order Vibrionales, but for absolute abundance, Mycoplasmoidales was the most responsive order. Thus, the absolute abundance of active bacteria is more suitable for analyzing bacterial dynamics in a community. Low water temperature affected the timeline of the diel pattern in bacterial activity by slowing down the response, probably due to a lower maximum growth rate of the bacteria and a slower gut transit time in fish.

The composition of active bacteria community in continuously fed fish kept an “active-feeder” profile throughout the whole day, with Vibrionales as the dominant order in terms of both, relative and absolute abundances. Sørensen similarity indicated significant differences in active bacteria community between “pre-feeding” and “active-feeder” fish.

Acknowledgements

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TISSUE EXPLANTS AS TOOLS FOR STUDYING THE EPIGENETIC MODULATION OF GH-IGF-I AXIS IN FARMED FISH

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Introduction

Growth rate in farmed fish is the phenotypic trait of major relevance for the aquaculture industry. Several strategies have been followed to increase growth rate in fish such as genetic selection and improvements in diet formulation. Somatic growth in fish, as in other vertebrates, is mainly controlled by the growth hormone (GH) / insulin-like growth factor I (IGF-I) axis. The molecular and physiological features of this axis have been thoroughly studied in several vertebrates. In fish, GH/IGF-I axis functionality in fish depends on endogenous and environmental factors such as genetic polymorphisms and nutritional status. There are now several evidences that fish GH-IGF-I axis can be modulated also by epigenetic mechanisms such as DNA methylation. However, the role of epigenetics in regulating this axis in fish is far from being understood as results, in addition to sparse, are poorly consistent among species regarding genes affected. The present study aimed to evaluate *ex-vivo* models for epigenetic studies in *Sparus aurata* and to provide the first assessment in this species of the putative role of DNA methylation on the modulation of GH/IGF-I axis.

Materials and methods

Gilthead seabream (*Sparus aurata*) fish (body weight 37 ± 0.8 g; mean ± sem) were maintained in 120 L tanks under optimal conditions for the species. Fish were fed by hand to apparent satiety (approx. 5% of body weight) twice a day with a commercial diet (Skretting). Fish were euthanized with overdose of 2-phenoxyethanol and decapitated. Pituitaries and liver explants were culture in microplates in sterile 80% (v/v) L15, pH 7.4, 10% FBS, 10 mM HEPES, 100 IU/ml penicillin, 100 µg/ml of streptomycin, 2.5 ug/mL amphotericin B, 5.5 mM D(+)-glucose, and 380 mOsm, for 48 h at 23ºC under 95% O_2 and 5% CO_2. Viability and functionality of explants were evaluated by histology and measurements of LDH, GH, IGF-I and antioxidant response. In the case of liver, several conditions were evaluated until good results obtained. The effects of DNA methylation effectors; decitabine (5-Aza-2'-deoxycytidine; DAC) and genistein (GEN) as DNA methylation inhibitors and S-adenosylmethionine (SAM) as the main donor of methyl groups for DNA methylation, were evaluated on the expression of several GH-IGF-I related genes in liver explants after 24 h exposure.

Results

Both tissue explants retained the morphology of corresponding tissues in control fish for 48 h. Culture of pituitaries for 48 h did not increase LDH in the media and glands retained its LDH content, kept GH values into the media indicating that GH cells retained its secretory activity, and only presented slight signs of oxidative stress. Initial attempts to maintain liver explant failed, with more than 50% of LDH recovered in the media after 24 h. Thus, we went through a series of optimization steps (changing agitation, number of washing steps, volume of media, size of explants and temperature) until explants kept their LDH and IGF-I content, and showed no signs of oxidative stress. The exposure of liver explants to SAM for 24 h did not produce variations in the expression of the genes studied. Likewise, the exposure of liver explants to GEN or DAC did not alter *igf1*, *igfbp1a*, *igfbp1b*, and *igfbp2b* expressions. However, GEN and DAC enhanced significantly *igfpb2a* expression, and decreased significantly the expression of *igfpb4*, *ghri*, and *ghrii* (**Figure 1**). Experiments are being conducted to assess the effect of SAM, GEN and DAC on the *gh* expression in pituitary.

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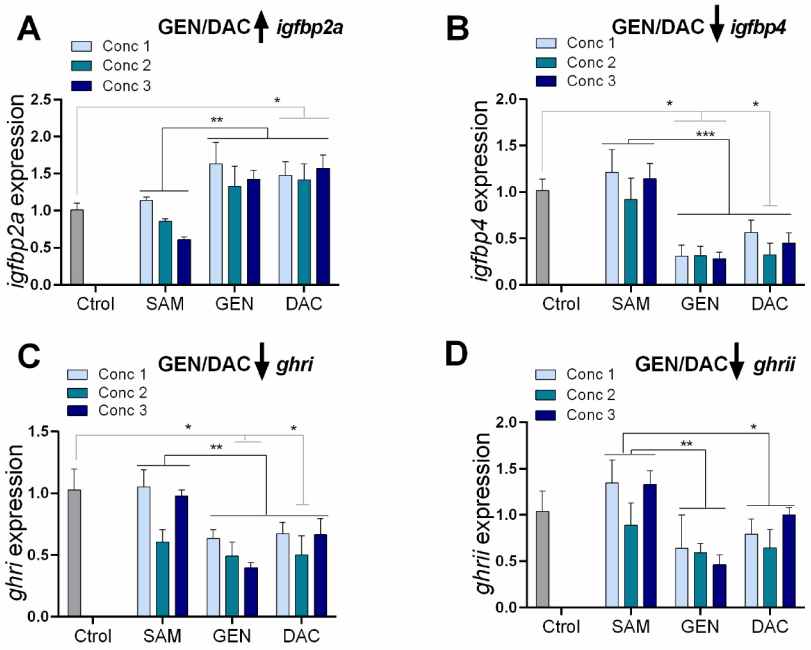

Figure 1. Effects of the methyl donor SAM and DNA methylation inhibitors (GEN, DAC) on the expression of GH-IGF-I related genes in liver explants of *Sparus aurata*. Concentrations are SAM (100, 200, 300 µM), GEN (5, 10, 20 µM), DAC (5, 15, 20 µM). Data are presented as mean ± sem. Asterisks denote differences among treatments (Two-way ANOVA, $p < 0.05$, with substance and concentration as sources of variation) and respect to controls (Dunnett's test, $p < 0.05$).

Discussion

Our results support the use of pituitary and hepatic explants in short-term (i.e., 24-48 h) assays, which is an experimental window equal or longer than that used, for instance, in 78% of research with other *in vitro* approaches such as precision cut liver slices. Given that *igfbp-2a* has been related with reduction in fish growth, that *igfbp4* seems to exert growth promoting effects in some fish, and that hepatic GH receptors are crucial for initiating GH signal transduction, the gene expression pattern obtained after a 24 h exposure to GEN and DAC is indicative of impaired growth. These results indicate that some genes involved in IGF-I production are directly or indirectly modulated by DNA methylation and that a methylation environment may increase growth in this fish or preclude negative impacts on growth of environmental stressors. Studies are ongoing to identify the genomic regions involved and the *in vivo* effect of surplus methyl donors in diets. Collectively, these studies will shed light on the potential to foster growth in this species.

Acknowledgements

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CAN THE GENETIC BACKGROUND OF GILTHEAD SEA BREAM CHANGE THE ACTION OF FEED ADDITIVES? ANSWERS FROM GUT MICROBIOME AND TRANSCRIPTOME INTERACTIONS

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Introduction

The sustainable growth of modern aquaculture must rely in the production of healthy and robust fish fed with diets overcoming the dependence on fish meal (FM) and fish oil (FO). Selective breeding and functional feeds should be keystones towards this development, though most of their synergies remains mostly unexplored. Certainly, recent studies in gilthead sea bream highlighted that selection for growth co-selects for a plastic microbiota capable of exerting a wide nutritionally-mediated metabolic response with less community changes (Piazzon et al., 2020). Otherwise, the use of feed additives as boosters of overall fish performance has expanded rapidly as an alternative to antibiotics and chemotherapeutics, with also the capacity to modify the gut microbiota and host transcriptional associations. However, we are far from establishing the ultimate mode of action of each feed additive for a given genetic background. To bridge this gap, we investigated the effect of a battery of feed additives upon gut microbiota and host transcriptomics in reference (REF) gilthead sea bream and genetically improved fish for growth (GS) within the PROGENSA® selection program.

Material and methods

Estimated breeding values ranged between -159.14 for REF fish and +223.18 for GS fish. The basal diet (CTRL; no feed additive) was formulated by Skretting to be a low FM diet (7.5%), completely devoid of FO. Feed additives (INVE Technologies) added to CTRL diet by oil-coating included a phytobiotic based on natural plant extracts (PHY), a mixture of organic acids (OA), and a *Bacillus*-based probiotic (PROB). After an acclimation period of two weeks with the CTRL diet, fish continued to be fed with either CTRL, PHY, OA or PROB diets until the end of the trial (97 days). At this end-point, tissue portions of anterior intestine (AI) were taken for transcriptional (RNA-seq) and AI scrapped mucus for adherent microbiota analyses, using the Illumina platform and RDP database. Additionally, AI and posterior intestine (PI) sections were used for histological survey.

Results

The GS fish presented higher growth rates and condition factors, lower feed conversion ratios, and an enhanced homogeneity in terms of microbiota composition, regardless of the additive. The PHY effects were especially remarkable in the intestinal transcriptome of higher growth GS-PHY fish, with a particular up-regulation of markers of epithelial integrity (*vil1*, *chmp2a-b*, *vps4b*), sphingolipid metabolism (*degs1*, *elovl1*, *sgpp1*, *plekha8*), high-density lipoproteins secretion to vascular tissues (*abcg8*, *abca1*, *nr1h3*), and bile salt-activated lipase and receptor (*cel*, *nr1h4*). Facing OA, the gut adherent microbiota of REF fish shifted towards a less pathogenic profile, with a reduction in *Staphylococcus*, *Streptococcus*, and *Neisseria* genera correlated with neutrophil degranulation genes. This profile showed inferred bacterial processes involving organic acids, such as ABE fermentation and TCA cycle, and was prone to exert vitamin K biosynthesis. The *Bacillus*-based PROB diet affected both microbiome and transcriptome features. *Bacillus* genus was stablished in the fish gut regardless of the fish genotype, and anti-inflammatory histology patterns were increased in the AI and PI of PROB fish. GS-PROB showed an increased proportion of the nitrate reducer *Kocuria*, and a reduction of the pathogenic *Photobacterium damselae*, in parallel with a better feed efficiency of GS-PROB fish than the rest of the groups. This group also showed the up-regulation of markers of epithelial regeneration and integrity (*ezr*, *ncstn*, *plec*, and *neurog3*) in concurrence with the down-regulation of markers of protein synthesis, that correlated with *Chromohalobacter*, *Enhydrobacter*, *Vibrio*, and *Acinetobacter*.

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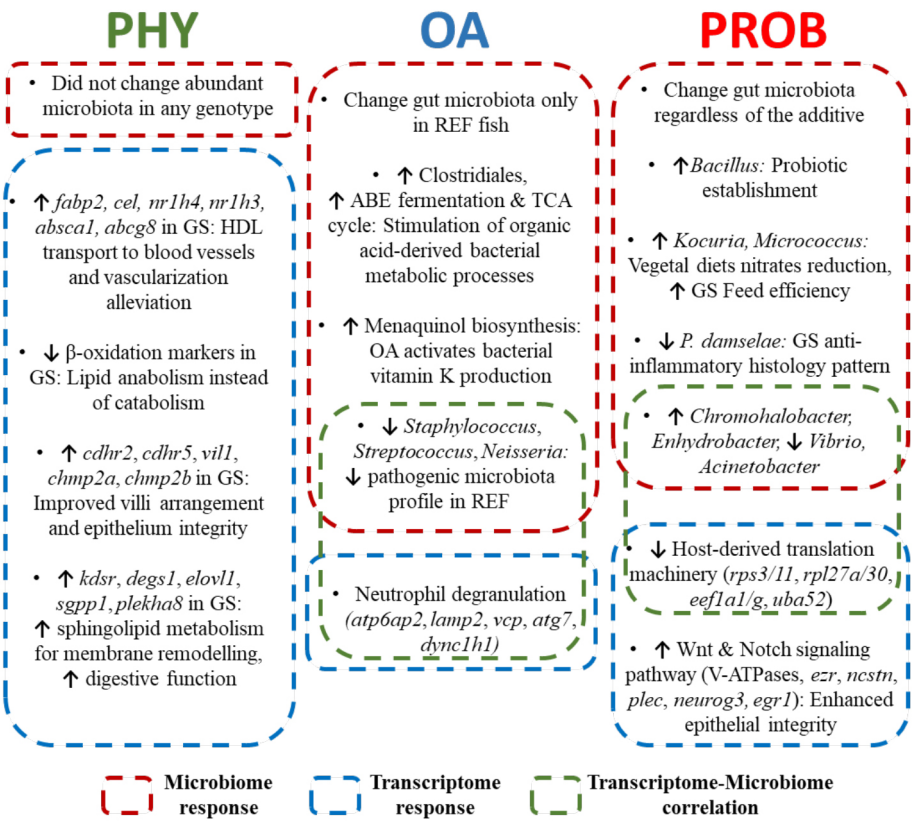

Figure 1: Proposed model for integrative associations of gut adherent microbiota, and intestinal transcriptome of gilthead sea bream fed with three additives.

Concluding remarks

As a general rule, it was confirmed that the gut microbiota variability among individuals was drastically reduced in GS fish (Naya-Català et al., 2022). The intensity and the specific effect of a given additive upon host transcriptomics and gut microbiota varied depending on the genetic background (Figure 1). Thus, PHY only shaped the transcriptome of GS fish. Conversely, OA shaped the gut microbiota of REF fish, whereas PROB triggered changes in both host transcriptome and gut microbiota of both GS and REF fish. Altogether, this work has generated a list of taxa and transcripts associated to a particular feed additive and fish genotype, which might help nutritionists, breeders and farmers to know which microbial and host elements are susceptible to be targeted in order to preserve and improve the gut function of PROGENSA® farmed fish.

References

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EFFORTS TO ADDRESS BIODIVERSITY IMPACTS OF AQUACULTURE IN INDUSTRIAL SYMBIOSIS

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Today, the strongest driver of increased demand for fish is growth in affluence, specifically in China and Southeast Asia, where population is no longer growing, but consumption is, now that consumers can afford it. As the natural fish stocks are exploited to the maximum, the supply of this seafood demand can only be satisfied through increased aquaculture production. It is essential that this is done through best practices to ensure the sustainability of the industry. WA3RM has initiated partnerships with aquaculture projects with the intention to play an important role in the development of modern sustainable industrial scale food production systems. We initially looked at it from the point of efficient energy use. Industrial waste, such as surplus heat, as well as CO2 and sludge are most often seen as costly problems. We look at them as very valuable resources. What we have realized though is that sustainable aquaculture must address more than this. The understanding of how the industry affects biodiversity is a necessity if we should develop land-based RAS as an alternative and as a complement to commercial fishing and off-shore aquaculture.

By making use of huge waste heat streams from large scale industry we will be able to invite fish farm operators to establish land-based operations in geographical areas previously not considered suitable. This will enable an all-year optimal production of several warm water species even higher up in the colder parts of the Nordic countries. WA3RM intends to assess any potential operator of fish farms at any of our project sites through a biodiversity and sustainability screening. This is one way to direct the aquaculture industry to think in a more structured manner to address the different sustainability impacts from the production, including the impacts from up- and down-stream activities. Such an assessment must of course also address the biodiversity related issues.

One of the major negative impacts of fish farming on biodiversity is that which is caused away from the farm operations, in the fish feed sector. Most of the commercially farmed species use formulated feed produced by the big feed manufacturers. All fish feed must be a complete feed with low feed conversion ratio to support healthy fish with a fast growth, minimizing volumes needed and effluent volumes produced. Most of the fish species farmed are carnivorous species and a portion of their feed must be based on fishmeal. This fish meal is to a large extent dependent on fishing of smaller pelagic species by large commercial fishing vessels. Such fishing is negatively affecting the biodiversity, as it is exploiting the wild stocks of fish that is part of the food chain of larger wild fish species and marine mammals. An additional biodiversity issue is that of by-catch of non-targeted species. It can be a direct threat to those species, but it could also mean that it is taking away a resource that could be used directly for human consumption. By discussing with operators and feed manufacturers/ suppliers we aim to support the continuous improvement in feed quality for best possible feed conversion ratio and trying to minimize the content of fishmeal-based ingredients.

We will also promote farming of alternative species for lower trophic level aquaculture, species that do not need the inclusion of feed ingredients from fish meal. This will need a change in consumer awareness and acceptance of non-traditional food-fish species, and it will only happen over time.

We know there will be different biodiversity consequences depending on what source the protein/oil will come from. By minimizing the need for fish meal in the diet the industry will create an increased demand for replacement ingredients from the terrestrial ecosystem. This necessary crop production will need huge areas under cultivation which might convert ecologically sensitive areas and farmland used for traditional agriculture into mono-crop environments with the use of pesticides. An article in Nature (A 20-year review global aquaculture, 24 March 2021), mentions that feed accounts for more than 90% of the environmental impact from fed aquaculture production.

With the aim to create a more circular economy that supports biodiversity, we will also seek opportunities for valorisation of generated waste streams from the fish farm projects (nutrient rich sludge and processing waste) by connecting with other production entities i.e., algae farming and insect farming aiming for an increased replacement of protein and oils in fish feed away from fishmeal.

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Another threat to biodiversity is the risk of farmed fish populations escaping and mixing with the natural populations, as is often the case with large cage farming operations. This will not be a risk with our projects as the operators will only farm in totally closed land-based systems. This means there will be no risk of introduction of new exotic species either. The operators will have their own breeding and hatchery operation, or alternatively will purchase egg/larvae/fingerlings from suppliers that are certified disease-free. Efficient filtration systems will remove almost all nutrients to minimize negative impacts to the receiving ecosystem and the biodiversity. Sterilization units will ensure that the effluent will not release any pathogens/parasites that can affect the natural fish stocks, and there will be no use of medication or hormones that could have a detrimental effect on the organisms in the recipient.

Our project for large-scale indoor RAS for shrimp culture will also address the stress on the natural environment. With the closed intensive systems, we will be able to minimize the physical footprint and we will not establish projects in any ecologically sensitive areas. In this way, we will help lessen some of the problematic land use issues in the tropical areas where the species is predominantly farmed, where it in many instances has led to physical habitat damage along large coastal areas.

WA3RM has created a novel business model that through industrial symbiosis can support operators that meet our sustainability criteria with investment finance based on their own production design needs. WA3RM will develop the project, finance, and build the infrastructure based on the operator's specific requirements, including the infrastructure needed for the most efficient utilization of the waste streams from the project processes. The WA3RM Developer Fund will own the real assets being infrastructure and real estate, while the professional operator will take full responsibility for the operations and pay a monthly rent.

DIGIRAS - A COMPREHENSIVE PROJECT ON MONITORING AND IMPROVING WATER QUALITY AND FISH WELFARE IN COMMERCIAL RAS PRODUCING SEA- AND FRESHWATER FISH

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Introduction

Land-based Recirculating Aquaculture System (RAS) technology has been recognized as a promising alternative for sustainable production of seafood and experienced a massive development in recent years. The technology also has the potential as an alternative to "capture fisheries production" since overfishing is a key driver of ecosystem collapse and biodiversity loss in aquatic environments. Such systems facilitate the production of fish in closed environments, which allows a high level of control on water quality and fish health, as well as recovery and refinery of valuable waste streams. Even though RAS technology has existed for over 65 years and experienced rapid development in technology and production volume, it is still considered to be in its infancy. Issues related to water quality and fish health prevent it from reaching its full potential.

DIGIRAS is a trans-European project funded by the BlueBio Cofund program, where six R&D institutions and five industry partners from five countries have joined forces to generate knowledge and develop new solutions for improving fish welfare, water quality and sustainability in land-based aquaculture. This goal is realized by systematic acquisition of biological, physico-chemical and operational data from different facilities, testing methods to remove unwanted compounds from rearing water, developing new sensors and water treatment techniques, conducting experiments to develop machine learning assisted fish behavior analysis and assessing fish welfare. Assessment and integration of the acquired data are conducted employing advanced statistics and artificial intelligence.

Main results so far

- Detailed monitoring of the microbiota in built rearing environments and the corresponding host allowed for RAS-specific spatio-temporal bacterial community mapping and detailed host-environment microbiota interaction analysis for Atlantic salmon, yellowtail kingfish, sea bream, sea bass and Arctic charr.
- Bioinformatic pipelines for standardized sequencing data processing and an aquaculture sequence database have been established, both for bacteria and protozoa. Full metagenomes from fish and built environments have been acquired using Oxford Nanopore Technologies (ONT) sequencing technology.
- Different H_2S and off-flavor compound analysis methods have been established and tested. A novel H_2S sensor prototype with high sensitivity has been developed. Covalent organic frameworks (COFs) have been demonstrated to be able to remove off-flavor compounds from water.
- A stereovision camera system has been developed and successfully tested. In addition, a surveillance camera system was successfully used to record and analyze fish behavior deviations related to stress.
- The histopathological studies made on samples of gilthead seabream and European seabass revealed no severe pathological observations. A comprehensive list of protozoa species present in seabream and seabass samples has been generated.

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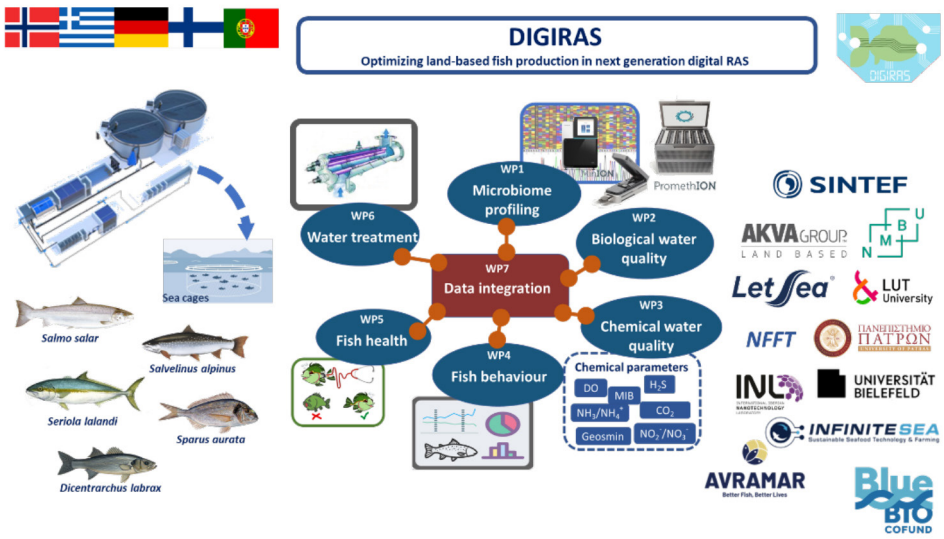
- Microalgae have been demonstrated as potent alternatives for the removal of nitrogenous compounds from RAS wastewater. AOP technology was shown to improve the degradation of off-flavor compounds in RAS wastewater.
- Fish behavior deviation is statistically correlated with increasing H_2S concentrations. Supervised machine learning assisted camera vision can be used to assess fish welfare and water quality.

Project overview



Impact

A major impact of DIGIRAS is to contribute to improving RAS technology towards sustainable production and improve fish welfare by parameterization of process ses and digitalization. So far, significant knowledge and microbiological, water quality and production data have been acquired in commercial RAS for the production of five commercially relevant fish species. In addition, experimental data on fish behavior and microbiota deviations in response to H_2S exposure has been acquired. Moreover, innovative water treatment technologies employing advanced oxidation processes and microalgae

AGRI BENCHMARK METHOD: TYPICAL FARM APPROACH REFERING TO DIFFERENT AQUATIC SHRIMP PRODUCTION SYSTEMS

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Introduction

Agri benchmark is an international non-profit network coordinated by the Thünen-Institute in Germany. Since 2001 cooperating partners have used the Typical Farm Approach to collect and analyse economic data from agricultural farms. The method has been adopted to aquaculture farms since 2015 [1; 2]. In 2019, the first steps were undertaken to apply the typical farm approach towards German beam trawlers fishing brown shrimps in the North Sea [3]. After the standard procedure of data collection via focus groups, a detailed profit and loss account provides the base for further analysis. In doing so, the collected production systems can be compared regarding their profitability, efficiency and productivity. Moreover, the application of scenario techniques enables valuable evaluations for policymakers concerning altering markets and framework conditions. Our presentation describes the method and evaluates its potential using examples of modelled fisheries and aquaculture.

Materials and methods

In the typical farm approach, a standard operational procedure (SOP) consisting of 5 steps allows identical international implementation across sectors. First, relevant regions and typical production systems must be identified by scientists. Statistics can give a first indication regarding regions and production systems. In step 3, data from the typical farm are collected preferably by conducting a focus group consisting of one or two scientists, one local expert and four to six practitioners, whose production system should be similar to the previously identified characteristics. A standard questionnaire is discussed within the expert group, aiming for consensus on each figure discussed. These figures feed into the Technology Impact Policy Impact Calculations model (TIPI-CAL) to analyse the data (step 4). The calculation takes into account physical, economic and environmental parameters. This results on a whole-farm level in a profit and loss account and on the enterprise level in a total cost calculation, which divides costs into cash costs, depreciation and opportunity costs. The data are adjusted until it is agreed on the dataset's realism, accuracy and consistency [4]. In step 5 all prices are updated annually, while the farm's or the vessel's basic characteristics are updated every three to five years [4; 2]. In 2015 the SOP was applied by scientific partner Le Thi Thanh Huyen to analyse the profitability of Vietnamese black tiger shrimp farms. Sulanke & Berkenhagen (2020) created a profitability time series for a German brown shrimp fishing vessel for the years 2014 and 2018 by using the typical farm approach [3]. The vessel model is currently being updated.

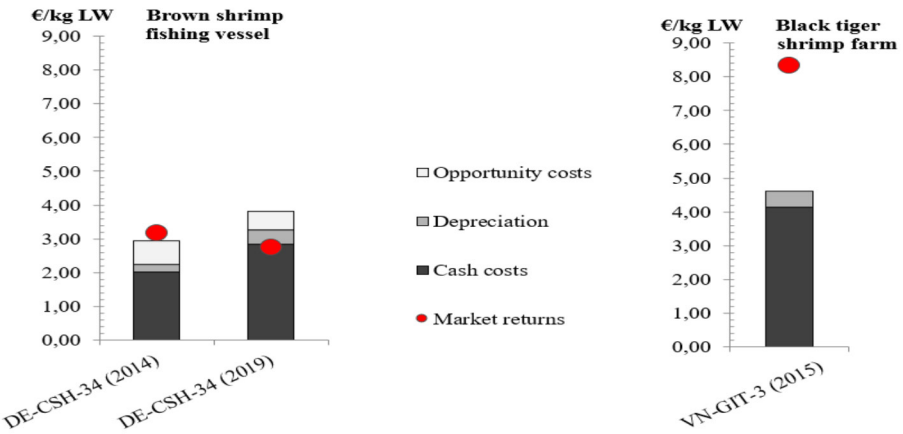

Figure 1: Profitability of a Vietnamese black tiger shrimp farm in 2015 (VN-GIT-3) and a German brown shrimp fishing vessel (DE-CSH-34) in 2014 and 2019.

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Results of different shrimp production systems

As an example, different studies of shrimp production systems applying SOP were chosen. Following the SOP brown shrimp was identified as the most valuable coastal fishery in Germany according to statistics. A focus group was conducted in 2020 to collect data for a typical brown shrimp fishing vessel in 2019 (DE-CSH-34) 2014 was considered a typical year by the participants so that prices and costs for this year were also recalled [3]. The costs and prices for the typical vessel were updated for 2022 (in validation) with almost the same participants from the first focus group. By the full cost analysis profitability can be represented over time (Figure 1).

When looking at the German brown shrimp fishing vessel (DE-CSH-34), all costs were covered by the revenues in 2014, which characterises long-term profitability. But just five years later, in 2019, the revenues did not cover the cash costs, which made the vessel not even temporarily profitable. Costs have increased over time, partly due to inflation. The data show that the varying catches and volatile prices are decisive factors in the profitability of the vessel. Another example where the typical farm approach has been applied is the black tiger shrimp farm in Vietnam (VN-GIT-3). The represented profitability results of the farm, which is located in Soc Trang, Vietnam, produces for the region typical in earthen ponds and encompasses 20,000m^2 (Le Thi Thanh Huyen). At first glance, the black tiger shrimp farm had a high profit margin in 2015. However, data for opportunity costs were not collected. Therefore, the farm was at least mid-term profitable. The data identify the cost of feed and the high black shrimp mortality rate as decisive factors for the farm's profitability.

Conclusion

A realistic model is only created when the costs incurred are discussed and recorded in the focus group. The substantiated results allow not only a status quo analysis but also the benchmarking of similar production systems in different countries. Furthermore, the influence of the introduction of new production techniques as well as new political framework conditions or economic shocks like COVID-19 on the company's profitability can be estimated if these are incorporated into the model [5]. This allows a before-after comparison regarding the farm-level results, but also an evaluation of the relative merits of different scenarios.

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UNRAVELING THE CORE GILL MICROBIOME OF *Sparus aurata* BETWEEN WILD AND FARMED POPULATIONS

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Introduction

In teleosts, skin, gills and gut are the major mucosal surfaces (MS) which also act as host-microbiota interface, where they can form mutualistic relationships. To date, numerous studies have revealed the importance of host microbiomes and the various factors (host associated or environmental) affecting their dynamics (Egerton et al. 2018). Despite that there is progress in research on fish microbiome there is limited knowledge on the microbial structure of natural fish populations and any differences that may exist in the composition between wild and farmed fish species. Wild fish microbiota can be used as a fish health indicator to improve production through innovative applications of microbiomes-tailored products, but also for other interventional strategies, such as conservation practices. In this study, we analysed the gill microbiomes of 20 wild-caught and 32 farmed Mediterranean gilthead seabream (*S. aurata*) specimens by 16S rRNA gene sequencing to detect structural and inferred functional differences between the two different sets of individuals.

Materials and Methods

In total 52 tissue samples of gills were collected from *S. aurata* individuals. The specimens obtained by experimental/scientific fishing in the Ionian Sea (Wild: n = 20; weight = 147.2 g ± 44.7) and by a distantly located (Aegean Sea coast in central Greece) commercial aquaculture open-sea unit [Farmed n = 32; weight = 127.4 g ± 185.7 (average ± SD)]. The farmed fish species were collected in 4 different time points across the aquaculture production cycle (between ~100 to 500 days after their transfer in the sea cages). After dissection with sterilized equipment, and prior to their storage, samples were rinsed with sterile particle free seawater, to reduce surrounding environment associated bacteria. Water samples (n=8) were taken from the sea cages and filtered by using 0.2-μm isopore membrane filters to enable comparisons of the microbial communities in the water column with those present on the fish gill. Total DNA was isolated from the collected samples using the DNeasy PowerSoil Pro Kit (Qiagen) and 16S rRNA gene sequencing was applied for the identification of their bacterial targeting the V3–V4 regions of the 16S rRNA gene. Sequencing was performed on a MiSeq Illumina instrument (2×300 bp). Raw data were analyzed on the MOTHUR v.1.48.0 (Schloss at al. 2009). PICRUSt analysis (Douglas et al. 2020) was applied to predict the genetic functions of the detected bacterial taxa. Statistical analyses performed in Past4 software (Hamme et al. 2001).

Results & Discussion

A total of 52 gill samples, collected from both wild-caught and farmed populations, along with 8 water samples, were analyzed for this study. The bioinformatic analysis yielded 4678 operational taxonomic units (OTUs), after subsampling to the size (n = 10313) of the smallest group. The wild group microbiota presented the highest number of unique OTUs, while 254 (5.9% of the total OTUs) were shared among the microbiomes of the two *S. aurata* groups. Overall, OTUs represented 33 bacterial phyla. Proteobacteria and Bacteroidota accounting for 75.78% of the OTUs abundance. The most abundant OTUs were identified as unclassified Burkholderiales sp. (Gills) and *Synechococcus* sp. (Water). PERMANOVA analysis detected significant differences (PERMANOVA: $p < 0.001$ using Bray–Curtis dissimilarity indices, 9999 permutations) in community composition between the gill microbial communities of the two *S. aurata* groups (Wild and Farmed) but also between host (Gill) and environmental samples (Water). The clear separation between *S. aurata* samples and Sea water, supporting previous findings suggesting that *S. aurata* and other fish species harbours distinct microbial communities compared to those found in their surrounding environment (Legrand et al. 2018; Quero et al. 2022). According to SIMPER analysis the average dissimilarity between Wild and Farmed group was 83.86% with only 6 OTUs presenting a cumulative contribution of 49.9%. It is noteworthy that in ordination of samples, based on the same distance matrix, all samples from the farmed group (except of those obtained during the initial sampling, which had spent the minimum time in the sea) clustered closely with those of the Wild group. The relative abundance of the predicted MetaCyc pathways from PICRUST analysis revealed that the two groups do not differ significantly (ANOSIM, $p = 0.0611$) and their most abundant (relative abundance ≥ 1%) pathways are involved in Aerobic Respiration, Fatty Acid, Lipid and Phospholipid Biosynthesis, suggesting that despite the structural differences of the gill's microbiota, these bacterial communities have no specific metabolic pathways. The same analysis revealed that the predicted metabolic pathways of the water samples shared similarities with the gill microbiota of the farmed group (ANOSIM, $p = 0.12$), but there were significant different when compared to the microbiota of the wild (ANOSIM, $p = 0.0001$).

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Conclusion

Knowing wild fish microbiota, could contribute to unravel the natural microbiome variation and enhance our knowledge on microbial diversity and host-microbe interactions. Our study revealed that gill microbial community composition differs significantly between Wild and Farmed populations. However, these differences tend to decrease as farmed populations spend more time in sea. These results suggest that host genetic background and the habitat status, due to human intervention, affect the bacterial dynamics in *S. aurata* gills. In addition, the existence of a core microbial community and the similarities in metagenomic functions strongly imply a coevolutionary relationship between *S. aurata* and its gill microbiome. These findings highlight the importance of thoroughly exploring these host-microbe interactions and taking them into account when developing conservation strategies and designing microbiome-based approaches.

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CAN FRESHWATER TREATMENT POTENTIALLY REDUCE ULCER OUTBREAKS IN LAND-BASED SALMON AQUACULTURE?

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Introduction

At low temperatures (<4 °C), ulcer formations cause welfare and economic issues in the production of Atlantic salmon. However, this problem also persists in land-based productions at higher temperatures, contrasting with what is established at sea production. Seawater flow-through systems in land-based production of salmon is gaining popularity, which escalates the issues of bacteria-mediated ulcers. This is especially true for smolt; the stage of fish which is more susceptible to bacterial infections. We hypothesize that the ulcer outbreak is related to the relative increase in surface area the fish is exposed to as well as a function of the higher density to water exchange rates in tanks compared to the traditional sea cages. Although susceptibility to marine bacteria such as *Moritella viscosa* and *Tenacibaculum sp*. may be increased in tank production, the self-contained nature of the production method may simplify treatment without pumping fish or using well boats or tarps.

This project aimed to establish a link between starting density, skin health, growth rates, and wound formation in the production of Atlantic Salmon in tanks using seawater. When wounds were observed in the experiment, we explored the potential of freshwater treatment to combat the pathogenic marine bacteria.

Material and Methods

The trial was conducted at LetSea's R&D Land facility in Northern Norway between January 2023 and May 2023. Smolts used for the trial were obtained from Kvarøy Smolt, Norway. At the start of the trial and with a mean weight of 135 g, the fish were randomly distributed amongst eight trial tanks (2 m^3) at an abundance of fish per tank that reflected 15 kg/m^3 (four tanks) and 20 kg/m^3 (four tanks). All tanks were given a continuous light regime and a 20-hour feed regimen aimed at 12% overfeeding daily. The tanks were all equipped with cyclone feed collecting systems. The feed chosen was a commercially available feed designed for strengthened skin health and minimizing wound formation. Water for the trial was derived from the facility's saltwater intake, located 140 m below the surface and 700 from the shore. The water was treated with UV before usage.

Five days after the start of the experiment, the first ulcer outbreak was observed, and a freshwater treatment was performed. All fish were starved 48 hours pre-treatment. On the day of treatment, 10 fish from each of the eight tanks were taken out and scored for ulcers on the body and fins and for the loss of scales (all scores 0-3 based on Fishwell welfare indicators)[1]. Samples were taken for bacteriology, and PCR on starting and open wounds were performed. After sampling, four tanks were randomly chosen for treatment (two high-density and two low-density tanks). 8 ml Aqui-S Vet. sedative (to a final concentration of 4 ml/m^3) was added to all four tanks. This was done to minimize the impact of the handling itself. Earlier trials also show Aqui-S reduce loss of blood chloride during freshwater treatments in Atlantic Salmon as well as reduces both cortisol and lactate formation during handling (results not published yet). The water column was reduced to 20 cm (20 % of the original volume), and 2 m^3 of fresh water was added. New salinity was measured, and the fish was kept under observation with continuous measurements of CO_2 and oxygen. The treatment lasted three hours. After the completed, treatment saltwater supply was turned back on, and new samples were taken from the handled fish.

Results and conclusions

Our results show that there were no clear differences in wound formation on the body or fins in tanks between low and high-density groups. It should be noted that body wound scores of 2 and 3 were only presented in high-density tanks. The freshwater treatment operations were successful, with little to no observable stress on the fish and only a drop in salinity to 2 ppt. PCRs taken from wounds before and after freshwater treatment showed little difference in CT values between pre-and post-treatment samples. Bacteriology showed positive results pre-treatment but no growth of either *M. viscosa* or *Tenacibaculum sp*. after treatment. No new wound formations were observed 30 days after treatment. The data on growth and FCR will be available in week 24, 2023, and will be presented at the conference. The current results suggest that freshwater treatments can potentially reduce marine bacteria-mediated ulcer formations and should be explored further.

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TECHNICAL EFFICIENCY OF TILAPIA (*Oreochromis niloticus*) CAGE CULTURE IN BADAGRY, LAGOS STATE, NIGERIA

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Introduction

Cage fish culture is gaining more grounds in Nigeria as a means of augmenting total fish production and supply. This study investigated the technical efficiency of Tilapia cage farming in Lagos State, Nigeria.

Materials and methods

Data were collected from ten commercial fish farms in Afowo Community, Apa, Badagry, Lagos State with the coordinates (6° 25' 672" N, 2° 50' 809" E). The farms used net cages to raise Nile tilapia (*Oreochromis niloticus*). Each farm had different cycle lengths because the time to achieve the final average weight of the traded fish (~800 g) was different. The cycles were 6 months, 5 months and 8 months.

Sampling and analytical technique

Random sampling technique was used to select farms where the data were collected. This technique ensured representative sample, reduced sample error, increased generalizability and ethical considerations. Data were gotten using well-structured questionnaires and personal interviews. A stochastic frontier production model using the Battese and Coelli (1992) approach in R programming was applied in data analyses. The model decomposes total factor productivity into a technical efficiency component and an error component. The production function involves the use of one output and six inputs and farm, including stocking density, feed, labour, fuel (energy), utility (operational) and other relevant production costs, as presented in Table 1. The model is of the form; $Yi = f(Xik ; \beta k) + \varepsilon i$ $i = 1, 2, ...n$.

Variable name	Definition	Measurement	Summary statistics			
			Mean	S.D	Min	Max
Output and input variables						
Y	Cage fish production per cycle	(kgm^{-2})	69.11	34.86	31.25	125.00
X_1	Fish fry	(pieces m^{-2})	81.59	27.16	62.50	125.00
X_2	Feed	(kgm^{-2})	69.01	29.01	24.38	121.88
X_3	Labour (owned and hired)	(days m^{-2})	13.99	9.44	4.50	31.00
X_4	Energy (fuel)	(litres m^{-2})	0.22	0.40	0.00	1.29
X_5	Utility (operational)	(USD m^{-2})	101.56	44.48	40.76	172.71
X_6	Other (maintenance)	(USD m^{-2})	33.38	42.21	2.72	140.40
Farm specific variable						
Z_1	Age	Year	33.05	8.92	21.50	30.50
Z_2	Education	Year	14.00	3.16	5.00	15.00
Z_3	Production cycle	Number	6.20	1.09	4.50	8.00
Z_4	Cage area	m^2	11.83	4.96	2.32	16.00
Z_5	Household size	Number	3.90	2.60	0.00	3.00
Z_6	Extension visit	Number	1.30	0.40	1.00	8.00

Table 1. Summary Statistics for variables in the model (*1USD = ₦460*)

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Farms	Technical Efficiency
1	0.969686
2	0.992047
3	0.397539
4	0.85724
5	0.888299
6	0.336105
7	0.995112
8	0.786254
9	0.782432
10	0.787624

Table 2: Technical efficiency of the sampled 10 fish farms
Log likelihood value: 4.5902
Mean Technical efficiency: 0.779234

GROWTH PERFORMANCE, INTESTINAL HEALTH, AND IMMUNE RESPONSE OF NILE TILAPIA (*Oreochromis niloticus*) FED AUTOLYSED BREWER'S YEAST

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Introduction

The Nile tilapia (*Oreochromis niloticus*) is one of the most common aquacultured species globally and is one of the most important farmed species in Nigeria. Despite the remarkable global growth of aquaculture, the sector still faces serious challenges in Nigeria and other sub-Saharan African countries (Barley, 2022), which undermines growth and sustainability. With this, there is a constant need to improve existing knowledge and develop new diets using sustainable additives such as yeast derivatives. Brewer's yeast is rich in nutrients and bioactive substances such as β-glucans, mannan-oligosaccharides, and nucleotides which have been demonstrated to improve growth performance, health, and immune response of farmed fish (Merrifield and Ringø, 2014). The present study was conducted to evaluate the potential of dietary supplementation of autolysed brewer's yeast (ABY) to improve the growth performance, intestinal health, and immune response of Nile tilapia fry.

Materials and Methods

A 35-day feeding trial was conducted in a freshwater recirculatory aquaculture system at the University of Plymouth with Nile tilapia fry (0.45 g). Four hundred and eighty tilapia fry were randomly distributed into twelve (15 L) tanks (40 fish/tank). Four diets were formulated to meet the known nutrient requirements (NRC, 2011) of Nile tilapia (Table 1). The control diet had no brewer's yeast while the other 3 diets were supplemented with ABY (CeFi® Pro, Leiber GmbH) at 1 g/kg (ABY1), 2 g/kg (ABY2), or 4 g/kg (ABY4). All diets were isonitrogenous and isocaloric. The fish were fed one of the four diets (n = 3 tanks) at 5% of biomass per day. Water quality parameters were monitored throughout the trial (temp. 26.1±0.5 ^{o}C, pH 6.93±0.41, dissolved oxygen 7.64±0.24 mg/L, NH_4 0.20±0.38, NO_3 24.73±13.52, and NO_2 0.03±0.03). At the end of the feeding trial, intestinal samples were taken for histological analyses and gene expression.

Growth performance was assessed by final body weight (FBW), mean weight gain (MWG), feed conversion ratio (FCR), specific growth rate (SGR), and survival (SUR) as described by Rawling *et al.* (2021). Intestinal samples were taken for histological appraisal of morphometry as described by Rawling *et al.* (2021). Data were analysed with one-way ANOVA and post-hoc LSD using SPSS v25 (significance accepted at $P < 0.05$).

Results

Fish fed ABY1 showed significant improvements ($P < 0.05$) of MWG, SGR, and FCR compared to fish fed the control diet (Table 2). All treatments displayed normal intestinal physiology characterised by extensive mucosa folds with simple lamina propria and intact epithelial barriers. No statistically significant differences ($P < 0.05$) of fold height or goblet cell count were observed (Fig 1).

Conclusion

Results obtained so far indicate that ABY at a low dietary inclusion has the potential to improve the growth performance and feed utilization of Nile tilapia fry. Ongoing analysis is being undertaken to assess expression of immunoregulatory genes and further intestinal morphometric assessments are being undertaken using light microscopy.

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Table 1: Experimental diet formulations and proximate composition (g/100g)

Ingredients (g/100g)	Control	ABY1	ABY2	ABY4
Soybean Meal 48	40.00	40.00	40.00	40.00
Sunflower meal	24.67	24.57	24.47	24.27
LT fishmeal	15.00	15.00	15.00	15.00
SPC60	9.98	9.98	9.98	9.98
Sunflower oil	8.67	8.67	8.67	8.67
DL methionine	0.17	0.17	0.17	0.17
Lysine	0.50	0.50	0.50	0.50
Fish premix	0.50	0.50	0.50	0.50
CMC	0.50	0.50	0.50	0.50
CeFi® Pro	-	0.10	0.20	0.40
Composition (%, dry matter basis)				
Protein	46.21	47.39	47.43	46.44
Lipid	11.14	10.85	10.73	11.32
Moisture	4.47	4.20	3.76	3.60
Ash	6.63	6.85	6.89	6.92

Table 2: Growth performance (mean ± SD) of Nile tilapia fed the experimental diets (values in the same row with different superscripts are significantly different $P < 0.05$).

	Control	ABY1	ABY2	ABY4
FBW (g)	1.13 ± 0.07^{a}	1.38 ± 0.21^{b}	1.21 ± 0.12^{ab}	1.12 ± 0.02^{a}
MWG (g)	0.67 ± 0.06^{a}	0.93 ± 0.20^{b}	0.75 ± 0.11^{a}	0.68 ± 0.01^{a}
SGR	2.64 ± 0.12^{a}	3.28 ± 0.36^{b}	2.86 ± 0.23^{a}	2.71 ± 0.04^{a}
FCR	1.99 ± 0.09^{a}	1.55 ± 0.23^{b}	1.78 ± 0.16^{ab}	1.91 ± 0.06^{a}
SUR (%)	90.83±3.82	94.17±3.82	91.67±6.29	94.17±1.44

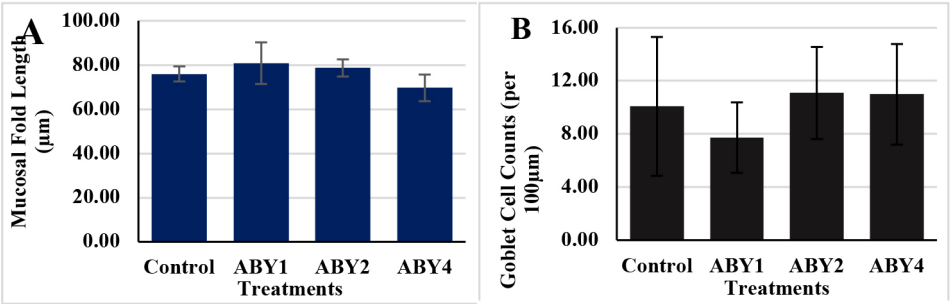

Fig 1: A] mucosal fold length and B] goblet cell abundance in the intestine of Nile tilapia fed various treatments (values are Mean ± Standard Deviation)

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WHAT INFLUENCES THE DEVELOPMENT OF NEW PRODUCTION SYSTEMS IN NORWEGIAN SALMON AQUACULTURE?

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Introduction

Norway is a leading producer of Atlantic Salmon (*Salmo salar*) globally. Despite ambitions of growth, production in conventional sea-based fish farming is curbed by challenges such as salmon lice and escape of fish. Technological innovation is seen as key to solving these challenges, and policies to develop new concepts for aquaculture have also been initiated by the government (Føre et al. 2022). In 2023, the Norwegian salmon aquaculture industry is becoming more technologically diverse with several new production systems including land-based, floating closed and semi-closed, and open ocean aquaculture systems emerging.

The objective of this study is to provide knowledge of industry actors' perceptions of key challenges and opportunities related to new aquaculture production systems in Norway. This knowledge is important for regulation and discussions of what influences the future development of the industry.

Materials and methods

To explore industry actors' perceptions of variables that influence the development of the salmon aquaculture industry in Norway, Fuzzy Cognitive Mapping (FCM) was used as a key method. The first FCMs were set up by the research group based on Vensim diagrams created during interviews with a selection of industry representatives (fish farmers and organisations). Fish farmers represented different production systems, and the interviews focused on key challenges and opportunities for each system, as well as key challenges related to the regulation of new concepts. Following the interviews, the FCMs were made using the software Mental Modeler which was used in a series of workshops facilitated by the research group in the period 2021-2023. Representatives from the same companies as the interviewees participated in the workshops. The workshops aimed at identifying key variables and evaluating their relation to other variables in the map. Mental Modeler is a software that allows us to show how one variable directly affects another variable by drawing arrows between them during the discussions.

Results

This presentation will include the FCM of more than 30 variables seen as important by the industry actors, as well as insight into the participants' reasoning as to why and how key variables would affect other variables.

In the initial workshops, some key variables were access to area, salmon lice, delousing, need for competent personnel and energy infrastructure. Discussions showed that all new production systems needed suitable areas to grow and that high salmon lice levels are driving the technological development towards solutions that may solve this issue such as land-based. Furthermore, all new technologies require competent personnel to operate the systems, and a solid infrastructure for energy.

Participants were concerned with geographical and seasonal differences in their discussions, for instance related to salmon lice levels and delousing that will vary in different parts of the country and at different times of the year. Variables such as delousing were seen as technology dependent, since there are several delousing methods that affect for instance fish welfare differently and new solutions are continuously being developed.

The workshops were spread out in time, which allowed participants to make changes to the maps during the project period. The predictability in framework conditions for the industry became highly relevant in September 2022, when the Norwegian government introduced a suggestion to a new resource rent tax (grunnrenteskatt) for sea-based aquaculture to be implemented from 2023. The variable taxes and fees, that was not part of the maps from 2021-2022 was thus introduced in a workshop in the spring of 2023. Furthermore, the variable political and regulatory predictability (both nationally and locally) was added. Both factors were seen as crucial for variables such as willingness to invest and economic results.

(Continued on next page)

Conclusion

The Norwegian aquaculture industry is becoming more technologically diverse. Using FCM, this study has identified relevant variables that industry actors think is important for the development of the salmon fish farming industry. Looking into the connections between different variables allows us to discuss different scenarios that can be useful for industry, regulators, and researchers moving forward.

Acknowledgements

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NEW TECHNOLOGIES AND APPROACHES FOR SUSTAINABLE SEAFOOD SUPPLY CHAIN

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Introduction

Two new projects will be presented - FishEUTrust: European integration of new technologies and socio-economic solutions for increasing consumer trust and engagement in seafood products and Sea2See: Innovative blockchain traceability technology and stakeholders' engagement strategy for boosting sustainable seafood visibility, social acceptance and consumption in Europe.

The projects were approved in 2022 within the Horizon Europe call "Sea to fork transparency and consumer engagement". More information about the projects can be found at https://fisheutrust.eu/ and http://www.sea2see.eu/.

Concept

A network of FishEUTrust co-creation living labs (CLL's) will be created in three diverse aquaculture regions: The Mediterranean, Atlantic and North Sea. CLLs actors will cooperate in developing and testing new technologies, products, services, policy instruments, planning tools, organizational forms, and governance arrangements. The technological development includes: (i) suite of tools for determining seafood quality, safety and traceability (Farm-to-Fork) integrating metagenomics, genetic biomarkers and stable isotope approach; (ii) digital technologies for traceability based on Product Passport and/or Blockchain, unique identification and labelling of product instances; and (iii) integrated smart control systems based on sensor technologies to monitor freshness and presence of pathogens, antibiotics and biotoxins along the seafood supply chain. Further FishEUTrust innovation platform will be design in an open way to integrate technologies for seafood transparency and connect consumers and other seafood supply chain stakeholders. It will be connected with other relevant European infrastructures, in order to enable an easy access to harmonised and validated data, tools and services needed to identify any socio-economic and cultural barriers in respect to increase the uptake of seafood as well as to support FishEUTrust businesses and education. The gathered knowledge will serve for educating and nudging the producers and consumers through gamification. The platform will provide access through two portals, one aimed for consumers and another for aquaculture and food producers from the EU region to provide data and to enable the promotion of their activities and the growth of new businesses.

SEA2SEE is an innovative European project which main goal is to make actors with sustainable seafood practices more visible to consumers thus giving them a competitive advantage. The objectives include: (i) to develop a co-creation approach to sustainable seafood transparency and traceability; (ii) leverage a set of awareness raising and educational practices to increase sustainable seafood consumption; (iii) develop a blockchain deployment model for seafood industry-specific traceability data collection; (iv) demonstrate the feasibility and advantages of the Sea2See blockchain model; (v) develop a standardized Life Cycle Assessment framework for identifying and quantifying the major sources of environmental impact. SEA2SEE's ambitious goal is pursued with the development, implementation and validation of a blockchain-based platform that consolidates and analyses data from the seafood value chain, in a system that is flexible enough to adapt to changes in the value chain. This will be completed with professional and consumer applications to increase trust and social acceptance of sustainably fished and farmed seafood. New strategies for stakeholders' engagement organised around site-specific but also seafood product specific value chains have been developed. A co-creation approach based on early-stage involvement of intended end-users, needs identification and feedback collection on the proposed Sea2See traceability innovation is being applied. Two feasibility demonstrators will be carried out - one for fisheries with one demo site (traditional octopus fishery in the Algarve, Portugal) and one for aquaculture with 3 demo sites (in Portugal, Greece and Spain). The use of innovative technologies of water quality and cloud-based software production management systems allow the farms to directly upload farm data to the blockchain.To secure the uptake and exploitation of Sea2See results and innovation technologies, Life Cycle Sustainability Assessment (LCSA), seafood safety and evaluation of the presence of certain non-frequent or emerging contaminants is carried out for each of the 5 case studies. The Sea2See project will contribute to significaltly increase Trust, Traceability and Transpoarency of the European Seafood Sector throughout the value chain.

GENOTYPE-BY-DIET INTERACTION (G×D) FOR FATTY ACIDS AND FILLET FAT IN EUROPEAN SEA BASS *Dicentrarchus labrax*

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Introduction

The content of essential ω-3 long-chain polyunsaturated fatty acids (LC-PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has proven to have beneficial health effects in human diets (Horn et al., 2020). An issue of major concern in the aquaculture industry is the replacement of fish meal and fish oil in aquafeeds with sustainable protein sources such as those coming from plants due to the fluctuating cost and the declining percentage of world fisheries production (Lupatsch et al., 2004). The purpose of the present study was to identify the presence/absence of genotype by diet interaction using a plant-based and a standard commercial diet for the fatty acids and fillet fat in European seabass.

Materials and Methods

In the Palairos area (Western Greece), 520 European seabass were divided randomly to be fed with two different diets, a plant-based (*N*=300) and a standard commercial one (*N*=220). From all the fish, fin-clips were collected and genotyped using the MedFISH SNP-array (Peñaloza et al., 2021) and the following fatty acids omega-3, EPA, DHA along with the fillet fat were recorded at 665 Days Post Hatching. The fillet fat was recorded using a Distell fat meter, (Distell FFM-692, Old Levenseat, Scotland, UK) and Fatty acid composition was measured using gas chromatography paired with flame ionisation detector (GC-FID, Variant 3800 GC, Agilent, California, USA). Genomic data were filtered using plink and a bivariate animal model for each possible pair of diets per trait (omega-3, EPA, DHA and Fillet fat) was performed in AIREMLF90. For each trait, two heritability estimates along with the genetic/genomic correlation between them were calculated,

$$Y = \mu + Xa + Zu + e$$

where Y corresponds to the matrix of each pair, μ is the mean of the above traits, Z and X are the incidence matrices, a is the tank, u is the additive genetic effect utilizing firstly, the Genomic Relationship Matrix (GRM) and it is illustrated as $\sim N(0, G\sigma_a^2)$(G is the GRM and σ_a^2 is the additive variance), and secondly, the Pedigree Relationship Matrix (PRM) and it is illustrated as $\sim N(0, A\sigma_a^2)$(A is the PRM and σ_a^2 is the additive variance), and lastly e is the residual. Then, the breeding values (EBVs) for each bivariate animal model using BLUPF90 were calculated and used to estimate the ranking correlation between them using R.

Results and Discussion

The correlation for the **ω-3**, EPA and DHA, between the two diets ranged from 0.94 to 0.99, which indicates a weak genotype-by-diet interaction independently of the relationship matrix. Albeit, for the fillet fat a strong genotype-by-diet interaction was detected since the genomic correlation was 0.83 and genetic correlation was 0.39. Additionally, only for the fillet fat, the ranking correlation firstly between Genomic EBVs for the plant-based diet and the standard commercial diet was 0.38 and secondly, between the EBVs was 0.16. Those findings indicate a strong re-ranking of selection candidates per diet for the fillet fat. In literature, the presence of genotype-by-diet interaction (from weak to moderate) has been identified for weight and fat in the European seabass (Le Boucher et al., 2013; Le Boucher, et al., 2011)”ISSN”:”00448486”,”abstrac t”:”In the last years, the increase of aquaculture production has led to the evolution of feed composition with an increasing

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Table 1. Genotype by diet interaction. Standard errors are illustrated in parentheses.

	Using the GRM			Using the PRM		
	Heritability		Genomic correlation	Heritability		Genetic correlation
	standard diet	plant-based diet		standard diet	plant-based diet	
Fillet fat	0.31* (0.13)	0.40* (0.11)	0.83* (0.37)	0.18 (0.12)	0.36* (0.13)	0.39* (0.95)
ω-3	0.19* (0.08)	0.26* (0.09)	0.99* (0.23)	0.22* (0.09)	0.27* (0.10)	0.99* (0.27)
EPA	0.17* (0.07)	0.24* (0.08)	0.98* (0.27)	0.27* (0.08)	0.26* (0.08)	0.94* (0.08)
DHA	0.17* (0.07)	0.23* (0.08)	0.99* (0.20)	0.20* (0.07)	0.28* (0.09)	0.96* (0.16)

*Significantly significant estimation

substitution of fish meal and fish oil with terrestrial plant products. In the meantime, selective breeding of fish has been widely developed. The ability to grow on plant-based diets has recently been proven to be genetically variable, pointing out the interest to increase knowledge on the potential consequences of substitution of fish meal and fish oil on current breeding programs. Moreover, heritabilities of major production traits other than growth also need to be estimated in this new environment. Experimental rainbow trouts (about 3000. In our study, a genotype-by-diet interaction for the fat has also been found and the estimated genomic correlation is within the reported range of Le Boucher et al. (2013; 2011).

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VALIDATION OF QTL ASSOCIATED WITH RESISTANCE TO *Lernanthropus kroyeri* IN EUROPEAN SEA BASS

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Introduction

Lernanthropus kroyeri is an ectoparasite that attaches itself to the gills of the European sea bass *Dicentrarchus labrax*. In a W. Greece site, the inheritance of the parasite count was previously estimated at 0.28 and showed a moderate genetic correlation with the body weight for the European sea bass (Papapetrou *et al*., 2021). A selected subgroup of the above population was genotyped and the genomic correlation with the body weight was harmonized with the above findings. Additionally, a GWAS analysis was performed revealing two putative QTL in linkage group 7 associated with the resistance to *L. kroyeri* (Oikonomou *et al*., 2022). In the present study, the significant effect of those putative QTLs in farmed fish stocks was validated, by genotyping healthy and infected fish from another open-sea trial using 30K MedFISH array (Peñaloza *et al*., 2021).

Materials and Methods

Fin-clips were collected from 222 infected fish with *L. kroyeri* (randomly selected), and 110 healthy fish from the same site (Astakos). The infestation of the fish was performed through natural cohabitation. The following logistic regression model was used in R to validate any significant effect of the putative QTL on the parasite infestation,

Outcome ~ AX_373127007 + AX_373218583 + Origin (Full Model)

Where *outcome* is the vector of the presence or absence of the *L. kroyeri*, the *AX_373127007* is the genotype of the highest SNP, based on -log(*p*-value), with 3 levels (CC, CT, TT), the *AX_373218583* is the genotype of the followed SNP with 3 levels (CC, TC, TT) (Oikonomou *et al*., 2022). Both were fitted as fixed effects in the model. Additionally, the *Origin* of the fish was used in the model as a fixed effect, since fish from two different genetic backgrounds were used (2 levels). Apart from the Full Model, Reduced ones were also utilized, in which each time one of the fixed effects was removed. In total, three *Reduced Models* were evaluated. For all the reduced models, the effect of the redundant fixed effect was estimated, for all the evaluating models, the Akaike Information Criterion (AIC) was used in order to validate the goodness-of-fit.

Table 1. Estimated Akaike Information Criterion (AIC) per model and the significance (*p*-value) of each of the three tested fixed effects after backward elimination from the full model.

Model		AIC	*p*-value *(Pr(>Chi)*
Full model		302.66	-
Reduced models	*AX_373127007*	306.77	0.017 *
	AX_373218583	305.40	0.034 *
	Origin	420.06	< 2e-16 *

*Statistically significant results with p-value < 0.05

(Continued on next page)

Results and Discussion

When removing the effect of AX_373127007 or AX_373218583 genotypes from the full model, the *p*-value was less than 0.05 (Table 1). Those findings indicate that both SNPs are important and significant factors to the model. Furthermore, the Full model showed the lowest AIC which means that this model showed the best goodness-of-fit compared to the reduced models (RM). Nevertheless, those SNPs could be considered to be linked to a putative QTL affecting the infestation with *L. kroyeri*, since both contributed significantly to the logistic regression which was performed using populations with different origin in the present analysis. Our next steps will be to calculate the probability to be, infected or healthy, for each SNP genotype as well as for the combined SNP genotype in order to identify the desirable "resistant" genotypes as well as their interaction.

Acknowledgments

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A PRELIMINARY QTL ANALYSIS FOR CAUDAL FIN ABNORMALITY IN GILTHEAD SEABREAM

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Introduction

Caudal fin abnormalities have been reported in reared fish with a variety of phenotypes (e.g., fin stricture and lack of rays, lateral bending, duplication) (Koumoundouros *et al.*, 1997). Their frequency has been shown to be affected mostly by the rearing environment and nutrition (Boglione *et al.,* 2013). In a recent study on the genetic basis of caudal fin in gilthead seabream, moderate to low heritability estimations were detected for the lack of caudal fin rays (Fragoulis *et al.*, 2020). In the present study, a subgroup of specimens (307) was selected from the data of Fragoulis *et al.* (2020) and used to identify any potential QTL link to the caudal fin abnormality.

Materials and methods

A de novo linkage map which was constructed and presented by Oikonomou et al., (2022) using 498 offspring who belonged to 153 full sib families (from 53 dams and 45 sires) was used. Out of the total fish, the 307 offspring (113 families) were used in the present analysis, in which the lower principal caudal ray (PCRlow_N) was recorded at 39 Days Post Hatching. Four categories were detected for the lower principal caudal ray (7, 8, 9, and 10). A Quantitative Trait Loci (QTL) analysis was performed in QXPAK 5.0 software (Pérez-Enciso and Misztal, 2011), to identify QTL related with the PCRlow_N, using a maximum likelihood Variance Component Analysis, the polygenic component (pedigree-based animal model) and the QTL effect.

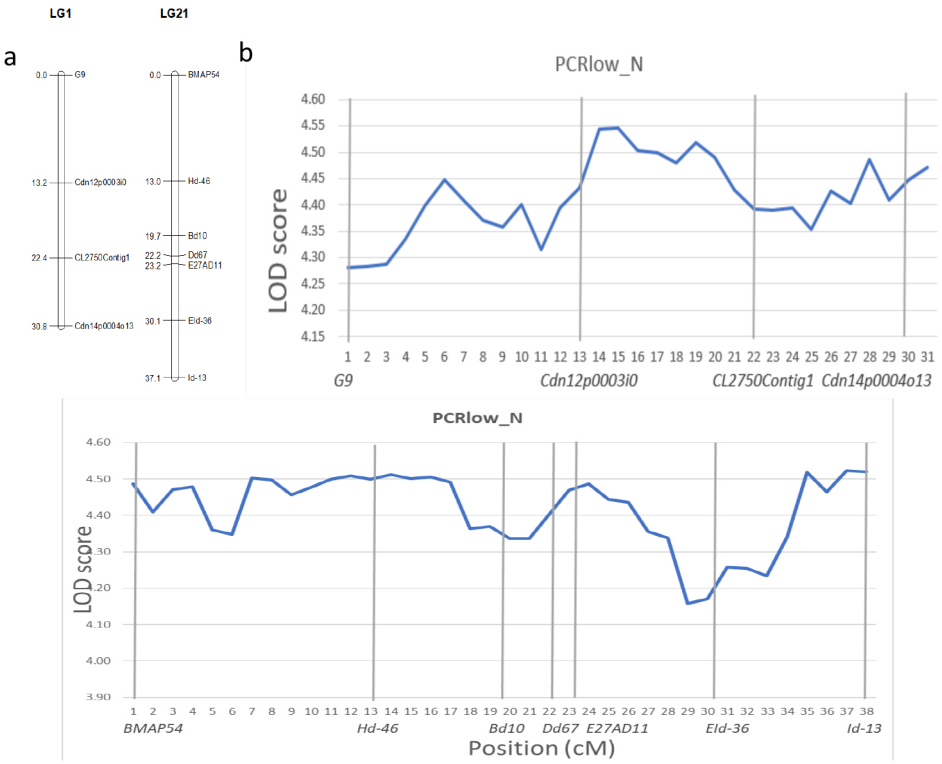

Fig 1 (a) Linkage groups 1 and 21 (Oikonomou et al., 2022) (b) Quantitative trait loci (QTL) analysis of PClow_N in LG1 and (c) Quantitative trait loci (QTL) analysis of PClow_N in LG21

(Continued on next page)

Results and Discussion

Two QTLs in LG1 and LG21 were revealed from the Quantitative Trait Loci (QTL) analysis explaining 13% of the phenotypic variation each. In LG1, the highest peak of the LOD score was close to the marker *Cdn12p0003i0* and in LG21 the highest peak of the LOD score was close to the marker *Id-13,* with *p*-value <0.05. In LG1, a QTL related to jaw-deformation (Inwards bending (bMa)) was also detected and linked to *Cdn14p0004o13* (Oikonomou et al., 2022). Even though the present findings are promising, further investigation must be done to the QTL linked to the caudal fin abnormality using a higher sample size before it can be used via Marker Assisted Selection in a production breeding program.

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SIMULATION MODELING OF SUBMERSIBLE OFFSHORE AQUACULTURE CULTIVATION SYSTEM

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Offshore aquaculture production technology production is promising and sustainable; it has the potential for food production security related to the global demand, circular economy biomass feed stocks, and protect and thereby preserve fragile ecosystems in the future. The major challenges are that the structure and species cultivation are subject to extreme ocean environmental forces, and lack of adequate system design to mitigate this risk, but also resists the forces at the rough sea itself. There is a need to develop robust cost-effective technologies and tools to ensure sustainability in offshore aquaculture cultivation of aquaculture species. Simulation modelling and advanced risk analysis of offshore technologies are conducted to introduce an efficient and improved method of cultivation that will meet challenging safety requirements and improve production. Submersible tube is a sustainable solution for offshore aquaculture. Simulation software Orcaflex is used for numerical modelling the design of the submersible system for marine aquaculture cultivation, that can withstand harsh ocean environmental force. The system comprises of a submersible tube connected through pulley arrangement to counterweight buoyancy devise, the tube can be controlled hydraulically inner tubes that can be filled with air or water. The system configuration is set up in the marine environment and is simulated with North Sea metocean data of designated farm sites. First principle modelling to determine equivalent Morison force of the system components, that is suitable for typical system design and analysis, is carried out for required for validation of the simulation to optimal performance of the system. The estimated load parameter is used in size the design of a culture system that will reduce the risk of system failure, potential loss of crop, and costly repairs, or replacement of the system components. This work presents the response of the system under the specified weather conditions of the North Sea. The work also presents the analysis of hydrodynamic loads, under varying waves and currents acting on the cultivation system and species. The model accounts for various environmental factors that may impacts the systems performance Figure 1 shows the simulation model of submerged system and Figure 2 presents the effective tension at the end of the tube. Figure 3 shows tubes buoyancy and pulley effort needed.

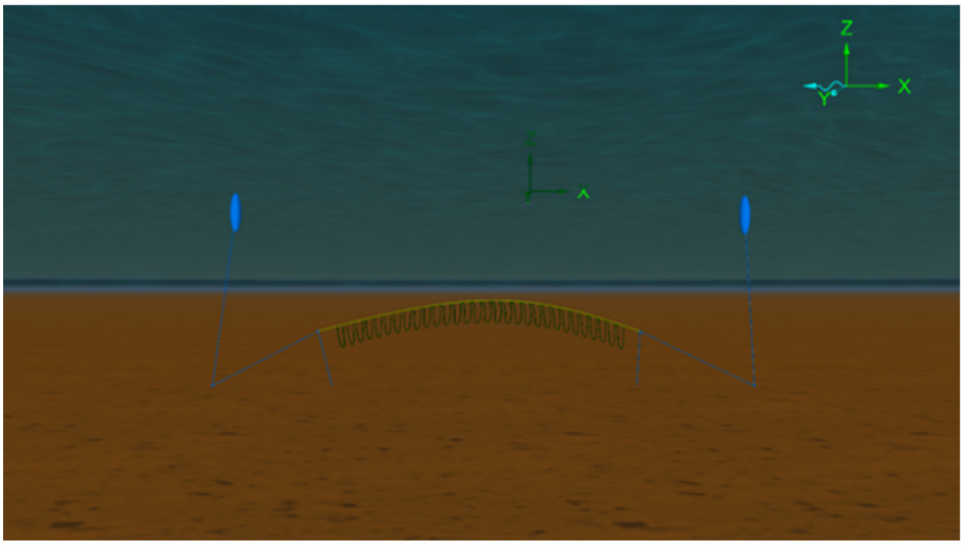

Figure 1: Simulation of the long tube

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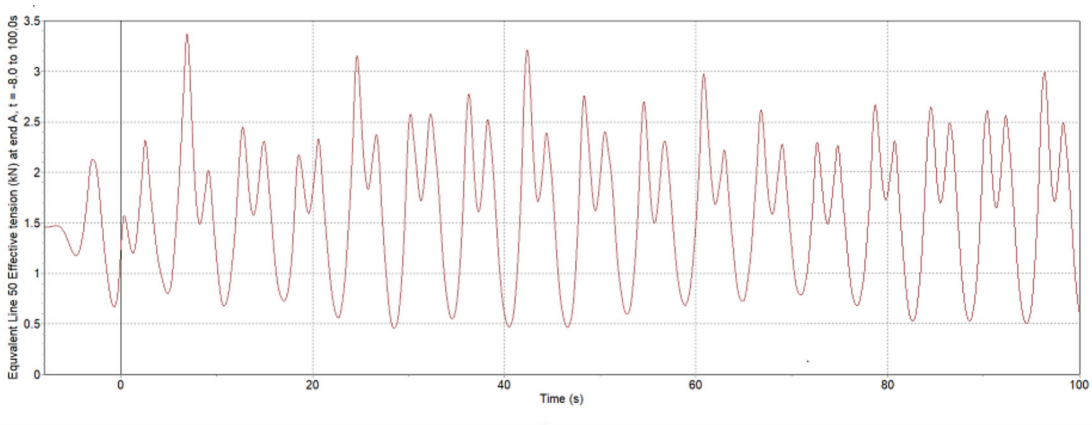

Figure 2: Effective tension at the end of the tube

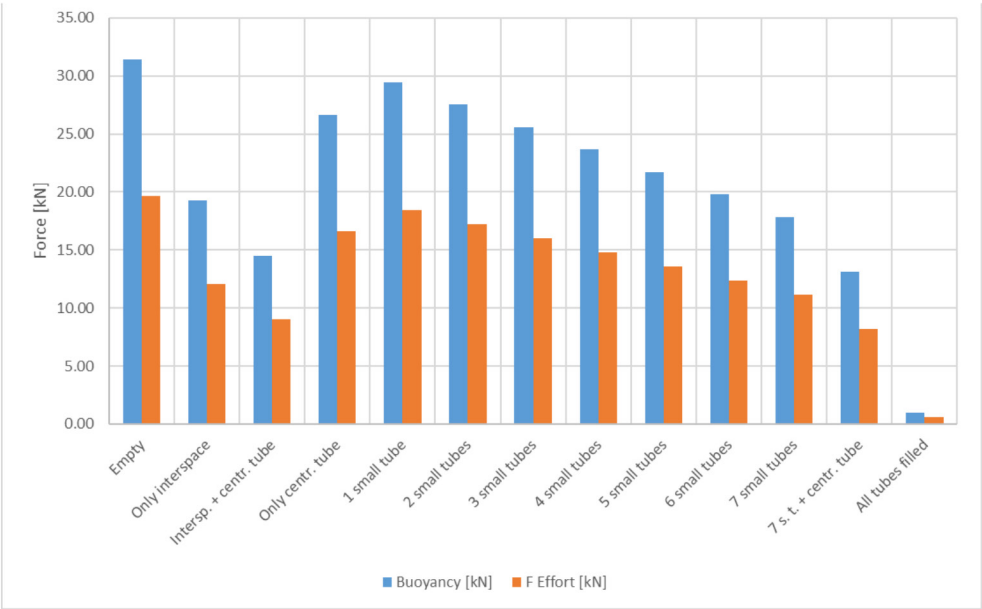

Figure 3: Tube Buoyancy and Pulley effort

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EFFECT OF DIETARY INCLUSION OF *Palmaria palmata* ON OXIDATIVE STATUS OF SEA BASS *Dicentrarchus labrax* JUVENILES

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Introduction

The aquaculture industry has been working to develop innovative aquafeeds that can enhance fish growth and health while ensuring aquaculture sustainability from social, economic, and environmental perspectives. Macroalgae have emerged as a plentiful source of bioactive compounds, such as antioxidants, polysaccharides, and pigments, which hold significant value for aquafeeds. Previous studies on several fish species showed the potential of macroalgae to provide essential nutrients for aquafeeds, leading to improved fish growth, immune response, and antioxidant capacity [1,2].

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species and their elimination, resulting in oxidative damage [3]. Fish possesses a robust antioxidant defense system to protect against oxidative damage. This system comprises both non-enzymatic components and enzymatic mechanisms. Enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR) play vital roles in this antioxidant defense mechanism, neutralizing reactive oxygen species and maintaining overall cellular balance and health [4,5]. The ability of seaweeds to function as antioxidants arises primarily from the presence of dynamic biomolecules. These biomolecules have the potential to mitigate cellular damage caused by oxidative stress [6;7].

Extensive research has been conducted to investigate the antioxidant properties of water- and ethanol-based extracts derived from *Palmaria palmata*, along with their potential to serve as natural antioxidants. The bioactivity of extracts can be enhanced by applying different treatments, highlighting the potential of *P. palmata* and its derived products for use as functional ingredients [8].

Thus, this study aimed to evaluate the effect of dietary *P. palmata* inclusion level (0, 7.5, and 15%) and submitted or not to an alkaline pre-treatment on oxidative defense mechanisms of European seabass (*Dicentrarchus labrax*) juveniles.

Material and Methods

Palmaria palmata was subjected to an alkaline hydrothermal pre-treatment, briefly: *P. palmata* was incubated with a 1N of NaOH, solid-liquid ratio of 4:3 (w/v) solution and autoclavated (121ºC) for 30 minutes. Triplicate groups of 16 European seabass (initial body weight of 38.0g) were fed five isoproteic (48% dry matter basis) and isolipidic (18% dry matter basis) diets, namely, a control diet, with no inclusion of *P. palmata,* and four other diets including 7.5% or 15% untreated or pre-treated *P. palmata* replacing a plant feedstuff mixture of the control diet. The trial lasted 11 weeks, and during the trial fish were hand-fed twice daily, 6 days a week, until apparent visual satiation. At the end of the growth trial, the liver and intestine from 3 fish per tank were collected and stored at -80ºC until enzymatic analysis. The enzymatic activity of glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), glutathione peroxidase (GPX; EC 1.11.1.9), and glutathione reductase (GR; EC 1.6.4.2) was determined. The concentration of malondialdehyde (MDA) was determined as a marker of lipid peroxidation (LPO) level.

Results

In the liver, G6PDH activity decreased with the inclusion of either 7.5%-treated or 15%-treated and untreated *P. palmata* compared to the control diet. Moreover, increasing dietary inclusion levels from 7.5 to 15% of untreated *P. palmata* decreased G6PDH activity. Regarding hepatic SOD, 15% of untreated *P. palmata* increased SOD activity compared with the control diet. At the 15% inclusion level, fish fed with treated *P. palmata* had lower SOD activity than fish fed the 15% untreated *P. palmata* diet.

In the intestine, G6PDH activity was lower in fish fed 15% treated *P. palmata* than in the control diet. Within the 15% *P. palmata* levels, the alkaline treatment promoted a reduction of G6PDH activity. Compared with the control diet, dietary inclusion of 7.5% *P. palmata* decreased intestinal GPX activity. Regardless of the dietary inclusion level, alkaline pre-treatment decreased GPX activity. LPO levels were increased by the dietary inclusion of 15% untreated *P. palmata*, while the pre-treated *P. palmata* promoted a decrease of LPO to levels similar to the control diet.

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In conclusion, the present results showed that dietary inclusion of *P. palmata* decreased G6PDH, SOD, and GPX activity in the liver and intestine of European sea bass. Additionally, dietary inclusion of 15% untreated P. palmata increased hepatic SOD activity and intestinal LPO levels, while the pre-treatment restored LPO levels. Overall, these results suggest that including 15% *P. palmata* could jeopardize the antioxidant response of European seabass juveniles, but *P. palmata* alkaline hydrothermal pre-treatment allowed the restoration of the antioxidant defense mechanisms to those of the control.

Acknowledgment
This work was supported by the project "MB4Aqua: Macroalgae biorefinery: a novel approach to produce sustainable feedstuffs and functional additives towards low carbon footprint aquafeeds", reference 2022. 06587.PTDC funded by Fundação para a Ciência e Tecnologia (FCT). Ramos-Oliveira C was supported by an FCT grant (2021.04809.BD), and Nicole Martins was supported by a research contract (2023_029_IS_MB4AQUA).

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DEVELOPMENT, TESTING AND VALIDATION OF MULTI-SPECTRAL CAMERAS (VIS+NIR) TECHNOLOGY TOOL FOR EGG QUALITY ESTIMATION

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Introduction

Among finfish, the diadromous subgroup (mostly Atlantic salmon and rainbow trout) account for 29.7 % of the overall aquaculture production and 37.8 % of the total aquaculture value. European aquaculture is a highly specialised sector. With the exception of rainbow trout, for each of the 10 major species in the EU at least a quarter of the total volume is farmed in one EU country. Among the 10 major species, rainbow trout (*Oncorhynchus mykiss*) is the most widespread: it is grown in 24 EU countries, either in inland freshwaters (84.2 %) or in the saltwater of the Northeast Atlantic (15.8 %), and mainly in tanks (64.9 %). Three countries together accounted for more than half of the total weight: Italy (17.9 %), Denmark (17.3 %) and France (16.8 %). Most of the productive cycle of rainbow trout has been optimized over the last years; however, the very early embryonic development (from fertilization to eye development) still represents the most vulnerable phase during which entire egg batches loss can occur. In fact, during this early developmental phase, several factors, pertaining to eggs, environment and operational procedures can influence on the hatching rate of fish. Egg infections of ubiquitous Oomycete species, *Saprolegnia* sp., other pathogens, as well as poor egg management may cause annually great losses in production of rainbow trout, becoming a major contributing factor to economical loss in aquaculture worldwide. Several methods including UV sterilizers, formalin, hydroxide peroxide, and ozone have been used to contrast microbial spread; however, most of hatcheries still rely on the manual removal of the whitish eggs. The production of a technological automatic system, able to identify and quickly remove the infected eggs is thus necessary to reduce egg (-food) losses.

Materials and Methods

The present study is aimed at developing and validating an automatic system based on multi-spectral cameras to precociously identify, remove, and count dead trout embryos. Good and bad just-fertilized rainbow trout eggs, obtained from an Italian supplier, were immediately fixed in Formol, and stored at 4 °C until use. Preliminary acquisitions in the visible (VIS) and near infrared (NIR) spectrum were performed on batches composed by good and bad eggs. After placing the eggs on a net in a single layer, both VIS and NIR acquisitions were performed for each batch with or without water. Two multi-spectral cameras manufactured by XIMEA using the IMEC technology were used. The cameras were the MQ022HG-IM-SM5x5 (NIR) and MQ022HG-IM-SM4x4 (VIS). The NIR camera had 25 different spectral bands while the VIS one had 16 channels. Figure 1 shows the set-up we used to acquire preliminary data.

Results and Discussion

Images were acquired using the equipment described in the above section. The pipeline was based on several steps as: 1) image acquisition; 2) extraction of multi-spectral images for each channels with calibrated reflectance data; 3) manual labelling / annotation of images performed by an expert to draw bounding-boxes over each egg also assigning a class as good/bad/uncertain (training set); 4) AI algorithm to detect eggs and assign label based on reflectance data obtained from the training set. Figure 2 shows an example of VIS data.

Figure 3 shows an example of detection starting from collected data. The bad eggs have reflectance values higher than the good ones. The cost of a multi-spectral camera is a key aspect that should be considered. In a real *scenario* (production environment) the use of multi-spectral camera could be challenging considering the overall cost of the solution. The idea is to select a set of wavelengths that maximize the sensitivity of the detection of bad / good eggs. In this way a classical pass-band filter could be used on a monochrome or RGB sensor reducing the overall costs.

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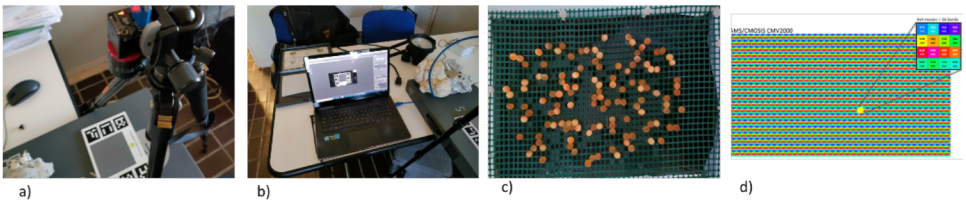

Fig. 1. a) set-up of camera with reference reflectance target; b) data-acquisition system; c) example of image with different eggs; d) detail on filter array for the VIS case (4×4 matrix).

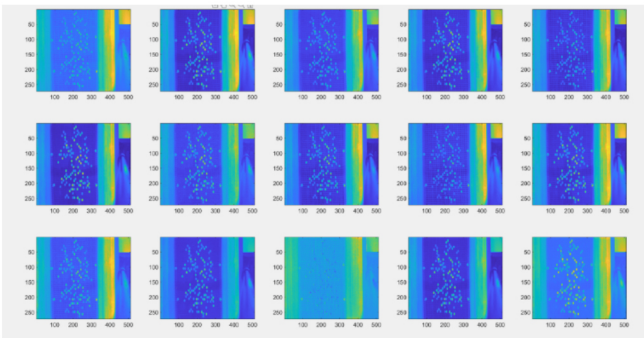

Fig. 1. Image in the VIS range over the 15 channels; in the processing pipeline the number of VIS channels is reduced from 16 to 15 due to internal calibration.

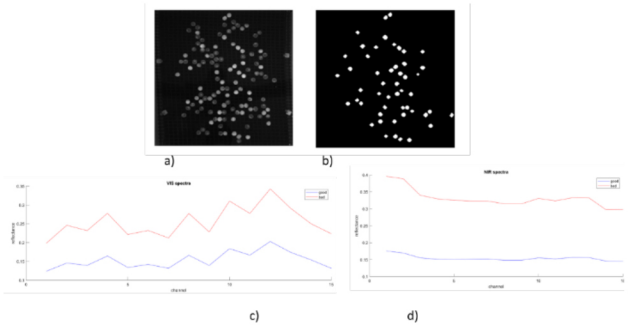

Fig. 2. a) gray-scale view of a channel; b) Identification of bad eggs; c) average spectra over the VIS range for good and bad eggs; d) average spectra of NIR range for good and bad eggs.

This work was supported by the Bringing knowledge and consensus to prevent and reduce FOod LOss at the primary production stage. Understanding, measuring, training and adopting project (FOLOU), HORIZON-CL6-2022-FARM2FORK-01-08, DOI: 10.3030/101084106

BIOLOGICAL PREMISES FOR ATLANTIC SALMON OFFSHORE FARMING.

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Introduction

Parts of future increased production of Atlantic salmon (*Salmo salar*) may take place in offshore environments. Companies have sketched several potential technological solutions to be put out in the open, harsh, oceans with a range of connected service management tools. Within Norway, politicians are positive and legislation authorities have been steered to develop new regulations and review areas for offshore farming.

But will the salmon cope in offshore farming installation? What are the biological challenges for Atlantic salmon in offshore compared to less exposed cages? And do we have enough knowledge to produce salmon offshore? Can new farming technology be based on biological premises?

Materials and Methods

Over the last decades several projects have addressed biological challenges to the salmon in exposed farming and a review was presented by Hvas et al (2021). Since then, more sophisticated trial setups in swim tunnels, observations in the field, development of new tag technology and farming methods have been carried out together with variable advisory reports. This presentation will show and summarize some key recent findings.

Results and discussion

Atlantic salmon is an athletic fish species with a surprisingly high sustained swimming capacity that last for >72 hours at 80% of U_{crit} (critical swimming speed). In fluctuating water speeds mimicking wavy conditions, salmon unexpectedly display higher swimming capacities compared to constant speeds. Fasting seem to have negligible negative effects on swimming capacity and periods of non-available feed may not become problematic. From observations within a limited number of cages, salmon display deeper swimming during strong surface wave actions. Submerged farming technology with airdomes for swim bladder filling may be a future successful offshore technology. Three offshore areas for farming outside Norway is being reviewed based on distance from shore, wave and current characteristics, temperatures, pathogen spread, bottom characteristics, wildlife and other conflicting interests together with fish health and welfare. Typically, offshore installations are large and most sketched future farming technologies are longer and involves 5-10× the number of salmon per cage compared to present farming. It is debatably how large offshore cages can become and still provide adequate environmental conditions for the fish. Even in present cage technology low oxygen conditions are regularly seen. For some of the sketched enormous offshore cages, hypoxia might become a future challenge for the well-being of the fish. Offshore cage farming needs to be based on biological premises.

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DIVERSITY AND ANTIBIOTIC RESISTANCE OF *Splendidus* CLADE (GENUS *Vibrio*) IN BIVALVE AQUACULTURE OF EASTERN ADRIATIC

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Introduction

Bivalves filter water as part of feeding process and accumulate large number of various particles and microorganisms, including the potential bacterial pathogens. One of the most relevant genera found in bivalve tissue is genus *Vibrio*, due to the fact that they cause mass mortality and huge economic losses in aquaculture. Among *Vibrio* pathogens, *Splendidus* clade is of particular concern due to numerus bivalve pathogens such as *V. splendidus*, *V. crassostreae*, *V. tasmaniensis* etc. (Destoumieux-Garzón et al., 2020). Additionally, one of the growing problems in marine environments is the emergence of antibiotic resistance due to the excessive use of antibiotics and anthropogenic discharges into the marine environment. However, antibiotic resistance in eastern Adriatic bivalve aquaculture has been poorly studied (Baralla et al, 2021).

Materials and methods

Focus of our research are two most important species in aquaculture in Croatia - European flat oyster, *Ostrea edulis* Linnaeus, 1758 and Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, 1819. These species were sampled in two protected marine reserves – Lim Bay and Mali Ston Bay, locations which are known for bivalve aquaculture. We sampled seawater, sediment and bivalve tissue (hepatopancreas and gills) on both locations. For bacteria isolation, *Vibrio* selective medium was used (*Tiosulphate Citrate Bile Salt Sucrose* - TCBS). Subsequently, we identified *Vibrio* clades with MALDI-TOF mass spectrometry (Culot et al, 2021). Due to large number of isolates, we applied BOX-PCR genomic fingerprinting method as intermediate step for determination of differences between species and identification of clones (Canellas et al, 2021; Culot et al, 2021). As final step, we preformed Multilocus Sequence Analysis (MLSA) with three selected phylogenetic marker genes (*gyrB*, *mreB* and *rpoD*) for species identification (Pérez-Cataluña et al, 2016). *Vibrio* isolates belonging to *Splendidus* clade, were tested for antibiotic resistance *via* disk diffusion on Mueller Hinton agar for 13 selected antibiotics which are frequently used in aquaculture and medicine.

Results

MALDI-TOF MS results showed that more than half of all culturable *Vibrio* bacteria belonged to *Splendidus* clade. As we isolated large amount of *Splendidus* clade samples (n=688), we applied BOX-PCR genomic fingerprinting method as intermediate step for identification of clones which eliminated numerous isolates from further molecular analysis. Our final step of identification to species-level we preformed MLSA which resulted in phylogenetic tree, displaying clusters of two or three species, namely, *V. splendidus-hemicentroti*, *V. tasmaniensis-atlanticus*, *V. crassostrea-gigantis-celticus, V. kanaloae-toranzoniae* and *V. chagasii-pomeroyi*. Results of antibiotic resistance showed that more than 94% of all isolated bacteria belonging to *Splendidus* were resistant to one or more antibiotics. Furthermore, almost all isolates were resistant to vancomycin (96%). However, there is rising concern for antibiotics erythromycin, oxytetracycline and oxytetracycline due to intermediate resistance for around half of isolates. This study shows first report on the diversity and antibiotic resistance of *Splendidus* clade *Vibrio* species from bivalve aquaculture in Croatia and shows rising concern that on-going increasing anthropogenic pressures, might negatively affect bivalve aquaculture both with bigger susceptibility of farmed bivalves to colonization by *Vibrio* pathogens and with more resistant *Vibrio* bacteria.

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Acknowledgements
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DEVELOPMENT OF A QUALITY SCALE FOR THE PHENOTYPIC CATEGORISATION OF PUGHEADEDNESS IN GILTHEAD SEABREAM

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Introduction

Skeletal abnormalities are a significant source of phenotypic variation in Gilthead seabream, which is mostly marketed whole (Fragkoulis et al. 2019). Deformities such as lordosis and pugheadedness exhibit a broad and continuous range of negative effects on external morphology, making the quality control and cull procedure cumbersome. This highlights the need for a precise and accurate quality scale linking the abnormalities' severity with changes in fish external morphology (Fragkoulis and Koumoundouros 2021). In the present study, a scale of quality was developed to quantify the effects of pugheadedness on the external morphology of Gilthead seabream.

Material and Methods

A total of 206 deformed and 70 normal fish (ca 400g mean weight) were selected from an unsorted experimental population, anaesthetized, and individually photographed. All deformed fish displayed pugheadedness that was not associated with severe malformations of the maxillaries and pre-maxillaries (*Par* type, Al Belbeisi et al. 2023). A geometric morphometric approach was employed to visualize and quantify shape differences among all fish. Specifically, Principal Component Analysis (PCA) was performed on the weight matrix (MorphoJ software package, version 1.07a) to study the deformity-induced body shape variation and test whether this variation could be related to *Par* severity.

Results & Discussion

Studied abnormality presented a continuous range of severity, mostly expressed as a distortion on the bending of the snout, without other obvious morpho-anatomical features (Fig. 1). The first principal component axis, PC1 (29.8% explained variation), contrasted between abnormal and normal fish, albeit with a slight overlap. Following their distribution along the PC1 axis, all the individuals were grouped into four classes and statistically compared. Differences in PC1 scores among the different classes were significant ($p<0.05$, Kruskal-Wallis and Mann-Whitney U tests), thus indicating the suitability of PC1 in classifying *Par* severity. Representative fish images for each of the Par severity classes are given in Fig. 1.

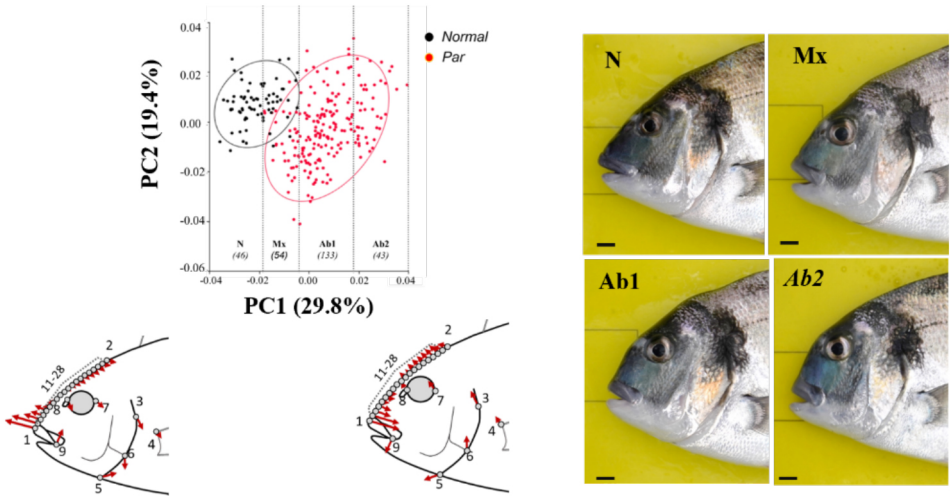

Fig 1. Left: Scatterplot of the first two principal component axes (PC1, PC2) depicting fish shape variation, including equal frequency ellipses. Spline diagrams demonstrate the components of shape change relative to the extreme values of PC1 axes (±X3). Right: Representative cases of the *Par* severity classes, as they were defined by PC1. Ab1, proximal part of PC1 range with only abnormal fish. Ab2, distal part of PC1 range with only abnormal fish. Mx, overlapping PC1 range of normal and abnormal fish. N, PC1 range with normal fish only. *Par*, Type I pugheadedness (Al Belbeisi et al. 2023). Scale bars equal to 1 cm.

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Deformities with similar phenotypic expressions but different ontogenetic and causative factors can develop in the same species, as is the case with pre-haemal and haemal lordosis. In Gilthead seabream, pugheadedness was shown to consist of two abnormality types, with significant differences with respect to their anatomy and recovery potential during the on-growing period (Al Belbeisi et al. 2023). The present study focused on the pugheadedness type that partially recovers (*Par*, Al Belbeisi et al. 2023) and developed a quality scale that classifies abnormal fish based on the severity of morphological deviation from the normal.

Our results could be applied by fish producers for the precise phenotypic scoring of pugheadedness in selective breeding programs and to improve precision for grading fish during packaging. In our next steps, we intend to test whether simpler morphometric indices (e.g., angles, bivariate ratios) could efficiently quantify the severity of *Par* abnormality.

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SOLVING THE RAW MATERIAL CRISIS: A NORWEGIAN PERSPECTIVE ON DEVELOPING FUNCTIONAL INGREDIENTS FOR NOVEL AQUAFEEDS

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Introduction

Today, Atlantic salmon is Norway's number two export in value, and the country wants to increase salmon production from 2 million tons today to 5 million tons by 2050. However, the industry faces multiple challenges, including a shortage of feed resources and high fish mortality due to exposure to multi-stressor conditions, such as sub-optimal nutrition, pathogens, environment, and handling stress, that need to be solved. To support further growth, we need to develop alternative feed ingredients from local underutilized natural resources that not only support high growth performance but also health and robustness of fish and that have low environmental impact. Microbial ingredients (MI) such as yeast and fungal proteins hold promise as alternatives; microbes have rapid growth rates, do not require agricultural land, use little fresh water, and can efficiently convert underutilized resources that do not compete with human food into high-quality proteins [1]. Thus, MI can meet the high protein demand of fish and they contain bioactive components with beneficial health effects. Foods of Norway, a Centre for Research-based Innovation at NMBU (**foodsofnorway.net**) and related projects, including NordicFeed, ForestFeed and Resilient Salmon are developing novel MI from local resources from forest by-products, animal by-products and food waste by biorefinery processing. Functions and applicability of these MI are evaluated during critical life stages of fish, such as during seawater transfer.

Results

Our results have shown that *C. jadinii* yeast produced from Norway spruce tree sugars contains about 50-58% crude protein and has a favorable amino acid composition. In general, diets based on *C. jadinii* supported high growth performance and improved health and robustness of Atlantic salmon [1-2]. We have also shown promising results on growth performance, health and product quality of *C. jadinii*-based diets under field conditions during the grow-out phase in seawater. In the Resilient Salmon project we have evaluated the use of hydrolysed *Debaryomyces hansenii*-based products (LAN4 and LAN6) in functional feeds during multi-stressor conditions to develop nutritional programming and to improve health and robustness of Atlantic salmon. Results showed that after exposure to an acute hypoxia, fish fed LAN4 were able to prevent the secretion of plasma cortisol and IL-10, which are both biomarkers associated with immunosuppression. In the gills, differential gene expression of metabolic pathways associated with stress tolerance and oxidative regulation were found. In the intestine, goblet cells maintained higher levels of mucin proteins, involved in the protection of the intestinal tract [3]. Interestingly, after the *M. viscosa* outbreak in seawater, plasma specific IgM levels against the bacteria increased in fish fed LAN6, in addition to an upregulation of genes related to humoral immune response and complement activation in liver.

Foods of Norway has developed a life-cycle analysis model on *C. jadinii* from Norway spruce tree sugars, which shows that this yeast has a low environmental footprint. However, a techno-economic analysis of yeast production shows that the price of the sugars has the largest impact on cost due to greater demand for biofuel. Considering this, we are developing a fungal protein *Paecilomyces variotii* (PEKILO®) that can be produced from a range of cheaper input factors to reduce cost. The fungal protein has a higher crude protein content of 60-70%. Our recent results suggest that *P. variotii* supports high growth performance and has immunomodulating properties.

Conclusion

To help solve the raw material crisis, we need to further develop biotechnology processing that enables the use of a broader range of cheaper input factors from various waste streams and gasses to produce MI. This will facilitate upscaling and reduce costs to meet requirements of the aquaculture industry.

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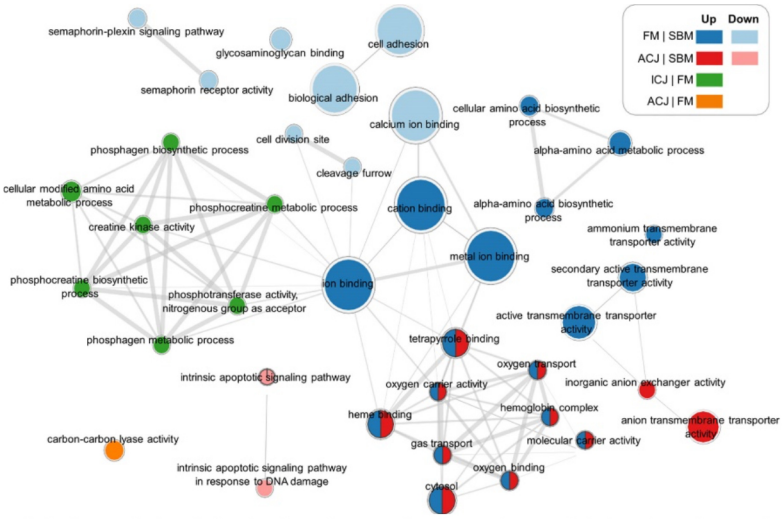

Figure 1. Modulation of physiological pathways in the spleen of Atlantic salmon fed *C. jadinii* yeasts and exposed to a SBM Challenge [2].

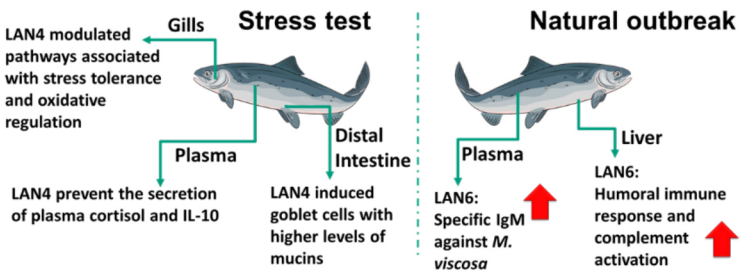

Figure 2. Dietary Inclusion of hydrolyzed *D. hansenii* modulates physiological responses in Atlantic salmon exposed to multi-stressor conditions [3].

SEASONAL STUDY ON THE REPRODUCTIVE STATUS OF MACROALGA *Codium tomentosum*

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Introduction

Macroalgae production has been gaining more support and recognition for its great potential, since these showed to have so many applications. However, the focus still remains in the same few species and diversification of seaweed production needs to happen, especially as other species have already been recognised for their potential.

Codium tomentosum is an important source of food and biomass with many bioactive properties [1;2]. This incites the interest of multiple industries, turning it into a highly desirable seaweed and consequently, raising the need for its cultivation. However, there´s a lack of knowledge when it comes to reproductive strategies and, therefore, cultivation approaches. This species experiences sexual reproduction as it releases gametes. Despite this, *C. tomentosum* is currently cultivated via fragmentation followed by vegetative propagation, which reduces the genetic diversity of the cultivated species.

In order to find alternative ways to produce this species, the gamete availability throughout the year should be studied. For this reason, this study focuses on the reproductive status of *C. tomentosum*.

Material and methods

Monthly, 20 specimens were collected in Praia da Paimó, Aguçadoura shore (41° 26′ N, 8° 47′ O), located in Póvoa de Varzim. The reproductive status was evaluated, through the observation (presence or absence) of reproductive structures, as well as their capability of developing to the next stages, gametes, zygotes, and early stages of development (germlings). These observations were followed through microscope. The specimens' length was measured with a standard measuring tape and the mean was calculated

Results

The samples were only considered reproductive (fertile), when it was observed the release of gametes, germination, and development of germlings. From 2020 to 2022, *Codium tomentosum* individuals were fertile from August to March and infertile from May to September, implying that this species follows a seasonal pattern.

From September to November, there was a significant increase in length, reaching its highest peak in November followed by a gradual decrease through the following months. The lowest peak was observed in April, gradually increasing until August.

Discussion

The natural population of *Codium tomentosum* collected from the Northern Portuguese coast showed to be reproductive from August to March as gametangia was present and germination occurred. As of April, the specimens collected were no longer fertile as germination did not occur. This coincides with the moment when the older *Codium* specimens on the shore are being replaced by new seedlings, immature ones, thus possibly explaining the infertility from April to July. Interestingly, the infertile specimens collected between March and July also presented lower observed thalli length, corroborating our hypothesis that these are immature juvenile specimens.

The lengths of the specimens for each month differs in both years (2020-2021 and 2021-2022). However, when analysing the pattern of the growth, for the entirety of the two years, the periods of time for the increases (Sept-Nov; Apr-Aug) and decreases (Jan-Apr) in length coincide.

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The months where the specimens weren't fertile and the smaller lengths were observed, (April to July), coincide with the spring and summer months. This suggest that the conditions that influence the reproductive status are, most likely, temperature and photoperiod. Excessive radiation can reduce photosynthetic activity, leading to reduction in growth. Many seaweeds can suffer photo-inhibition during times of strong sunlight [3]. These occurrences happen mostly during spring/summer when the days have more sunlight exposure and temperatures increase. Therefore, the fact that the older *Codium* specimens start being replaced by the new seedlings during this time leads to believe that this seaweed uses this pattern as a strategy to grow and mature during the more favourable months. Corroborating even more the theory that *Codium tomentosum* does indeed follow a reproductive pattern through the year.

Acknowledgments
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BIOLOGICAL AND ECOLOGICAL FEATURE OF TILAPIA (*Oreochromis niloticus*) IN THE CHICKAN LAKE DISTRACT DADU

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Tilapia (Oreochromis niloticus) is an invasive fish species in Indonesia. A study on biological feature is expected to be used as an assessment to implement fisheries mitigation, restoration, and enhancement. This study aimed to analyze the ecological l conditions, biological feature of the sex ratio, total length-weight relationship, maturity stages, and tilapia fecundity in the Chickan lake. Fish samples were collected four times in April to Dec 2022 using gill nets (mesh size of 3 inches). The sampling sites were 4 stations located in Chickan lake. Ecological parameters were measured, including water temperature, pH, Salinity, dissolved oxygen., Fish were measured for total length, weighed for gonads, and total weight. water temperature, pH, Salinity, dissolved oxygen ranged from 18.0-30.9 ^{o}C, 6.51-7.70, and 5.04-8.39 mg/l, and 7-12ppt respectively. The sex ratio of tilapia was 2:1.5 (females: males). Total length ranged from 90 to 360 mm, and total weight ranged from 15 to 780 g. The growth pattern of tilapia was positive allometric. The condition factor of male and female fish was 0.94-1.16 and 0.95-1.21, respectively. 36.8% of the female fishes were ripe stage (IV), and 65.8% of the male fishes were immature stage (I). The fecundity ranged from 28 to 7,852 eggs.

DOES THE LIGHT COLOR MODULATE STRESS DURING OUT-OF SEASON REPRODUCTION OF EURASIAN PERCH FEMALES – FIRST STEP TO UNDERSTANDING

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Introduction

Since the 1990s, the Eurasian perch (*Perca fluviatilis* L.) has been considered one of the key species for diversifying aquaculture in Europe (Fontaine and Teletchea, 2019). Despite the fact that commercial production of this species began over 30 years ago and is already established in some countries, research on its biology, larviculture, and reproduction technology in controlled conditions is still being optimized and developed (Żarski et al., 2017; Palińska-Żarska et al., 2020). One aspect that still requires in-depth research is determining the optimal lighting conditions, especially during out-of-season reproduction in Eurasian perch. This is especially important when considering the modulation of light as a tool to reduce stress during this critical period. In the present study, selected genes related to stress and immune response were investigated in response to different light colors (white, blue, and red) used during the controlled out-of-season reproduction of this species.

Material and methods

Pond-reared Eurasian perch females, obtained in early November, were transferred to a recirculating aquaculture system (RAS). They were then subjected to a 40-day wintering period before spawning. Throughout this entire period, the fish were exposed to three different light colors: white (W), blue (B), and red (R). At five specific sampling points (S), the females were anesthetized, and their livers were collected for RNA extraction (n=6 for each S) (Fig. 1.). Subsequently, qPCR was conducted to assess the expression of selected genes: *hif1α* (hypoxia-inducible factor 1α), *tnfα* (tumor necrosis factor α), *hsp70* (heat shock protein 70), *hamp* (hepcidin), and *lyz* (lysozyme). The obtained data were compared using a two-way ANOVA, and differences were further analyzed through Tukey's *post-hoc* test ($p<0.05$). All statistical analyses were performed using Statistica software developed by StatSoft.

Results

Two-way analysis of variance ($p < 0.05$) indicated significant interactions between light color and the expression of the genes encoding *tnfα*, *hsp70*, and *hif1α*, while there was a lack of significant interactions ($p > 0.05$) between light color and the expression of the genes encoding *hamp* and *lyz*. Furthermore, Tukey's *post-hoc* analysis revealed significant differences ($p < 0.05$) in the expression of *hif1α* at S2 for all tested light colors, as well as *hsp70*, but solely in the case of R light. Additionally, a higher expression of *hif1α* in S3 was observed, specifically in W light (Fig. 2).

Discussion

One of the factors that significantly impact the induction of stress reactions in fish is the light color during rearing conditions (e.g., Maia and Volpato 2013). Therefore, the aim of this study was to address the question: Can different applied light colors during the out-of-season reproduction of pond-origin Eurasian perch, kept in RAS, somehow influence their stress levels? The used light colors did not yield significant differences in the maturation of females. However, the analysis of selected genes related to fish reactions to stress and their immune responses indicated that the light colors in which the spawners were kept significantly affected the expression of the *hif1α* and *hsp70* genes (see Fig. 2). It was revealed that these light colors stimulated stress reactions in fish at the beginning of the wintering phase, before reaching the final oocyte maturation. Importantly, just before spawning, the levels of all tested genes were comparable. The results obtained suggest that varying light colors indeed significantly influence stress reactions in fish during their adaptation to rearing conditions in RAS. However, these light colors do not directly impact the final reproductive outcome. Similar stress reactions to different colors were observed in goldfish (*Carassius auratus*), where B and R colors induced chronic stress and immunosuppression (Eslamlo et al. 2013). These preliminary observations need in-depth exploration of additional stress and immune-related parameters. Considering the distinct reactions of Eurasian perch females to different light colors and their welfare, it is advisable to employ W light to minimize stress associated with fish adaptation to RAS conditions, whenever possible.

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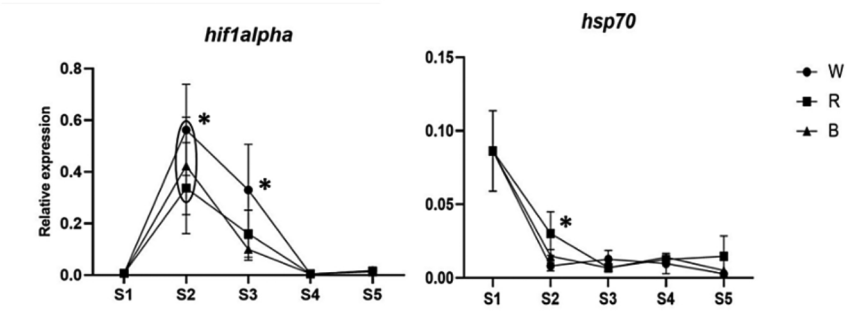

Fig. 1. Diagram of sampling points (S) during out-of sezon reproduction of perch. S1 – acclimation; S2 – beginning of wintering faze; S3 – beginning of "heating" faze; S4 and S5 – hormonal stimulation, first and second dose, respectively.

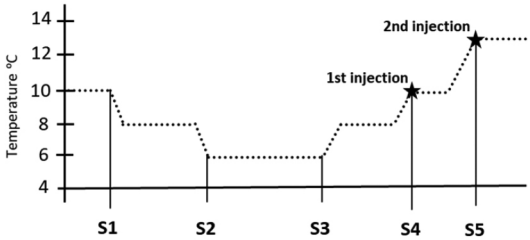

Fig. 2. Relative expression of chosen genes, during rearing Eurasian perch females in three different light colors. Data (mean ± SD) marked with asterisk were significantly different ($p < 0.05$). W – white, R – red, B – blue, *hif1alpha* - hypoxia inducible factor 1 α, *hsp70* – heat shock protein 70, S – sampling point.

Acknowledgment

This work was performed in the project " Diversification of pond-based production through semi-intensive aquaculture of Eurasian perch, *Perca fluviatilis* " (Grant agreement no 00002-6521.1-OR1400004/17/20, acronym: PRO-PERCH), financially supported by Polish Operational Programme "PO RYBY 2014–2020" within European Maritime and Fisheries Fund.

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THERMAL TOLERANCE IN CLEANER FISH (*Labrus bergylta*): THERMAL LIMITS, GILL TRANSCRIPTOME AND DNA METHYLATION

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Introduction

Ballan wrasse (*L. bergylta*) has become a popular choice of biological control against sea lice infestation in salmon farms in the UK and in other salmon-producing countries in Europe. They are stocked in cages with salmon where they stay throughout the ongrowing period of up to 2 years. This consequently subjects ballan wrasse to seasonal changes in environmental conditions specifically water temperature which affects their delousing performance and welfare (Brooker et al, 2018). During winter, ballan wrasse has been observed to enter dormancy, exhibiting stunted growth and increased susceptibility to diseases (Erkinharju et al 2020). However, the basic understanding of thermal tolerance limits and tolerance range of ballan wrasse is lacking. In the present study, we determined the thermal tolerance limits of ballan wrasse and analysed the molecular changes following acclimation to different temperature and exposure to their thermal limits.

Materials and Methods

Hatchery-bred juvenile ballan wrasse were acclimated at a range of water temperature (6°C, 10°C or 14°C) for one week. Critical Thermal Methodology (CTM) was then performed wherein ballan wrasse from each group were individually placed in a challenge container and subjected to either increasing or decreasing water temperature until fish exhibit loss of equilibrium, indicating their upper (CTMax) and lower (CTMin) thermal tolerance limits. Gill tissues were collected from fish before and after they were subjected to CTM and analyzed for changes in transcriptome and methylation analyses.

Results and Discussions

CTM has shown that thermal tolerance of ballan wrasse shifts depending on acclimation temperature (Fig. 1). CTMax and CTMin values are lowest in fish acclimated to 6°C and highest in those acclimated to 14°C. Consistently, the thermal tolerance range was positively correlated with acclimation temperature. No prior estimate is available for the thermal tolerance limits of ballan wrasse although it has been shown that decrease in temperature is associated with significant decline in metabolic performance and aerobic scope which limits their tolerance to environmental conditions (Yuen et al 2019).

Preliminary results from transcriptome analysis show that acclimation temperature has an effect on the overall gene expression profile in the gills of ballan wrasse. Differential gene expression analysis between fish exposed to CTMax and CTMin is ongoing. In addition, DNA methylation data is being analysed to determine the epigenetic effects of acclimation temperature and exposure to thermal tolerance limit to ballan wrasse. This study will provide relevant information to improve our understanding of the physiological responses associated to temperature changes in ballan wrasse.

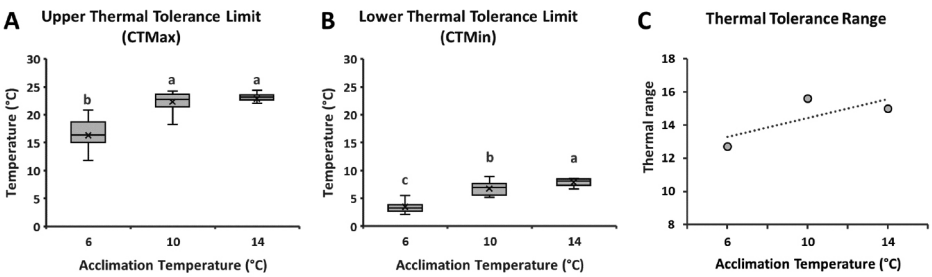

Fig. 1. Thermal tolerance of ballan wrasse at acclimated to different temperature. (A) Upper limit, (B) Lower limit, and (C) Range. Values that share the same superscript are not significantly different ($P<0.05$).

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POPULATION GENOMICS SCREENING OF *Ruditapes decussatus* IN THE ATLANTIC AND MEDITERRANEAN EUROPEAN COASTS: SIGNATURES OF SELECTION TO THE PARASITE *Perkinsus olseni*

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Introduction

Ruditapes decussatus, commonly known as the grooved carpet shell clam, is a commercially important bivalve species distributed throughout the Mediterranean Sea and the NE Atlantic Ocean. Its production has declined due the introduction of Manila clam (*R. phillipinarum*) and to environmental factors such as heat waves, low salinity and ocean acidification, as well as to several diseases, such as perkinsosis, caused by the parasite *Perkinsus olseni*.

P. olseni outbreaks have produced significant economic losses in the shellfish industry due to reduced growth rates and increased mortality. Clams can acquire the parasite through ingestion of infected water or through contact with infected individuals. Once inside, the parasite replicates within different organs, including the digestive gland and gills (Choi & Park, 2010). Control and prevention of *P. olseni* infections in *R. decussatus* has relied on a combination of management strategies, including monitoring and surveillance of infected populations, proper handling and disposal of infected individuals, and minimizing stressors that may weaken the immune system of clams (Ramilo et al., 2016). Despite management and prevention strategies will continue to play a critical role in reducing the impact of perkinsosis on the shellfish industry, understanding the genetic basis of the resilience to perkinsosis can facilitate developing breeding programs to obtain resilient strains for its control.

The aim of this study was carrying out a populations genomic screening to analyse the genetic structure of the grooved carpet shell clam to know its genetic structure across the Atlantic and Mediterranean areas and to use this information to identify outlier loci pointing to specific genomic regions associated with perkinsosis resilience.

Materials and Methods

High molecular weight (HMW) DNA was obtained from abductor muscle of one individual from the Atlantic area. Long-read Nanopore (50x) and short read Illumina (150 bp pair-end; 120x) sequencing was carried out for genome assembly. Furthermore, RNA from digestive gland, gill, mantle, haemolymph, and muscle of five individuals was extracted and equimolecular pooled by tissue for RNA-seq for genome annotation.

The Ray's fluid thioglycollate medium (RFTM) histological method was used to evaluate the degree of perkinsosis infection from 0 (non-infected) to 5 (heavily infected).

Seven shellfish beds (~30 clams / bed) distributed across the NE Atlantic (NO: Noia, PO: Pontevedra, ALG: Algarve) and the Mediterranean Sea (SAR: Sardinia, VEN: Venice, NAP: Naples, TU: Turkey) coasts were sampled (Fig. 1). One sample was never in contact with the parasite (naïve), while the other seven were long-term affected. 2b-RADseq genotyping was performed using STACKS program taking the assembled draft genome as reference. The whole genotyping data was used to ascertain genetic diversity and population structure considering the geographical hierarchy (GENEPOP, ARLEQUIN, STRUCTURE). Outliers showing signals of divergence over the neutral background were identified across the species distribution range (BAYESCAN, ARLEQUIN), but also considering the different degree of perkinsosis infection of samples estimated by histology.

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Figure 1. Sampling map of *R. decussatus* in Atlantic and Mediterranean coasts.

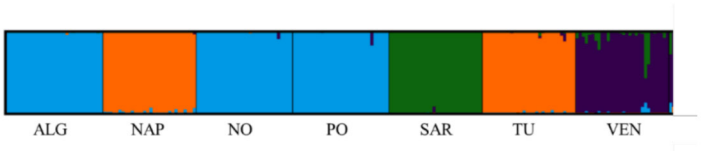

Figure 2. STRUCTURE analysis of *R. decussatus* (K=4)

Results

The draft genome *R. decussatus* rendered a C-value of 1.77 Gb, assembled into 1,220 contigs (N50 = 1.83 Mb) and BUSCO annotation close to 95%.

A total of 23,968 consistent SNPs were identified after filtering using the reference genome. Genetic diversity was significantly lower in the Atlantic than in the Mediterranean Sea shellfish beds (He: 0.137 *vs* 0.179). Genetic structure analyses differentiated two main clusters including the Atlantic and the Mediterranean beds, respectively (Fig.2). Within the Mediterranean cluster, the Venice sample seemed a population in-between the main clusters, suggesting restocking with seed of both origins. Using the neutral structure as background, 56 outliers showed consistent signals of divergent selection across different levels of infection, suggesting their potential for marker assisted selection programs.

Conclusions

Genomic resources are very valuable to manage and restore depleted beds of molluscs. Here we present a first draft genome of *R. decussatus* that was applied for RADseq genotyping across the distribution range of the species. Two main regions were identified to be considered in restoration programs and a set of outlier markers could potentially help in the production of strains with improved resistance to perkinsosis.

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IDENTIFICATION AND VALIDATION OF GENETIC MARKERS ASSOCIATED WITH *Marteilia cochillia* RESILIENCE IN THE COMMON COCKLE (*Cerastoderma edule*).

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Introduction

The cockle shellfish beds of the Ría de Arousa (Galicia, NW Spain) were devastated in 2012 by the protozoan parasite *Marteilia cochillia* responsible for marteiliosis. The annual outbreak of this parasite threatens production and the ecosystem services provided by cockles In 2018, Villalba et al. (2021) suggested the presence of naturally resilient stocks, when compared their survival to naive cockles (never in contact with the parasite) from a northern estuary (Ría la Noia).

Generating preventive measures against parasitic infection is complex due to the lack of knowledge of the parasite's biology and the difficulty of fighting against a parasite in a natural open environment. Therefore, developing resilient strains against *M. cochillia* through breeding programmes is an appealing approach, previously applied in other molluscs to control the impact of the parasite and restore cockle beds.

This study aimed to identify candidate genetic markers, single nucleotide polymorphisms (SNPs), associated with resilience to marteliosis for developing and validating a cost-effective genomic tool to assist in the restoration of cockle beds and preventing economic losses due to parasite spreading to other areas. For this, a common garden experiment involving naive and long-affected cockle stocks was carried out in Ría de Arousa in 2021 using families founded at hatchery.

Materials and Methods

To identify markers associated with *Martelia* resilience we followed two complementary approaches: (i) population genomics (PGA) using 77 samples, 39 collected in 2018 from a long term affected shellfish bed in the Arousa estuary, Lombos de Ulla, before and after the annual outbreak of marteiliosis, and 38 naive from 2012 before the first report of marteliosis in the area. Samples were genotyped using a 2b-RAD panel of 9.154 SNPs [2] mapped on the cockle's genome and used to identify outlier loci . (ii) transcriptomic (TA), where cockles sorted by infection degree were compared to identify SNPs associated with 767 differentially expressed genes (DEG) [3] according to the infection status . SNPs from both approaches were filtered according to missing data, heterozygosity (He) and technical criteria, to select the most consistent SNPs to be genotyped using a MassARRAY platform [4].

For the common garden experiment, mature breeders (400 individuals) from naive and long-affected shellfish beds were used to produce families at hatchery, After a pre-growing stage in a raft, 300 cockles from each stock were settled in two shellfish beds in Ría de Arousa under marteiliosis pressure. Prevalence and mortality were recorded through histopathology. Individuals were histologically classified according to the level of infection and genotyped with the SNPs panel selected (183 naïve, and 197 long affected).

Results and Discussion

From the TA, a total of 121 DEG-SNPs showed significant differentiation depending on the cockle's infection degree and another 110 outlier SNPs from the PGA were selected and mapped in the cockle's genome. Finally, a panel of 45 SNPs including both approaches were combined in two multiplexes to be genotyped in a MassARRAY platform. Results from the common garden experiment showed that the naive stock was fully depleted after four months in both shellfish beds, while

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the affected stock barely showed marteiliosis.. Among the 45 selected SNPs, 28 showed significant divergence between naive and long affected beds (p value <0.0012; Bonferroni correction), despite no significant differentiation was detected with neutral markers, suggesting they were involved in directional selection after eight generations of marteiliosis pressure (long-term selection). Furthermore, 11 SNPs showed significative differentiation in the short period affecting the naive stock (short-term selection), most of them shared with those from the long-term comparison. Several SNPs were located within relevant immune genes pertaining to families such as proteasome, ubiquitine and glutation S-transferase.

Conclusions

Common cockle became resilient to marteiliosis after eight generations of selection. The genomic approach followed to identify SNP markers associated with resilience proved to be useful and a total of 28 SNPs are candidates to be applied in marker assisted selection programs to manage common cockle shellfish beds. Some of these markers are within genes involved in critical immune functions. A SNP panel can be applied to recover common cockle shellfish beds in Galicia.

Funding information

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VALIDATION OF A METHODOLOGY FOR APPARENT NUTRIENT DIGESTIBILITY AND FAECAL COLLECTION BY SEDIMENTATION IN PIKEPERCH (*Sander lucioperca*)

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Introduction

In aquaculture, the evaluation of feed ingredients is essential for the development of high quality aquafeeds. The determination of nutrient digestibility is the first step in evaluating the potential of an ingredient for use in the diet of an aquaculture species. This information is very useful for formulation of diets that maximize the growth of fish, by providing appropriate amounts of available nutrients, but also to limit fish waste products.

Over the last decades, apparent digestibility coefficients (ADC) of dry matter, crude protein, crude lipid, gross energy and phosphorus of different ingredients have been determined for different aquaculture species. However, different methods of feces collection (dissection, stripping or sedimentation) and different inert markers (chromic oxides, celites or yttrium) have been used, which often makes it difficult to compare results. Moreover, there is a lack of information on the ADC of ingredient in emerging aquaculture species. To our knowledge, no reports are available for the ADC of nutrients in pikeperch (*Sander lucioperca*). The objective of this work was to validate feces collection by sedimentation for the determination of ADC in macronutrients and phosphorus in pikeperch using yttrium as inert market.

Material and methods

The trials were conducted in a recirculating aquaculture system (RAS) consisting of 12 circular tanks (750-L/tank), a drum filter, moving bed filter, UV filter and an oxygen tower. Each tank was connected to a 200-L settler for feces collection.

A practical diet, 52% crude protein, 17% crude fat, 20.5 MJ/kg gross energy and 0.02% Yttrium (Y_2O_3) as inert market, was used in two trials. In the first trial, fifty pikeperch (body weight: 109 ± 9.8 g) were stocked on each tank for ADC determination. For three weeks, pikeperch were fed once a day until apparent satiation. The feces were collected after 18 hours, in cold plastic tubes connected to the bottom of the settlers, and stored at -20°C until analysis. The feed and the feces were analyzed for proximal composition of macronutrients, phosphorus and yttrium. The ADC was calculated using the formulas described by Glencross BD et al. (2007).

In the second trial, feces collection using three different strategies was determined. 1) one feeding per day-feces collection after 18 hours; 2) one feeding per day-feces collection after 21 hours; 3) two feedings per day-two feces collection: after 6 hours and after 18hours. The percentage of feces recovery; feces production and dry weight of feces were determined.

The results of both trials will be presented and discussed during the EAS2023 percid session.

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CRYOPRESERVATION AS A TOOL TOWARD EXPLORATION OF PATERNAL-EFFECT GENES IN EURASIAN PERCH, *Perca fluviatilis*

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Introduction

Sperm cryopreservation is one of the leading, yet sophisticated techniques to employ both in research purposes and aquaculture production. It has already been reported that sperm cryopreservation may affect the transcriptomic profile of the progeny (Wang et al., 2022). It is, therefore, justified to hypothesize that by implementation of cryopreservation technology, possibly modifying cell physiology and/or affect structure of the cells retaining fertilizing capacity, a paternal-effect genes – being poorly explored in Teleosts – can be identified. Such strategy has a potential to elucidate paternal contribution to progeny quality what may be used by aquaculturists to fine-tune the selective breeding operations.

This study aims to explore the transcriptomic and phenotypic consequences of progeny obtained following usage for fertilization either cryopreserved or fresh semen in Eurasian perch (*Perca fluviatilis*), our model of commercial interest, in order to identify paternal-effect genes in this species.

Materials and methods

The semen from wild males (n=6) were stripped, checked for their motility and concentration; divided a portion (~ 1.5 ml) of semen was cryopreserved as described by Judycka et al. (2021), and the second portion (~1.5 ml) was used as fresh semen. Fertilizations were done with eggs coming from each female (n=3) portioned equally, one for cryopreserved semen (group C) and the other with fresh semen from the same male (group F). Advanced larviculture was carried out as described by Palińska-Żarska et al. (2020) till 16 days post hatch (dph), while noting zootechnical parameters. Larvae at mouth opening (MO) stage and weaning stage were sampled for transcriptomic analysis.

Sequencing of RNA of larvae at MO from both groups were compared using RStudio (version 4.1.3) using the package DESeq2 (Love et al., 2014). Differences were considered significant when corrected p-values were inferior to α (α=0.05).

Quantitative real-time PCRs (qRT-PCR) were performed for the differentially expressed genes (DEGs), normalized using 5 housekeeping genes, to verify expression levels of those genes at MO stage and at the weaning stage.

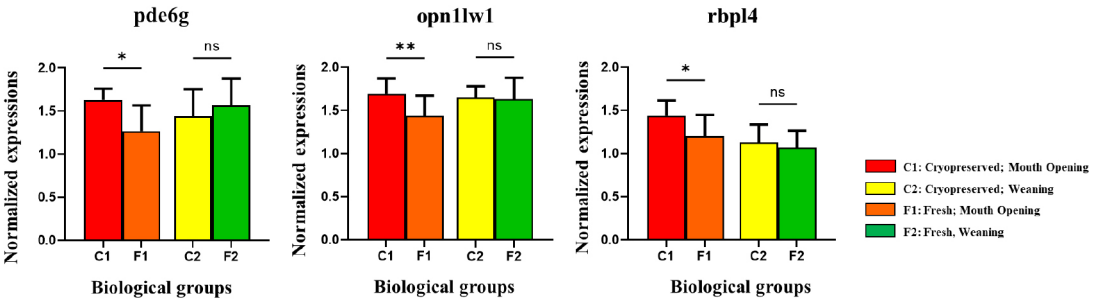

Figure 1: Relative expression level of successfully qPCR-validated genes at mouth opening stage and their relative expression ath the end of the rearing trial. * – p<0.05; ** – p<0.01; ns – non significant.

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Results

Embryonic survival rate was significantly lower in Group C than in Group F, and the Group C larvae had higher weight wise at their weaning stage. The remaining zootechnical parameters were similar in both groups. Transcriptomic analysis revealed 11 DEGs, out of which 3 genes were further successfully validated by qRT-PCR, namely *pde6g*, *opn1lw1* and *rbpl4* (**Figure1**). Interestingly, all the genes are responsible for the development of the eye and had higher expression at MO stage in Group C but later at weaning stage, the expression levels were similar in both groups.

Discussion

This experiment was done with the intent to not just check the effect of cryopreservation on progeny quality, but also to use the technique as a condition to reveal paternal-effect genes. Phosphodiesterase 6 Gamma (*pde6g*) is expressed in rod photoreceptors and functions in the phototransduction signaling cascade (Dvir et al., 2010). Long-wave sensitive Opsin (*opn1lw1*) codes for red opsins and are abundant in cone cells (Crespo et al., 2018). Retinol Binding protein 4 (*rbp4l*) has a role to play in retina development. Examples of signalling pathways for *pde6g* and *opn1lw1* is G protein mediated phototransduction, for rod and cone cells (Zang n.d.). Abola et al., (2015) found *rbp4l* gene being important in pathways like retinoid signalling. Thus we may conclude that development of an eye and potentially other sensory capabilities as well as its functioning during the first days post-hatching is under the influence of paternal genome, probably via the methylome status of the sperm (Jiang et al., 2013).

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PRODUCTION GROWTH, COMPANY SIZE, AND CONCENTRATION: THE CASE OF SALMON

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The largest companies in salmon aquaculture are rapidly getting bigger due to organic growth as well as mergers and acquisitions, and the largest are now multi-national companies. There are two main explanations for this growth: 1) An attempt to become large enough to exploit market power, or 2) New technologies that increase the efficient scale. In this paper, we investigate the degree of concentration in each of the main production countries for Atlantic salmon, as well as globally for Atlantic salmon, all farmed salmon, and all salmon to account for the global nature of the market using Herfindahl-Hirschman Indexes. The results indicate a high degree of concentration in the smaller producer nations but not in Chile and Norway. Globally, the Atlantic salmon industry can be characterized as unconcentrated, and it becomes even more so when the supply of other farmed salmon and wild salmon is accounted for.

THE SAFE SOLUTION TO CAPTURE AND VALORISE WASTE STREAMS FROM COMMON CARP (*Cyprinus carpio* L.) POND FARMING

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Introduction

Traditional pond aquaculture in CE Europe currently faces numerous challenges (e.g. water shortages, soaring production costs, subsidies-dependent production) that significantly affect profitability of the farms. To overcome this difficult situation farmers must use some ponds to secure production water when available in excess (e.g. heavy rains), diversify produced species or seek for solutions to use waste streams already available at a farm (SCAR-Fish 2020). A good example of a waste stream are suspended solids, widely available in discharged water when ponds are annually emptied to harvest market-size fish for the market or to transfer fish between the ponds for grading or wintering. Emptying the ponds is a traditional on-farm procedure. However, it releases a significant pulse of organic and inorganic matter to the creeks downstream the farm. One of the objectives of SAFE project is to reduce the environmental impact and improve the viability of the freshwater aquaculture by applying circular economy approaches to the valorisation of solid and liquid wastes from pond farms. Therefore, to reduce the impact of the farms on the river systems and to increase profitability of the farms the aim was to build a low-cost and low-tech system to capture suspended solids from the discharged water for further use in a production of beta-glucan-rich oyster mushroom (*Pleurotus ostreatus*).

Material and methods

To sequestrate solids suspended in the water discharged from drained ponds a multilayer barrier made of straw bricks (rye) was installed in the channel below the common carp farm (Fig. 1).

After 2 weeks of filtering out suspended solids, straw bricks were removed from the discharge channel and its suitability for oyster mushroom production was analysed (e.g. nitrogen content, organic matter, pH, fibres, pesticides). Subsequently, straw bricks with sediment were mixed with the conventional substrate for oyster mushroom production in ratios 25%/75% (A), 50%/50% (B) and 75%/25% (C). Additionally, two substrates made of 100% conventional medium for oyster mushroom production (CTR) and made of 100% of straw with captured suspended solids (D). Polypropylene bags with a gas exchange filter were filled with 3 kg of each substrate (10 replicates), and were sterilised at 121ºC for 2 hours. Sterilized bags were inoculated with *P. ostreatus* H9 (Gurelan) spawn and incubated at 25ºC. After 20 days, bags were placed in a cultivation room at 18ºC. From each bag mushrooms were collected and weighed separately without removing stems.

Results and Conclusions

No pesticide residue was detected in the analysis of the straw bricks. Results of the physicochemical parameters and fibres are shown in table 1 and 2.

The mushrooms obtained from all the bags have been collected and the average kg of production of each bag has been calculated for the kg of substrate that each bag initially contains. The partial results obtained to date are those shown in Fig. 2.

As the freshwater ponds can accumulate from 0.76 to 3.2 t of sediments per hectare along the production season, the valorisation of suspended solids in mushroom production is a sound solution to curb release of sediments into river systems and at the same time increase profitability of the freshwater farms (Eymontt et al. 2017). Therefore, further studies should evaluate whether the oyster mushroom caps can be offered as safe and nutritious food items for human consumption. Whereas a typical by-product of *P. ostreatus* production, stalls, can be used as a beta-glucan rich ingredient of feed used to mitigate commercial fish production bottlenecks, such as increased mortality of young common carps during wintering.

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Fig. 1. Low-tech and low-cost technology for collection of suspended solids and subsequent valorisation as a substrate for cultivation of oyster mushroom.

Table 1. Physicochemical parameters and content of fibres in the tested cultivation materials.

	Humidity (%)	Ashes (dw%)	Nitrogen (dw%)	pH	Organic matter (%)	Lignin (dw%)	Cellulose (dw%)	Hemicellulose (dw%)
Sediments with straw	78.84	24.78	0.72	7.62	75.22	10.95	31.86	22.48
Sediments	49.80	93.77	0.31	8.15	6.23	1.98	1.59	2.60
Straw	17.84	4.84	0.44	8.21	95.16	5.22	52.37	31.22
Substrate	70.68	10.27	0.87	8.69	89.73	9.79	42.10	24.40

Abbreviation: %dw - dry weight.

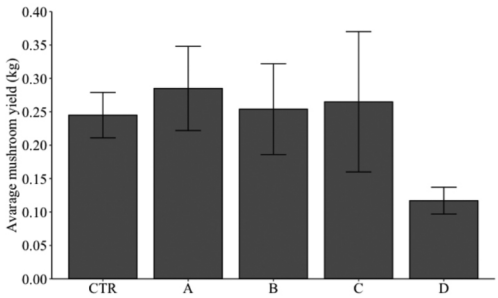

Fig. 2. The average yield of *P. ostreatus* produced on substrates with different share of straw with suspended solids.

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FUNCTIONAL CHARACTERISATION OF TILAPIA LAKE VIRUS (TiLV) PROTEINS

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Introduction

Tilapia lake virus (TiLV) is an emerging RNA virus posing a severe threat to global tilapia aquaculture and the food security of millions of people. The viral genome comprises 10 segments of negative-sense, single-stranded RNA, with segment 1 showing sequence similarity to PB1 of influenza C virus, while the remaining sequences show no sequence homology to any other known sequences[1]. Due to its relatively recent identification, there is limited molecular characterisation of the viral proteins, with currently no specific treatments or commercial vaccines available to combat the infection. In this study, we aimed to characterise and elucidate functional properties of the TiLV proteome. Firstly, to confirm the presence of the major viral polypeptides and identify the protein composition of virus particles, samples prepared from infected cells and partially purified virus pellets were analysed by liquid chromatography and tandem mass spectrometry (LC-MS/MS). Secondly, we investigated the expression and cellular localisation of wild type and mutated TiLV proteins *in vitro* and in eukaryotic cells using synthetic cDNA constructs as a means of examining individual protein function and a platform for mutagenic assessment of potential functional domains. Overall, we identified 11 major TiLV polypeptides: one from each segment plus a potential accessory protein with a Crm1-dependent nuclear export signal. We identified the segment 5 (S5) polypeptide as likely to occur in multiple isoforms, at least one of which is a N-glycosylated membrane protein, and S4, S7, S8 and S10 polypeptides as likely structural components of the virion. We also identified a potentially secreted TiLV polypeptide, as well as a nuclear localisation signal in S2. Overall, our data provide a foundation for further functional characterisation of the TiLV proteome and the design of intervention strategies.

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iFishIENCi FEED AND FEEDING TECHNOLOGY DEVELOPMENT IN RAS

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Introduction

The iFishIENCi project aims to demonstrate the viability of sustainable feeds that support the development of new production species with improved growth and feed utilization efficiencies. This involves the implementation of effective monitoring systems for fish health and welfare, as well as the adoption of efficient feeding practices that reduce the pressure on sources of fish-feed ingredients such as agricultural crops and wild-caught fish for fishmeal and oil. The project targeted several species, including Asian seabass (Lates calcarifer), rainbow trout (Onchorynchus mykiss), and catfish (Clarias spp.), and employed a multidisciplinary and holistic approach at AquaBioTech group to develop feed and feeding technology in RAS.

Experiment 1

The Asian seabass, Lates calcarifer, is a popular farmed species with an established market, and its potential for expansion of barramundi farming worldwide has been noted (Lawley, 2010). However, the cultivation of this fish in a recirculating aquaculture system (RAS) needs further development (Larkin, 2000). The trial performed within the project compared the growth and body quality traits of Asian seabass reared at two different salinity levels in RAS, and tested the potential benefits of a microalgae extract (Nannochloropsis gaditana) for fish growth, health, and fillet traits. The study found no significant differences in weight, specific growth rate, and most body quality traits between the different treatments, but fish in higher salinity (S1) had a significantly higher visceral-somatic index (VSI) compared to those in lower salinity (S2). The microalgae extract did not have a significant effect on the growth, health, or fillet traits of the fish. These findings suggest that further research is needed to optimize the RAS culture of Asian seabass and to determine the potential benefits of microalgae extracts in improving fish growth and health.

Experiment 2

Catfish is one of the fastest-growing sectors in the aquaculture industry, and its production has increased significantly over the past few years (FAO 2020). Clarias is one of the most popular types of catfish, and its hybridization has been studied to improve growth and disease resistance (Rahman et al., 2018). Sustainable aquaculture production is crucial, and aquaculture sustainability requires reducing dependence on traditional raw materials (Shah et al., 2018). The experiment compared the growth performance, morphometric indexes and body colour intensity of hybrid catfish (Clarias gariepinus ♀ x Heterobranchus longifilis ♂) fed with a control diet consisting primarily of land-based and animal-based ingredients with two experimental diets containing Candida utilis at 10% and 20% inclusion levels. The results showed no significant difference in growth performance among the treatments. The bio-morphometric indexes also showed no significant difference. However, the red/green coordinate in the body colour intensity of the 20% inclusion diet was significantly higher than the control diet. The inclusion of C. utilis reduced the plant-based ingredients in the diets by about 14% and 28% but did not affect growth performance. The study demonstrates the potential of C. utilis as a novel and sustainable alternative protein source for the aquafeed industry. Although the results are promising, further investigation is required to evaluate the interaction among the ingredients in formulated diets and the relative effect on digestibility and gut impact in hybrid catfish. The study suggests that catfish farming is a valuable candidate for further investigation due to its growing interest worldwide and the development of related technology.

Experiment 3

To ensure economic benefits and efficient growth in aquaculture, appropriate feeding management is crucial. Inadequate feeding strategies can lead to low growth and feed conversion efficiency, resulting in increased labour costs (Wu et al., 2004). Temperature is also a crucial factor affecting fish growth and survival, as it affects physiological and biochemical functions. The gastric evacuation rate (GER) in fish is a significant factor affecting feed intake and digestion and it can be estimated through the serial retrieval of gastric contents, and the optimal feeding frequency is closely related to GER conditions (Seymour, 1989). Overfeeding can lead to negative consequences such as gastrointestinal overload and reduced food utilization efficiency (Jobling, 1986).

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This study aimed to determine the optimal feeding strategies for rainbow trout (Onchorynchus mykiss) at different temperatures (16±1°C, 18±1°C, and 20±1°C) by conducting a gut transit study after starvation for various time frames. The digestive tract of the sampled fish was divided into three parts (stomach; anterior + mid intestine, and posterior intestine), and the collected gastric content was weighed and stored separately. The wet and dry weights obtained after drying the samples at 60°C for 24 hours were used to calibrate a digital twin model (FishMet) for rainbow trout under different temperature. The results will be presented as part of the project implementation.

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FROM RAS TO SMARTRAS – FEEDING OF ATLANTIC SALMON, *Salmo salar* BASED ON BEHAVIOUR

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Introduction

The development of new technologies has transformed aquaculture from a traditional labour-intensive farming to a mechanized production with a gradual evolvement to automated systems (Fore et al., 2018). However, automated production requires highly skilled workers, affecting cost-effectiveness and resources such as water and feed are still impacted (Engle et al., 2019). The integration of AI in the aquaculture industry represents a new business form of modern aquaculture development (Wang et al., 2021). AI in aquaculture involves aspects like monitoring and control; feeding optimization; disease detection and management; water quality management and predictive analysis to improve profitability and sustainability.

The study aimed to improve the production control and management in aquaculture to maximise feed utilisation through smart feeding, providing continuous monitoring of fish behaviour, health, and welfare through the iFishIENCi Biology Online Steering System (iBOSS). The study was conducted in Atlantic salmon, *Salmo salar* in RAS at AquaBioTech Group.

Materials and Methods

The trial was performed in a 12 x 650L RAS system equipped with UV disinfection (TMC Pro Pond 110); Drum filter (BaseDrum 15, Ratz); Protein skimmer (Tornado, Aquosis); Automatic feeders (Arvo-Tec TD 2000); swirl separator and camera (JideTech P2R-20X).

The development of the technology, testing and monitoring of the fish lasted for 16 weeks in which three phases were conducted, each serving a specific purpose.

Phase 1 aimed to develop and calibrate the real-time detection, tracking, and monitoring of fish, using 400 juvenile Atlantic salmon (mean weight of 13.6±0.01g) allocated to one experimental tank for 10 weeks. The fish were fed four times a day (at 9:00, 11:30, 14:00, and 16:00) with commercial feed by automatic feeder until apparent satiation.

Phase 2 focused on the assessment of the fish activity under different feeding regimes and development of a scoring system ranging from 0 to 2 to measure it. Fish were fed four times a day with a fixed feeding rate using commercial feed. The experiment lasted for three weeks, and the feeding rate was changed weekly (WK1 FR of 1.5%; WK2 FR of 0.75%; WK3 FR of 3%).

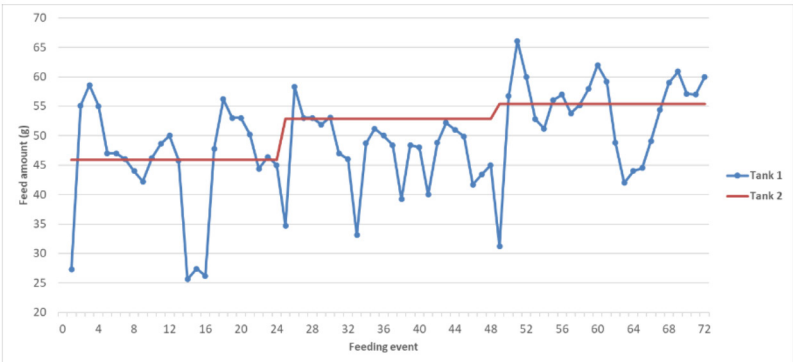

Figure 1: Feed amount provided per feeding event during phase 3 of the trial.

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During phase 3 of the experiment, the technology developed was tested comparing two different feeding strategies. One based on fixed feeding at 1.5% of body weight and the second based on the scoring system developed in phase 2. 200 fish of 60.58 ± 0.92g were allocated into two experimental tanks. The fish were fed four times a day using automatic feeders, as previously described.

Results

In phase 1, the fish detection and tracking was carried out using YOLOv5, a deep learning object detection model implemented in Python, and Norfair, a Python library that implements a tracker using Kalman filters. During phase 1, around 100 out of 400 fish were detected while at rest, and between 20 and 40 fish were detected during feeding. However, since the goal was to estimate the behaviour of the entire fish group, the percentage of undetected fish did not impose a limitation on the technology's development.

In phase 2, fish movement was estimated globally by comparing two successive frames of the video. The difference between the frames indicated the level of activity exhibited by the fish. The algorithm used for this purpose was relatively lightweight and capable of running in real-time throughout the day. The raw difference between successive frames was calibrated during the preliminary trial to ensure its value could be centred around 1. The measured movement was then averaged from the start of feeding until 15 minutes after it ended, taking into account the resting time after feeding. The resulting average value represented the feeding score.

In phase 3, the feeding based on the scoring system (T1) showed multiple daily fluctuation in the amount of feed provided, reflecting the variations in hunger exhibited by the fish as showed in the figure below. The growth performance showed a higher growth rate and a lower FCR of fish fed at 1.5% of body weight (T2) compared to the fish fed using the scoring system (T1).

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BLUEFIN TUNA, *Thunnus thynnus* SIDE STREAMS FROM MALTESE FARMS AS POTENTIAL RAW INGREDIENTS FOR AQUAFEED

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Introduction

Fish and seafood represent an important source of protein to support the increasing world population, which is set to reach 9 billion in 2037 [1]. The expansion of fishery and aquaculture sectors will produce, along with the proteinaceous biomass, increasing quantities of side streams and residuals, such as skins, heads, viscera, and fillet cut offs. These side streams account for approximately 70% of processed fish [2] and can be used as a source of fish oil and fishmeal [3)] as well as other bio-compounds that can be used by nutraceutical, pharmaceutical and cosmetic industry [4]. Some of the valuable fractions of fish side streams are collagen, enzymes, chitin, and minerals [5]. Fish side-streams are also used for biofuel production, and as a source of nutrients in fertilizers [6]. However, the utilization of this side streams is often hampered by logistical barriers. For example, the limited refrigerated space on the vessels is used for the fillets and the side-streams are stored at air temperature for several hours before being transported to a processing plant.

The aim of this study was to investigate the quality of different tuna side-streams derived from a tuna farm in Malta in the hours following the harvest. The oxidation state of the side-streams transported to a processing plant was assessed and the histamine content was predicted using a model based on temperature changes.

The study is part of the BlueBio-funded project PROFIUS that has the goal to valorise underutilized fish biomasses.

Materials and Methods

Samples of tuna side streams were collected during the tuna harvesting season, from October to December 2022. A temperature logger (Tinytag, Gemini Data Loggers, Chichester, UK) was inserted in multiple samples of side-streams immediately after being removed to monitor the temperature from harvest to processing (between 3 and 6 hours). The software FSSP [10] was used to predict histamine formation based on the temperature profile. Other samples of tuna side-streams were taken to evaluate the nutritional composition and the oxidative status. The samples were frozen at -20°C within five hours of collection. Three different tuna side streams were identified and separated (head, liver and mixed stream). A total of six samples, two for each type, were analysed for proximate compositional analyses, following the methodology indicated by AOAC International (2000)[6], for Dry Matter (DM) (AOAC #934.01), Crude Protein (CP) (AOAC #984.13), Ash (AOAC #942.05), Ether Extract (EE) (AOAC #2003.05). Additionally, the fatty acids profile was determined following the protocol described by Schmid et al. [7]. The oxidative status (Peroxide value and thiobarbituric acid reactive substances) were assessed using the methodology described in AOAC #965.33 and the modified Thiobarbituric acid (TBA) method according to procedure described by Witte et al.[8], respectively.

Results

The results of the temperature profile recorded, analysed with the FSSP software, predicted no histamine formation by *M. psychrotolerans* and *M. morganii*, in any of the samples collected.

The nutritional composition showed a variation of the DM content across the side streams ranging between 25.65% of the head to 53% of the liver; the latter with the highest CP content as it of approximately 16%, while head and mixed samples accounted for 2.8% and 11.5% as it respectively. In terms of crude fat expressed as Ether Extract (EE), the liver showed the highest concentration of 35.9%, while the head had the lowest at 20.04% as it.

The primary oxidation expressed as peroxide value showed the highest value in the liver (between 5.48 to 6.74 meq peroxide/kg oil), while the lowest value was found in the tuna head samples (1.45 meq peroxide/kg oil). Regarding the secondary oxidation, the TBARS levels ranged between 1.89 and 49.00 ng MDA/g, with the lowest value found in the tuna head and the highest in the liver. After 72 hours at -20°C, all the values of PV and TBARS increased, highlighting the occurrence of a slow oxidation.

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TOXIC EFFECTS AND HEALTH RISKS OF THE SYNTHETIC ACTION OF DIETARY MYCOTOXINS IN GILTHEAD SEABREAM

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Introduction

Plant proteins have been widely used in aquafeeds as a substitute for fish meal diets to support aquaculture development. However, contamination of plant ingredients with toxicogenic fungi is common and occurs in a wide range of these raw materials worldwide. Accidental mycotoxin consumption by fish, can result in various toxic actions and raise concern for both animal health and food safety. In aquaculture, diets contaminated with mycotoxins is a burden on health of cultured fish (Zhonghao *et al*., 2023). This study aimed to evaluate the impact of co-exposure to the emerging dietary mycotoxins including Aflatoxin B1 (AFB1), Fumonisin B1 (FB), and Deoxynivalenol (DON) in graded contamination levels, on zootechnical parameters and health indices of gilthead seabream (*Sparus aurata*).

Materials and Methods

Triplicate aquariums received five experimental diets: A (DON:500, FB:1000, AFB1:5 ppb), B (DON:150, FB:650, AFB1:2 ppb), C (DON:3000, FB:40, AFB1:2 ppb), D (DON:150, FB:40, AFB1:10 ppb), E (DON:150, FB:100, AFB1:2 ppb). The control group (CTRL) was fed with a marine-based, mycotoxin-free diet. The feeding trial lasted 12 weeks. Fish were hand-fed "ad libitum", two meals per day, 6 days a week. Feed intake and mortalities were daily recorded. Water physiochemical parameters were maintained within the standard levels. Endpoint assessment included evaluation of haematological and immunological parameters, energy utilization (feed-intake and growth) and induced genotoxicity by Single Cell Gel Electrophoresis assay. Liver samples were collected for the determination of hepatic lesions through histological analysis. One-way ANOVA was used to determine the variance among treatments, followed by Tukey's post hoc test at a significance level of $P < 0.05$.

Results and Discussion

All treatments containing mycotoxins caused significantly lower food consumption compared to the control group. The group with the highest DON concentration (Group C) showed the lowest feed intake, followed by the group with the highest AFB1 concentration (Group D). Inferior growth (lower mean weight, length, and total biomass increase) was also recorded in group C, followed by groups D, E and B (Table 1.), as mentioned before in the case of rainbow trout after dietary exposure to DON (Koletsi *et al.*, 2022). Significantly lower haematocrit was recorded in all groups compared to the CTRL, while the lowest value was estimated for the group with the highest FB concentration (group A). Significant decreases in the erythrocytes, leucocytes, and the haematocrit values were previously observed in fish fed with diets with a mixture of FB and AFB1 (Adeyemo *et al.,*2022). Diets C and D showed strong effects with significantly elevated Hb and reduced complement, trypsin inhibition and/or alkaline phosphatase activities. The inhibitory action of AFB1 was already shown in Indian major carp with a significant immunosuppressive effect including reduced serum total globulin and reduced bactericidal activities (Sahoo & Mukherjee, 2001). Diet A also showed significantly reduced trypsin inhibition. Concerning genotoxicity induced by the mycotoxins, no significant differences on DNA damage were confirmed between examined groups. Liver sections from all mycotoxin groups revealed pathological signs, such as focal to extensive degenerative changes in liver parenchyma and early necrotic changes in some hepatocytes, that showed pyknotic and shrunken nuclei (group C). Mild to extensive hepatocyte cytoplasmic vacuolation, indicating hydropic and fatty degeneration of liver cells was also detected in all mycotoxin groups. In contrast, histopathological examination of liver sections from the CTRL group showed normal hepatic parenchyma and hepatopancreas.

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Table 1. Growth and haematology parameters.

Parameters/ Dietary groups	A	B	C	D	E	CTRL
Feed consumed per fish (g fish^{-1})	48.30±1.20^{de}	46.03±0.50^{cd}	37.20±2.17^{a}	41.10±1.53^{ab}	42.20±2.68^{bc}	51.97±2.33^{e}
IBW (g fish^{-1})	7.63±0.06^{a}	7.60±0.00^{a}	7.63±0.06^{a}	7.60±0.00^{a}	7.60±0.00^{a}	7.63±0.06^{a}
FBW (g fish^{-1})	49.21±2.01^{c}	47.53±1.56^{bc}	38.93±1.86^{a}	43.43±1.43^{ab}	41.98±3.22^{a}	51.01±2.46^{c}
FCR	1.16±0.03^{a}	1.15±0.03^{a}	1.19±0,02^{a}	1.15±0.03^{a}	1.23±0.06^{a}	1.20±0.01^{a}
SGR	2.49±0.05^{ab}	2.62±0.05^{bc}	2.44±0.11^{a}	2.33±0.07^{a}	2.66±0.05^{c}	2.72±0.05^{c}
HTC	30.67±5.90^{a}	33.87±5.95^{ab}	36.67±3.87^{b}	34.93±7.09^{ab}	37.47±4.21^{b}	44.40±5.85^{c}
RBC (10^{6}μL^{-1})	1.49±0.33^{a}	1.39±0.26^{a}	1.39±0.32^{a}	1.02±0.19^{a}	1.10±0.20^{a}	2.59±0.79^{b}
WBC (10^{3}μL^{-1})	35.86±11.48	28.00±5.77	41.75±13,65	50.67±19.75	33.33±16,48	58.33±45,10

Values are presented as means ± standard deviation of the triplicate groups of each treatment. In each parameter examined, symbol with a different letter indicates a statistically significant difference between treatments (P<0.05).

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QUALITY AND SAFETY STANDARDS FOR READY-TO-EAT RAW CONSUMPTION OF FARMED GREEK FISH

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Introduction

The popularity of ready-to-eat raw fish (such as sushi and sashimi) has been significantly increased over the last decade. Raw fish is very appreciated worldwide and has become a major component of human diet because of its fine taste and nutritional properties. Possible hazards concerning fish safety and quality are classified as biological and chemical hazards. They may be contaminants (mainly bacteria and parasites) that often accumulate in edible tissue of fish and transmit to humans via the food chain affecting the consumer's health (Lehen et al., 2020). The perception of health, quality and safety benefits is considered as one of the motivational factors that could explain fish consumption patterns; however, the issue of perceived convenience or discomfort of fish has often emerged as influential in consumer choice too (Masi et al., 2022).

The objective of the study was to establish a verification method for the absence of live parasites in farmed fish, to evaluate and define the quality and safety standards of whole fresh fish and fish fillets, and finally to design an efficient production and transportation/storage system for ready-to-eat raw fish obtained from the Greek aquacultures. The results were validated in actual fish production environment by implementing a standard production plan to control the potential risks (bacterial and parasitic infections) while retaining the high quality in terms of microbial spoilage and sensory profiling. The proposed protocol for the verification of absence of contaminants, expected to exclude farmed fish in Greece from the obligation of prior freezing fisheries products, intended to be consumed raw or undercooked according to Reg. (EEC) 853/2004. Currently the part of the assessment for bacterial contaminants and the validated self-life predictive model will be presented.

Materials and methods

Fish (gilthead seabream, European sea bass, red sea bream and meagre) was harvested from 6 different fish farms within the period November 2022-February 2023 from western Greece (Thesprotia and Kefallonia), central Greece (Phthiotida), northeastern Greece (Lesvos) and southeastern Greece (Rhodes). 80 fish from each batch was delivered to the Agricultural University of Athens (Department of Food Science and Human Nutrition, Laboratory of Food Process Engineering) for quality evaluation and shelf life modelling. Time and temperature was continuously monitored from harvesting upon receipt of samples at the laboratory for shelf life evaluation, using electronic data loggers (RC-5 USB temperature recorders, Elitech, London, United Kingdom). Quality evaluation was based on microbial spoilage (enumeration of total viable count, *Pseudomonas* spp. and Enterobacteriaceae) and sensory evaluation. Bacterial microbiota of fish flesh initially and during refrigerated storage was characterized by 16S metagenomic analysis. A sample of 600 fish (450 European sea bass and 150 Meagre) from 4 different fish farms was delivered to the Hellenic Centre for Marine Research for microscopic and molecular parasite examination.

Results

Initial microbial load of fish flesh (without skin - skinless) ranged 2.4-4.6 logCFU/g for total viable count (N=50). The required time from harvesting to the delivery of fish to the laboratory for shelf life evaluation was 2.5-52.5 hours and the effective temperature during harvesting and transportation ranged 0.2-1.6°C. A logarithmic equation was developed to describe the effect of time and temperature during harvesting and transportation of fish on the microbial load of fish flesh. Based on the shelf life experiments, the dominant spoilage microflora in fish stored at 2 and 4°C was *Pseudomonas* spp., in agreement with relevant studies on Mediterranean fish stored aerobically at refrigerated conditions (Gram and Huss, 1996; Papaharisis et al., 2019; Koutsoumanis and Nychas, 2000).

Discussion and conclusion

The definition of the dominant microflora and the development of a validated shelf-life predictive model is a prerequisite for the definition of the spoilage process and the quality and safety assurance of ready-to-eat raw fish products. A fish production system has been provided in the form of a detailed guide for fish farms and processing facilities, to ensure the availability of safe and high quality ready-to-eat raw farmed fish and develop distribution channels with focus on export market Mediterranean and the European region.

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EXPLORING THE GUT MICROBIOME MODULATION BY CIRCULAR AQUAFEED INGREDIENTS IN GILTHEAD SEA BREAM AND EUROPEAN SEA BASS

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Introduction

The study of the gut microbiome has received great attention in the aquaculture sector as an indicator of productivity and fish health, although many interactions among gut microbiome and fish host are still unknown. The promotion of a healthy gut microbiome by dietary intervention is currently of relevant interest, especially in the context of circular ingredients such as single-cell protein, insect meal and marine by-products, which are candidates to replace fishmeal and vegetable protein. Indeed, besides their optimal nutritional values, these novel ingredients may promote gut health by favouring beneficial gut microbiota taxa. The aim of this study is to explore the gut microbiome modulation induced by circular aquafeed ingredients in gilthead sea bream and European sea bass by analysing several dose-response feeding trials. Implications on gut microbiome functions and correlation with fish welfare parameters are discussed.

Materials and methods

Three feeding trials in gilthead sea bream and two in European sea bass were conducted in a closed recirculation aquaculture system (RAS). Gilthead sea bream (initial weight 75, 25 and 98g for trial 1 to 3, respectively were fed: 1) dietary increasing level of bacterial single-cell protein (SCP) from *Corynebacterium glutamicum* (0%, SCP, 10% SCP, SPC10; 15% SCP, SCP15; 20% SCP, SCP20) to replace vegetal ingredients; 2) dietary SCP from *Candida utilis* to replace fish meal, FM (0% CTRL, 5% SCP5, 7.5% SCP7.5, and 10% SCP10) and 3) dietary *Hermetia illucens* (HI) larva meal (0% CTRL, 5% HI5, 10% HI10, and 15% HI15) in partial substitution of FM. European sea bass (initial weight, 75g) were fed dietary increasing levels of fishery and aquaculture by-products and microalgae to totally replace wild-caught FM and soy protein. At the end of each trial, distal intestine content was collected for 16S rRNA gut microbiota analysis on 15 individual fish per treatment. Sequencing was performed on the Illumina MiSeq platform. Growth, feed efficiency parameters and welfare parameters by plasma biochemistry were also assessed. Microbiota biostatistical analysis was produced using R software (https://www.r-project.org/)

Results and Discussion

At the end of the trials, gilthead sea bream fed SCP from *Corynebacterium glutamicum* and HI larva meal showed a significant ($p<0.05$) variation compared to their respective control group, in terms of overall gut microbiome composition (Fig.1A and B). In the first case, SCP, even at the lowest content, led to an increase in *Bacillus* and *Oceanobacillus* relative abundance, which were the most relevant taxa responsible for a significant separation in the PCoA analyses. Similarly, HI induced a general gut microbiome reconfiguration with significant enrichment of *Bacillus*, *Oceanobacillus* and *Paenibacillus*. As also observed for animals fed with *Corynebacterium glutamicum*, no dose-response was detected for the ability of both raw materials to shift the gut microbiome structure, which was evident from the lowest dietary inclusion level. In both trials (especially with HI) a general reduction ($p<0.05$) of alpha diversity indices (i.e., PD_whole_tree, number of ASV and Shannon index) was also detected. In gilthead sea bream fed SCP from *Candida utilis* and in European sea bass fed with by-products from fisheries and aquaculture (Fig.1C and D), no significant overall impact of diet on the gut microbiome was observed. A common feature of these latest growth trials was a slightly decreasing growth performance (in terms of FCR and SGR) observed at the highest dietary inclusion level of the circular ingredients tested.

Conclusion

The inclusion of novel circular ingredients, such as bacterial single-cell protein and insect meal, clearly modulated the gut microbiome of gilthead sea bream promoting bacteria taxa belonging to *Bacillaceae*, which may exert beneficial host functions. Specific compounds characterizing these ingredients such as nucleotide/nucleic acids in SCP and chitins in insect meal might induced this modulation. This data match literature on other fish species and confirm the potential role of these ingredients to exert functional properties on gut health besides being a valid alternative for FM and vegetal ingredients at nutritional level.

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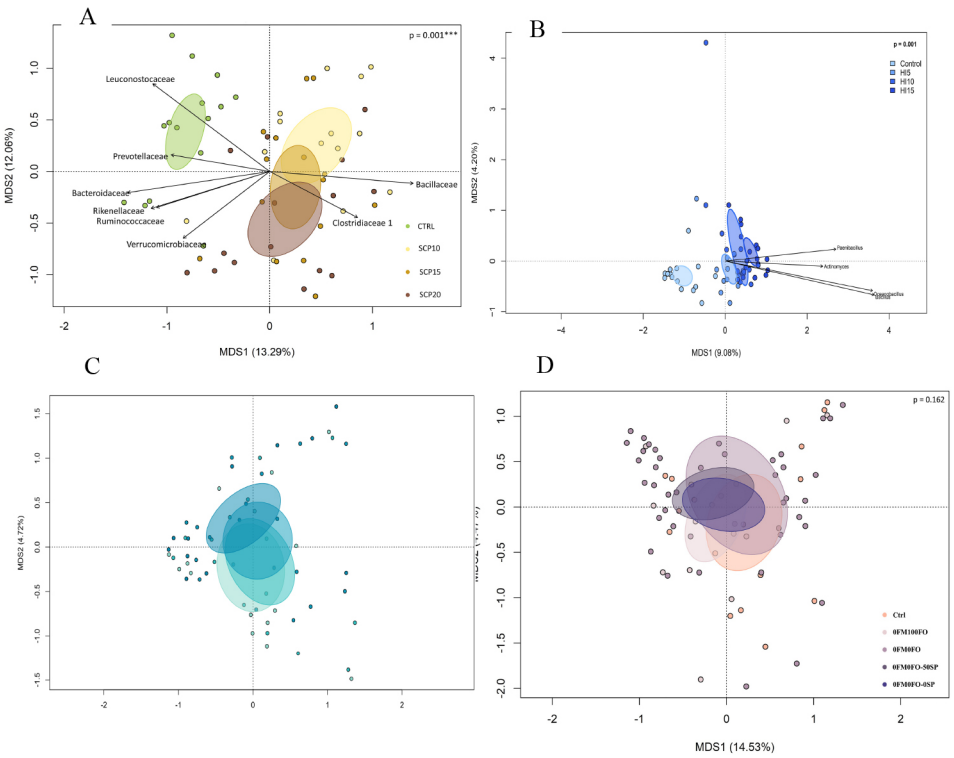

Figure 1. Unweighted UniFrac Distances between gut microbiota composition of different groups. A) gilthead sea bream fed with bacterial single-cell protein from *Corynebacterium glutamicum*; B) gilthead sea bream fed with *Hermetia illucens* (HI) larva meal; C) gilthead sea bream fed single-cell protein obtained from *Candida utilis*; D) European sea bass fed with of fishery and aquaculture by-products and microalgae.

Acknowledgements

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CHEMOSENSITIVITY OF THE SEA URCHIN *Paracentrotus lividus*: IMPLICATION ON FEEDING BEHAVIOR AND DIETS FORMULATION

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Introduction

The sea urchin *Paracentrotus lividus* is considered a delicacy seafood item in many countries and the wild population is suffering due to the overexploitation of the resource and inadequate fishery regulation. Pilot-scale hatcheries acquired adequate biotechnologies for their successful breeding in captivity to counterbalance the fishery pressure on wild populations and for restocking purposes (Giglioli et al., 2021). Nevertheless, it is necessary to acquire knowledge of their feeding behavior, aimed at formulating diets with higher palatability, and able to improve their growth under rearing conditions (Prato et al., 2018). The aim of this study is to shed light on the chemosensitivity of *P. lividus* and its behavioral response to different feeding stimuli. Here, we evaluate the chemical sensitivity of the sea urchin *P. lividus* to several stimuli possibly related to food, such as a few sugars and amino acids, the seaweed *Ulva* sp., compared to the blue-green algae.

Materials and methods

To explore the ability of sea urchins to detect the feeding stimuli we developed a simple, innovative method based on the recording of "urchinograms" estimating the movements of spines, pedicellariae, tube feet, and eventually of the whole sea urchin, in response to chemicals, while keeping both the whole animal and the stimulus in their natural environment, underwater (Solari et al., 2021; Addis et al., 2023). We considered *Ulva*-, amino acids-, sugars-, and green-blue algae based experiments, using a defined concentration of each stimulus. The experimental setup consisted of a small rectangular Plexiglas tank containing about 350 mL seawater (SW), which was connected to two different channels of a peristaltic pump (Gilson, Minipuls Evolution) which ensured a constant flow within the tank. The inflow and outflow terminals allow the SW and chemical stimuli to be delivered into and removed from the tank. Trials were video-recorded for later analysis, using a digital camera (Samsung SMX-F34, Korea) mounted above the experimental tank. The behavioral response was determined by measuring the movement rate of spines, tube feet, and the fully coordinated locomotion, if any, by which the whole animal moves toward or away from the outlet of the stimulus supply.

Moreover, to define the attractivity of the stimuli, we performed trials in an experimental arena consisting of ten circular plastic tanks (30 cm in diameter, 8 cm high) where sea urchins were individually exposed to fresh *Ulva*, whereas green-blue algae were supplied to the animals by using the polyvinyl chloride (PVC) dispenser technique. The animal movement in the tank was recorded by means of a color digital camera (Samsung SMX-F34, Samsung, Korea) positioned 60 cm above the tank. The attractiveness was estimated by taking into account the following measurable parameters: a) percentage of tested animals that found the micro-algae within 1 h and remained in contact with it for at least 10 min; b) distance and time (min) traveled (mm) to find it; c) mean speed (mm/min), determined as the ratio between the distance traveled to reach the microalgae and the time to the target and; d) tortuosity of the sea urchin's route to the microalgae substrate, determined as the ratio between the distance (mm) traveled to find the item and the shortest distance (mm) from the center of the tank and the targeted item.

Results

Our results show that Spirulina is a highly stimulating compound for the sea urchin, by acting in a dose-dependent manner. The animals resulted also sensitive, even if to a lesser extent, to some sugars, such as the monosaccharide glucose, but not to its isomer fructose, while among disaccharides, they sensed cellobiose, but not sucrose or trehalose. The results showed that all forms of *Ulva* (fresh, defrosted, and fragmented) resulted in an effective stimulus, evoking in sea urchins strong responses with robust activation of spines and tube feet, where the defrosted one was the most stimulating. Among the amino acids tested, glycine, alanine, and glutamine produced a significant response, highlighting for the latter a concentration–response relationship. Sea urchins also displayed a sensitivity, even if to a lesser extent, to leucine, threonine, arginine, and proline.

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Discussion and conclusion

From an applied point of view, any insight into the chemical sensitivity of sea urchins toward potential food-related compounds may lead to the discovery of key chemicals that would help improve the efficiency and reduce the costs of dietary substrates for optimization of intensive rearing strategies. Although this method has been developed for *P. lividus*, it will be suitable to evaluate the chemical sensitivity of other echinoderms and other marine invertebrates characterized by low mobility.

Major results indicate the role of *Ulva* as a chemostimulant and strongly attractant for such herbivore species. From an applied point of view, the presence of potential *Ulva*'s feed-related compounds, acting as chemoattractants (to reduce food searching time) and/or feeding stimulants (to stimulate ingestion), would improve the several applications of *Ulva* in the formulation of the feeds for sustainable aquaculture. Besides, our results show that blue-green algae with high nutritional value, are very attractive for this sea urchin species. These findings gain further importance considering the commercial potential of echinoderms for human consumption and their growing importance in aquaculture. Moreover, green-blue algae combine high nutritional profiles with great stimulating and attractive effectiveness and may be considered as potential valuable feed supplements in aquaculture.

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EFFECTS OF DIFFERENT CULTURE MEDIA ON GROWTH, COMPOSITION AND QUALITY OF *ULVA* SP. CULTIVATED IN CYLINDRICAL PHOTOBIOREACTORS

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Introduction

Ulva sp. is a valuable seaweed with numerous recognised commercial applications, including food, feed, and ecosystem services. Ensuring a sustainable and consistent supply of biomass with targeted and desirable biochemical profiles, aligned with intended uses, is fundamental for the successful applications of this species (Mantri et al., 2020). This study investigates the cultivation by propagation of *Ulva* sp. in indoor cylindrical photobioreactors, considering different sources of nutrients (N, P) and their effect on the growth, composition and quality of the biomass.

Material and Methods

Wild *Ulva* sp. was collected in June 2023 from a coastal lagoon of South Sardinia, Italy (Santa Gilla, Lat 39°13›50.67»N, Long 9° 4›49.34»E) and transferred to laboratories for experiments. The collected seaweed was acclimated in indoor tanks for one week before the trials. On the day of the experiment, the biomass was rinsed with freshwater to remove attached organisms and biofouling. The central sections of the blades were cut into discs of 8-10 cm to ensure homogeneity of the cultured biomass. Approximately 20g of blotted dry weight per bioreactor was placed, resulting in a final density of 1g/L. Twelve 20L photobioreactors (plexiglass cylinders) were incubated in a controlled temperature room set at 20°C, under artificial light, and provided with aeration from the bottom. Each photobioreactor contained a 20 L volume of media. Four types of media were tested and compared in triplicate: lagoon water (LW), lagoon water enriched with F/2 (LF/2), lagoon water enriched with sea urchin wastewater (LU), and lagoon water enriched with cow digestate (LD). The experiment spanned a duration of one week. At the end of the experiment, the biomass was blotted dry and weighed to calculate the specific growth rate (SGR), expressed as percentage per day. In addition, the nitrogen content (nitrite, nitrate and ammonia expressed in ppm) and phosphorous (total, phosphate, and phosphorus pentoxide) in each treatment's media were analysed both at the beginning and at the end of the experiment. This analysis aimed to understand the specie's preference for biosorption of N and P sources. The colorimetric analyses of *Ulva* sp. were conducted using a digital colorimeter (Chroma meter CR-400, Konica Minolta, Tokyo, Japan), which specified the color as L*=lightness, a*=red/green and b*=yellow/blue (CIELAB). Light absorption (calculated by subtracting the absorbed light and expressed as photosynthetically active radiation, PAR) were recorded for subsamples of each treatment using a quantum sensor, connected to a quantum/radiometer/photometer (Li-Cor Inc., Lincoln, NE, USA). Moreover, the biochemical composition of the produced *Ulva* sp. was compared across the different culture media used in the cultivation trial. This comparison encompassed proximate composition (ash, fiber, moisture, total carbohydrates, lipid, and protein) polyphenols and pigments. The goal was to understand the effect of the treatments on the overall morphology and biochemical profile.

Results

There were significant differences in SGR among treatments ($P<0.001$). The highest SGR was recorded for the biomass cultivated in LF/2, which was double (9.86 ±0.52 % day^{-1}, mean ± SE) compared to cultures grown in LD (4.53 ±0.74), followed by LU (4.25 ±1.04) and finally LW (3.74 ±0.29). At the end of the trial, there were no significant differences for the light absorbed by the blades ($P=0.813$). However, the highest value was reported for the biomass cultivated with lagoon water enriched with LD, followed by LF/2, LW, and LU. While a noticeable difference in colour was observed among the treatments, specifically, the L* and a* parameters were higher in samples cultivated with LF/2.

The biochemical analyses showed that the lipid content in *Ulva* sp. did not vary among treatments (2-3 % DW), whereas the protein content exhibited significant variation among treatments ($P<0.001$). LF/2 displayed the highest average, 21 % DW, followed by all other treatments (9-6 % DW). Conversely, carbohydrates and fiber content showed an opposite pattern, with significantly lower content in LF/2 (16% and 30% DW, respectively) compared to other treatments (27-34% and 41-48% DW, respectively). Carotenoid concentrations varied among treatments, being double in concentration in LF/2 and LU compared to LW. Moisture, ash, and polyphenols contents did not vary among treatments. Significant differences

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in nutrient concentration were observed between treatments and time (T0 vs T*final*) for phosphorous (as Ptot, PO^{-3}_4, and P_2O_5 in µg/L). Ptot exhibited an increase of up to 20-fold, while both PO^{-3}_4 and P_2O_5 doubled at T0 compared toT. Ammonia levels (in ppm) did not vary significantly, and nitrate levels (in ppm) varied over time but not between treatments, revealing a 7-fold decrease in the LF/2 treatment from T0 to T*final*. Conversely, an opposite trend was reported for nitrite (in ppm),which was nearly 4 times higher at T*final* in LF/2, with no observable differences in the other treatments.

Discussion and conclusion

Seaweeds belonging to the genus *Ulva* exhibit high plasticity and adaptability to environmental and growth conditions, which can significantly modify their quality (Simon et al., 2022). In this context, we assessed the quality of *Ulva* sp. produced using different culture media. At the end of the trials, *Ulva* sp. effectively reduced and absorbed the nutrients in all media given. In our experiment, the quality of *Ulva* sp. was affected by the culture medium. In detail, those produced using recycled sources of nutrients, such as sea urchin wastewater and cow digestate, showed similar overall quality and characteristics in terms of color and photosynthetic activity. Conversely, *Ulva* sp. produced with LF/2 showed greater protein content compared to other treatments and to previously reported values (Shuuluka et al., 2013). This was accompanied by reduced carbohydrate amounts, a darker green colour, and a higher SGR.

The growing interest in *Ulva* genus includes a multitude of applications such as human consumption and the food industries. These require a biomass with consistent nutritional qualities and reduced variability in product characteristics. Our study shows how different nutrient sources can change the nutritional composition and overall quality of biomass. *Ulva* cultivated under LF/2 exhibits characteristics better suitable for human consumption, although requiring a higher economic investment for production. On the other hand, biomass derived from an excess of nutrients, confirms as *Ulva* may have applications in wastewater treatment services and such potential biomass could be used for biodegradable (bioplastic) packaging production.

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REPLACING VEGETABLE OIL IN THE FEED OF ARCTIC CHAR (*Salvelinus alpinus*) BY MICROBIAL OIL FROM THE OLEAGINOUS YEAST *Rhodotorula toruloides* GROWN ON LIGNOCELLULOSE HYDROLYSATE - CONSEQUENCES ON SAFETY, SENSORICAL OUTCOME AND ENERGY BALANCE OF THE PROCESS

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Introduction

Aquaculture is the fastest growing food industry for decades, a large user of fish oil (FO) and fishmeal for fish feed production. FO resources are found in finite supplies, and partially replaced by vegetable oils (VO). The addition of VO to fish feed has limitations such as competition with food production, high carbon footprint and may indirectly result in rain forest cuttings (note that in most European aquaculture industries, rapeseed oil is used, rarely or not palm- or soya oil). Single cell oil produced by cultivation of oleaginous yeasts on lignocellulose sources has the potential to solve several of the problems related to VO. However, utilisation of lignocellulose for growth of oleaginous yeasts requires thermochemical pretreatment, which requires energy. Energy is also required for fermentation and other processes required for production of microbial oil. Moreover, pretreatment of lignocellulose may generate persistent organic pollutants (POPs) from the polyaromatic compound lignin. POPs can also be taken up by the plant from the environment. These compounds could be biomagnified through the food chain and result in metabolic changes/unwanted side effects. The aim of this study was to evaluate food safety aspects, as well as energy use, of a novel sustainable fish feed ingredient produced from yeast cultivation on lignocellulose (wheat straw) hydrolysate and to determine whether its use would be advantageous compared to using VO.

Materials and Methods

Cultivated yeast was screened for the presence of persistent organic compounds of concern by GC/MS. Analytical methods were optimized and validated. POPs quantification was performed by the isotope dilution method; isotopically labelled standards were be used for each compound.

Arctic char (*Salvelinus alpinus*) was fed a diet in which VO and parts of the protein content were replaced by biomass of the oleaginous yeast *Rhodotorula toruloides* (Brunel et al. 2022). Growth of the fish was measured and the lipid composition of the fish was determined and compared to a control. Additionally, impact of new feed on fish was identified by measuring the catalytic activities of cytochromes 1A1 enzymes. Sensory analysis was performed with the triangle test method (Sinkinson, 2017) at the Swedish University of Agricultural Sciences (SLU) in November 2019 with 34 untrained volunteers.

In the system analysis, the outcome was a mass and energy balance for the yeast oil-producing biorefinery (Karlsson et al. 2016). The mass balance was represented by the yield of farmed salmon, yeast oil and other valuable outputs per tonne of straw input. The energy balance covered the fossil primary energy demand per tonne of salmon, per tonne of yeast oil produced and per tonne of wheat straw used, which was the sum of all non-renewable primary energy used for inputs in the manufacturing process. Four scenarios representing different process designs of the biorefinery were also tested and themass and energy balance modelling was carried out in Aspen Plus.

Results and Discussion

The investigated heavy metals and organic pollutants in the yeast biomass were below the limits of the regulations defined by the European Union. Moisture and ash contents, fatty acid profile and protein contents were similar between feed containing yeast oil and the control, confirming previous results with another oleaginous yeast species (Blomqvist et al. 2018). No significant differences were found in the hepatic activity of 7-ethoxyresorufin-O-deethylase. Sensory analysis did not reveal any perceptible sensory difference.

System analysis showed that production of 1 tonne of yeast oil would require 9.2 tonne of straw, 14.7 GJ in fossil primary energy demand, 14.6 GJ of process electricity and 13.3 GJ of process heat, while 21.5 GJ of biomethane (430 kg) and 6 GJ of excess power would be generated simultaneously. By applying economic allocation, the fossil primary energy demand was estimated to 11.9 GJ per tonne oil. In comparison with the commonly produced rapeseed oil, the primary energy demand of the oil among the scenarios tested, was 10-38% lower.

The results show that at least partial replacement of VO by oil from oleaginous yeasts grown on lignocellulose hydrolysate in aquaculture feed is safe and can be a sustainable alternative.

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EARLY LIFE PROGRAMMING AND MOLECULAR REGULATION OF GROWTH IN GREATER AMBERJACK (*Seriola dumerili*) LARVAE

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Introduction

The growth and development of farmed fish depends mostly on genetic, environmental and social factors, as well as on the production system and husbandry practices. Variation in growth rate between different populations of the same species and/or between different individuals of the same population is a challenge for scientists and producers. Farmed fish show great variability in size, ranging from 20-35% or even 40% in the early stages (Barki et al. 2000). Greater amberjack (*Seriola dumerili*) shows even greater size variability during pre-growing and on-growing stages, reaching 200 to 300% respectively, resulting in unreliable larval rearing and unpredictable production performance (Papandroulakis unpublished data). Water temperature is an important environmental factor that affects feed intake, food conversion efficiency and growth rate. The aim of the study was to investigate the impact of early life temperature on the molecular programming of growth rate in greater amberjack's larvae.

Materials and Methods

To investigate the role of water temperature on the molecular programming of growth in greater amberjack larvae, we applied two different temperature conditions (20°C & 24°C) from the embryonic stage to first feeding. The growth rate was monitored during the whole larval period and mRNA expression of genes, involved in growth and appetite regulation, was determined at first feeding (FF, 4.0-4.5 mm), notochord flexion (FLX, 5-6 mm), and middle metamorphosis (MM, 15-20 mm). Hypothalamic-pituitary axis genes involved in growth-promoting and developmental effects were analysed: growth hormone-releasing hormone (GHRH) that stimulates growth hormone (GH) secretion, which in turn initiates insulin-like growth factor I and II (IGFs) secretion. In addition, we analyzed leptin (leptin) and neuropeptide Y (NPY) genes that are responsible for the regulation of appetite and long-term energy storage (i.e. fat deposits). Gene expression analysis was performed using real-time PCR quantitative technology and their expression levels were calculated and normalized to a reference gene (Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the $\Delta\Delta$Ct method. Data were analyzed using the statistical package SigmaStat 3.1 (Systat Software, Inc., San Jose, CA, USA). Two-way ANOVA was used to estimate the effects of temperature on the three different developmental stages while Tukey's multiple comparison test was used to confirm statistically significant differences.

Results & Discussion

Analyses showed variation in gene expression of the six analysed genes: growth hormone, growth hormone-releasing hormone, insulin-like growth factor I and II, leptin and neuropeptide Y, among the three developmental stages (Figure 1). The variation in gene expression was influenced by temperature, with increased expression at 20°C compared to 24°C. Results indicate that larvae maintained during the embryonic and autotrophic stages at 20°C show increased expression of both GH and NPY, a major appetite stimulator, at the FF stage where the exogenous feeding starts and leptin, which is a multifunctional hormone in fish and is involved in the food intake regulation and the body weight via anorexic actions presents increased expression for the same temperature regime. Results show that early life temperature affects the expression of genes implicated in the regulation of growth at subsequent stages of development, and that incubation of embryos and pre-larvae at 20°C is more prominent compared to incubation at higher water temperature (24°C).

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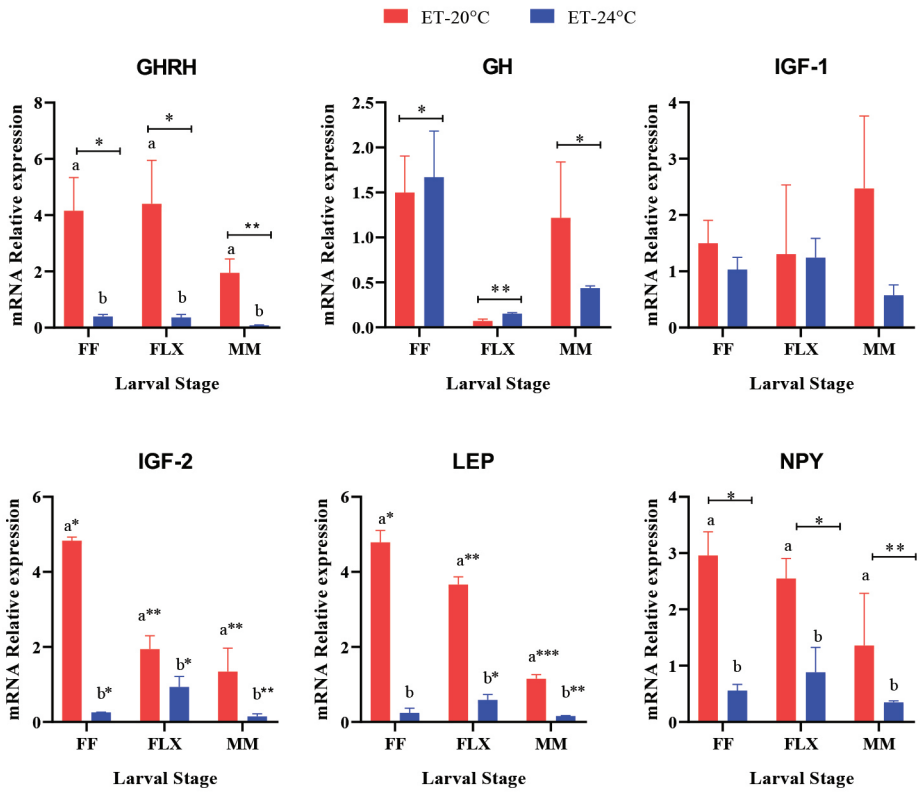

Figure 1. Changes in mRNA expression levels of genes involved in the regulation of growth and appetite, during early ontogeny (FF: first feeding, FLX: notochord flexion, MM: middle metamorphosis) in larvae exposed to different incubation temperatures (20ºC vs. 24ºC) from embryos to FF. Different letters indicate statistically significant differences among the different incubation temperatures within each respective developmental stage. The asterisk denotes statistically significant differences among the different developmental stages within each respective incubation temperature.

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EFFECTS OF ACUTE DISTRESS AND EUSTRESS ON EXPRESSION OF STRESS- AND IMMUNE-RELATED GENES IN THE PITUITARY AND HEAD KIDNEY OF JUVENILE KOI CARP (*Cyprinus carpio* L.)

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Introduction

The stress responses in teleost involve a variety of complex physiological processes in the organism, including alterations of immune functions [1]. Activation of hypothalamus-pituitary-interrenal (HPI) and hypothalamus-sympathetic-chromaffin (HSC) axes following acute stress plays a substantial role in adaptation to challenges fish encounter. Besides a clear role in stress responses, the head kidney has crucial functions in fish immunity, and due to its organization enables direct signaling between the endocrine and the immune system [2]. A more detailed knowledge about differential effects of acute stress on the immune system in fish is still scarce. The study intends to show that distress and eustress have different downstream effects on immune responses in koi carp.

Materials and methods

In total 60 juvenile koi carp trained to receive mosquito larvae as a daily feed reward were kept either in a group tank, from which they were sampled immediately, or kept in pairs in 50 L aquaria surrounded curtains, where they underwent experimental treatments after acclimatization. The treatments were as follows – opening curtains and lids of aquaria (C) as a control, receiving the feed reward (F) as a positive stressor, or netting the fish above the water surface for one minute (A) as a negative stressor. The fish were sampled 10, 30 or 60 minutes after each treatment. Each group consisted of 6 fish. During sampling, blood, brain (including pituitary) and head kidneys were collected. Blood analysis included measuring plasma glucocorticoid levels (cortisol, cortisone, corticosterone), as well as glucose and lactate. The plasma parameters were then analyzed using ANOVA. Gene expression analyses of HPI-related genes in the pituitary gland, immune-related genes, as well as genes related to isotocin and vasotocin signalling in the head kidney were analyzed by qPCR. Furthermore, the gene expression data was subjected to a principal component analysis (PCA), as well as bootstrapped (n=2000) and used for ElasticNet regression allowing treatment classification.

Results

The analysis of the plasma steroid values revealed that one minute air exposure caused a significant increase in cortisol levels in fish 30 min after treatment. The gene expression analysis of the pituitary gland suggests that both treatments, feed rewarding and air exposure, are perceived as stressors, however they are processed differently. The head kidney transcriptome profile shows that acute eustress and distress induce differential immune-related responses. The principal component analysis revealed that HPI-axis and immune genes have the highest impact on the outcome. Moreover, the ElasticNet regression analysis of the gene expression of both pituitary and head kidney was able, with high accuracy, to classify the different treatments and indicate genes with stronger association to certain treatments.

Acknowledgements

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IMPROVING LIVEFEED AND MICRODIET FORMULATION FOR ATLANTIC COD (*Gadus morhua*)

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Introduction

Atlantic cod aquaculture is still faced with some production challenges which can be partially tackled through the optimization of rearing and feeding protocols. In particular, feeding and nutrition during early larvae stages are paramount for ensuring the quality of juveniles. Although rotifers and *Artemia* are the most common protocols for cod larvae cultured by intensive methods, these have been shown to have lower growth rates and result in more severe and frequent skeletal deformities, than larvae fed on copepods in extensive and semi-extensive rearing systems. Barnacle nauplii have been proven to be suitable live prey for first-feeding cod larvae, being naturally high in essential polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Olivotto et al. 2010). And these have been identified to be a requirement for neural development, pigmentation, growth and survival of marine larvae (Puvanendran et al. 2021).

On the other hand, developing more suitable microdiets is also a relevant strategy to improve the quality of cod juveniles (Puvanendran et al. 2021) as it is for early weaning into inert diets. Although much progress has been made concerning feeding protocols for the intensive culture of cod larvae, further research is needed to optimise microdiets and a better combination with live feed. This work presents a trial conducted to evaluate the effect of two microdiets and one live feed protocol on Atlantic cod's survival and growth performance.

Material and methods

Cod larvae at two days post-hatching (dph) were randomly distributed in 400 L tanks at Ode facilities (Stadsbygd, Norway). The light regime was 24 hours, and water parameters were monitored daily, maintaining oxygen saturation above 90 %, salinity at 35 psu and temperature at 8,8 ± 0,2 ºC until 30 dph followed by 10,8 ± 0,2 ºC until 66 dph.

Two experimental groups, in triplicate, were created differing on the feeding regime: 1) D1 group (D1) consisting of Cryo-micro (plankton eggs) and both small (Cryo-S) and large (Cryo-L) barnacle nauplii (Planktonic AS, Norway) co-fed with rotifers, followed by Diet 1; 2) D2 group (D2) consisting of Cryo-micro, Cryo-S and Cryo-L (Planktonic AS, Norway) co-fed with rotifers, followed by Diet 2.

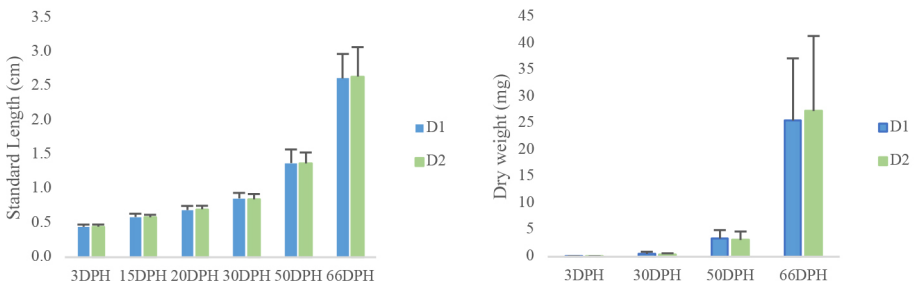

Figure 1. Standard length (cm) and Dry weight (mg) of cod larvae fed with protocols D1 and D2. Results are expressed as means ± standard deviation.

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From 27 to 45 dph, the protocol consisted of co-feeding with two experimental formulated feeds provided by SPAROS Ld. (Olhão, Portugal) for D1 and D2. After this period, feeding was done exclusively with dry feed until the end of the trial (66 dph). The main ingredients of the two experimental diets were squid meal, krill meal, fish hydrolysate, lecithin and krill oil, D1 being lower in marine phospholipids and D2 richer in marine phospholipids and with higher content of n-3 PUFA, namely DHA and EPA.

Results

At day 66 ph no significant differences were found in dry weight or standard length between the treatments D1 and D2, despite the trend for increased parameters in D2 treatment (Figure 1). Moreover, larvae survival, FCR and RGR weren't significantly affected by the different experimental diets.

Discussion and Conclusion

Overall, both protocols resulted in good growth performance and better survival rates, when compared to other studies (Puvanendran et al. 2023). Despite no significant differences between the two groups and although requiring further testing, at the end of the trial larvae from the D2 protocol showed a propensity for enhanced performance in terms of dry weight, standard length, RGR and FCR. This protocol included an experimental formulated feed richer in marine phospholipids, DHA and EPA which has been proven relevant for cod larval development and growth (Wold et al. 2007; Puvanendran et al. 2021). In future studies, there will be scope to test feeding protocols combining live feeds with novel microdiet formulations to further optimize the growth performance and survival of cod larvae.

Acknowledgements

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DIETARY TRYPTOPHAN SUPPLEMENTATION MITIGATES STRESS- AND INFLAMMATION-INDUCED GENE EXPRESSION ALTERATIONS IN THE HPI AXIS OF A TELEOST FISH

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Introduction

Currently, immune nutrition is used to enhance animal health by incorporating functional ingredients into aquafeeds. These ingredients are designed to stimulate or modulate fish immune system, and might serve as a complementary strategy alongside vaccination in aquaculture. In line with this, the objective of the present study was to investigate the connections between tryptophan nutrition and the complex network that regulates the bi-directional pathways between neuroendocrine and immune systems in European seabass.

Materials & Methods

European seabass juveniles (12.02 ± 2.77 g) were distributed in two independent recirculating seawater systems with a density of 5 kg/m^3 (control) or a stress-inducing density (10 kg/m^3). Fish were fed a control diet (CTRL) and a CTRL-based diet supplemented with tryptophan (0.3% DM basis; TRP) in quadruplicate for 15 days. Fish were sampled at the end of the feeding period and at 4, 24 and 72 hours post intraperitoneal injection with *Photobacterium damselae piscicida*. Plasma was used for the quantification of cortisol levels and the hypothalamus, pituitary gland and head-kidney were sampled for gene expression analysis.

Results & Discussion

Changes in transcription rates of several genes, including anti-inflammatory cytokines (*il10*) and others related to the HPI axis (*gr1*, *htr2a*, and *tph1α*) where observed in fish exposed to an ongoing stress response, that was translated into elevated plasma cortisol levels. When tryptophan was included as a dietary supplement, it played a key role in modulating the HPI axis response to stress, as observed by further alterations in expression levels of anti-inflammatory cytokine IL10 and glucocorticoid receptors 1 and 2. Additionally, tryptophan supplementation led to decreased *pomcb* mRNA levels, that was accompanied by a decrease in plasma cortisol levels in stressed fish. Moreover, when stress and inflammation were combined, tryptophan dietary supplementation attenuated suppressive effects of stress by modulating the neuroendocrine response, leading to a gene expression profile more similar to that of non-stressed fish. In particular, fish given a tryptophan surplus showed decreased expression of the anti-inflammatory cytokine IL10 compared to the control group. It also resulted in reduced stress-induced cortisol production and down-regulation of key components in the HPI axis, including serotonergic receptor (*htr2a*), tryptophan hydroxylase (*tph1α*), glucocorticoid receptor gr1, and pro-opiomelanocortin a-like (*pomca*). Such changes might have inhibited the release of ACTH and subsequent cortisol production.

Conclusion

Results unveil modulatory effects of tryptophan dietary intervention in central (brain) and peripheric (head-kidney) molecular patterns that might have sustained changes in cortisol release. These results highlight the importance of both this amino acid and serotonergic activity in pathways that orchestrate neuroendocrine-immune communication.

Acknowledgements

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OPTIMIZATION OF THE CONSERVATION PROCESS OF MATURE BIOFLOC FOR THE PRODUCTION OF *Penaeus vannamei*

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Introduction

In recent decades, with a growing world population, aquaculture has increased its activity to levels where environmental sustainability is compromised. In response to this problem, Biofloc technology has emerged. This technology consists of the use of microbial aggregates with the capacity to maintain water quality and provide food for cultured aquatic organisms. In this way, the toxic forms of nitrogen are reused while acting as a probiotic, providing a partial replacement of commercial food. The availability of stable mature Bioflocs for the reactivation of production tanks represents a clear competitive advantage. For this, it is essential to know the most appropriate cryopreservation technique for these microbial inoculums. Under this perspective, the present work has focused on the study of the microbiota of mature Biofloc subjected to 5 different cryopreservation methods.

Material and Methods

The effectiveness of refrigeration, freezing, freezing with addition of glycerol (10%), freezing with addition of glucose (10%) and lyophilization of the samples in skimmed milk was evaluated. First, samples of mature and concentrated Biofloc were generated. Subsequently, they were subjected to the different cryopreservation scenarios and analyzed by cultivar microbiological techniques. Total, heterotrophic and nitrifying bacterial populations were evaluated in this study. Total bacteria were measured with the Guava Muse® Cell Analyzer cytometer (Luminex Corporation, USA); Heterotrophic bacteria were cultured at 30 °C for 48 h in Marine Broth medium (Panreac AppliChem); Nitrifying bacteria, were cultured at room temperature for 14 days in a medium with specific components with a minimum mineral composition described by Rodriguez et al., 2017. Finally, we performed a metataxonomic analysis, in order to know the biodiversity and metataxome present in the Biofloc of each cryopreservation treatment. To know the uncultured microbiota of the samples, sequencing of the variable regions V3-V4 16S rRNA gene was carried out. DNA extraction was performed with the Power Soil Kit, Quiagen (Qiagen N.V., Hilden, Germany). After amplification of the genetic material by PCR, sequencing of the samples was performed with a MiSeq PE300 kit (Illumina Inc., San Diego, CA, United States). The quality of the demultiplexed FASTQ files was checked through FastQC software; and pairwise assembly of R1 and R2 reads was done with FLASH. Sequences were analyzed using QIIME2 software version 2021-04. Regarding biodiversity indices, two alpha biodiversity indices were calculated: the Chao1 index (richness of samples) and the Shannon index (diversity of samples).

Results and discusión

When analyzing the culture, it was observed that both freeze-drying and refrigeration resulted in a reduction of the microbial load, and, therefore, it was not an effective method when trying to preserve the viability of the microbiome (Figure 1). Freezing at -80 °C, both with the addition of 10% glucose and 10% glycerol, ensured the maintenance of a high microbial diversity, with a predominance of marine heterotrophic bacteria over nitrifying bacteria.

On the other hand, meta-taxonomic analysis revealed that the preservation methods that provided the highest richness to the biofloc were freeze-drying (Figure 2), followed by treatment with 10% glycerol. The result of the meta-taxonomic analysis coincided with the fact that refrigeration was not an optimal method for preserving the total microbiota, but also indicated that the use of 10% glucose generated a lower richness in the microbial community. The biodiversity present in the mature Biofloc was high, being constituted by a high number of diverse species. The Flavobacteriaceae family was the most representative microbial group, presenting a higher relative abundance in half of the preservation methods analyzed.

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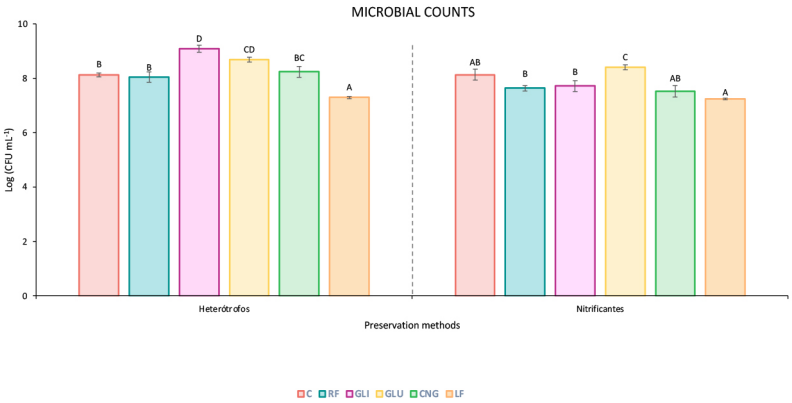

Figure 1. Representative bar graph of heterotrophic and nitrifying microbiota counts using the logarithm of colony forming units per milliliter of Biofloc versus different preservation methods. C: initial concentrated sample; RF: refrigerated concentrated sample; GLI: frozen concentrated sample with 10% glycerol; GLU: frozen concentrated sample with 10% glucose; CNG: frozen concentrated sample; LF: freeze-dried concentrated sample. Results are mean (n = 3) ± standard error (vertical bars). Above the error bars, the different homogeneity groups (LSD=95%), obtained by multiple range analysis, are shown.

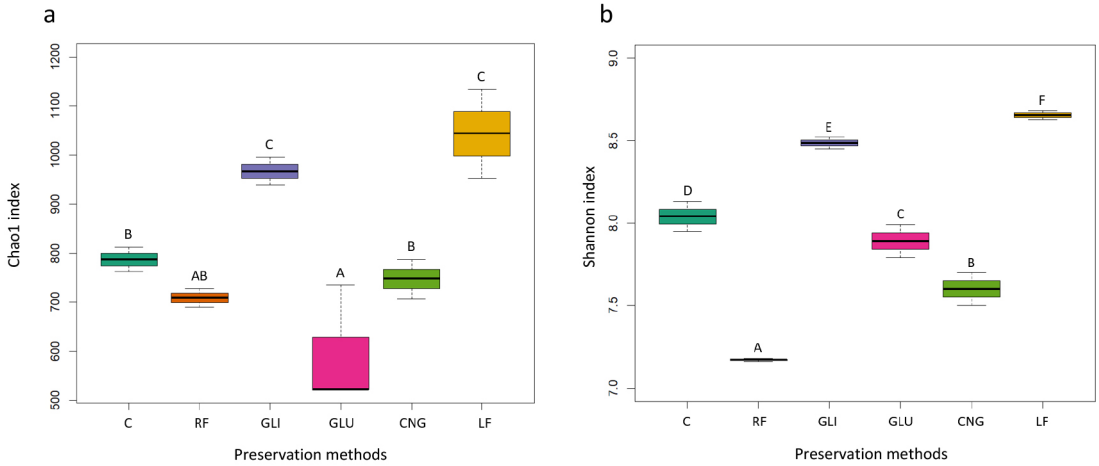

Figure 2. Chao1 index (a) and Shannon index (b) of Biofloc samples subjected to different preservation methods (Box and whisker plot). C: initial concentrated sample; RF: refrigerated concentrated sample; GLI: frozen concentrated sample with 10% glycerol; GLU: frozen concentrated sample with 10% glucose; CNG: frozen concentrated sample; LF: freeze-dried concentrated sample. At the top of each maximum value, the different homogeneity groups (LSD=95%), obtained by multiple range analysis, are shown.

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OPTIMIZATION OF MICROALGAE COMMUNITY FOR *Penaeus Vannamei* PRODUCTION UNDER BIOFLOC TECHNOLOGY

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Introduction

Biofloc technology (BFT)is presented as a sustainable aquaculture method due to its low water exchange and increased stocking density compared to conventional methods. The bacteria population present at the biofloc is responsible of nitrogen recycling, maintaining the total nitrogen at safe levels for the cultured species. Furthermore, bacteria form protein-rich flocs, resulting in an improvement of growth rate and associated feed costs. On the other hand, microalgae are a source of beneficial fatty acids that improve the health and growth of cultivated species. Studies have demonstrated that continuous supplementation with different types of microalgae has enhanced BFT in different ways, such as increased survival rates and growth in *P. vannamei* (Dong et al., 2022). The main objective of this study is to find the ecological balance between the bacterial population and the inoculated microalgae. It is a known fact that microalgae require a light/dark regime for productive photosynthesis (specific photoperiod). Therefore, this experiment was conducted at different photoperiods to determine the most suitable for microalgal growth and *P. vannamei* in BFT.

Material and Methods

Two microalgae, *Chlorella sp*. (CH) and *Phaeodactylum tricornutum* (PH), with a final absorbance of 0.15 AU (λ= 680 nm) were added into a biofloc system (total suspended solids (TSS): 150 mg/L) under three different photoperiods (PP) (16:8, 12:12, and 8:16 light:dark). Additionally, a control group was included that consisted in a biofloc without microalgae inclusion and under darkness conditions (0:24). All groups had 3 replicates, with a total of 21 experimental units of 90 L. The experiment was carried out with a density of 123 animals/m2, and an initial weight of 3.6 g and 5.1 g for CH and for PH, respectively. *P. vannamei* were fed twice a day with commercial feed (Le Gouguessant Aquaculture, France). The experiment had a duration of 14 days. Water parameters were monitored daily by the multiparametric HANNA equipment HI19829 Model, with salinity values of 19±4 g/L, temperature of 28 ºC, pH of 8-8.8, dissolved oxygen >5 mg/L, and alkalinity of 207 ± 40 mg/L. Ammonium, nitrite, nitrate, TSS < 500 mg/L values were measured twice a week. Microalgae population was quantified with total chlorophyll extraction and spectrophotometry (DLAB SP-UV1100). Finally, the animals were weighed at the end of the experiment. The results of the study were analyzed using multiparametric ANOVA and One-way ANOVA (p-value <0.05) with the Statgraphics 19 software.

Results and discussion

The concentration of chlorophyll showed different dynamics based on the PP, as can be seen in Figure 1. Using CH as microalgae, mostly, the control group showed lower levels of chlorophyll until 7th day. PH group showed significantly higher levels after microalgae inoculation respect to control, but only four days later decreased dramatically, remaining low the rest of the experiment. Except 16:8 group, which increased on day 14, showing differences with the rest of the groups. (Figure 1).

Regarding water quality, meanwhile CH group was able to maintain the ammonia, nitrite and nitrate concentration to non-toxic levels, in PH group, ammonium and nitrate concentration increased until the 11th day, to decrease later on, but never achieving toxic levels.

In relation to growth performance, no significant differences in weight gain were observed between PP within each experimental group (Table 1).

Nevertheless, when we compared PP and microalgae, generally, higher growth was obtained using *Chorella spp* than *P. tricornutum*, with significant differences in the PP 12:12 and 8:16 PP. Thus, it seems that medium bacterial and microalgae growth are the best conditions for shrimp production using *Chorella spp*. On the other hand, likely, due to the bad adaptation of PH to the BFT, the highest growth was obtained in the control group, without microalgae inoculation. The poorer adaptation of *P. tricornutum* could be due to being a diatom, it may face issues of insufficient silica for its growth.

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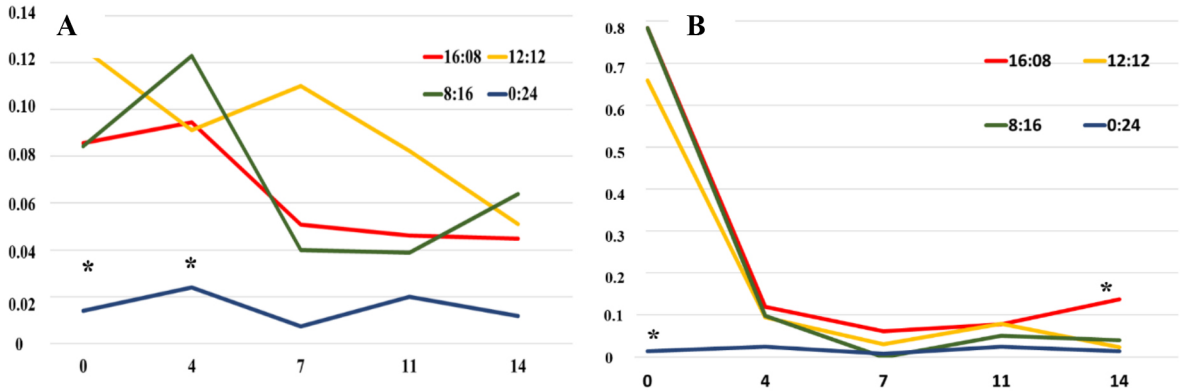

Figure 1. Evolution of concentration of chlorophyll over time (days). A) *Chlorella sp* and B) *P. tricornutum. Asterisks indicate significant differences between photoperiods (p<0.05).*

Table 1: Weight increase of P. vannamei. Different letters indicate significant differences between rows (p-value < 0.05).

	Chrolella sp.	*P. tricornutum*
16:8	2.06 ± 0.78	1.12 ± 0.69
12:12	2.20 ± 0.38[a]	0.54 ± 0.48[b]
8:16	2.21 ± 0.52[a]	1.21 ± 0.04[b]
0:24	1.55 ± 0.09	1.55 ± 0.09

Therefore, we can conclude that based on the major stability of water parameters, minor decline in chlorophyll concentration and the greatest weight gain in animals indicates that *Chlorella sp.* may be the most optimal candidate to use under BFT. Similar effects were also found in the study by Ge et al. (2016), with better shrimp growths using microalgae. Longer experimental trials are necessary to confirm these results, but it is the first study under our knowledge that is able to maintain a stable microalgae population to produce *P. vannamei* under BFT.

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Acknowledgments

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EVALUATION OF DIFFERENT FEEDING FREQUENCIES IN RAS-BASED JUVENILE PIKEPERCH (*Sander lucioperca*) AQUACULTURE

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Introduction

Feeding is crucial in aquaculture, accounting for 60% of production costs. Reducing overfeeding and increasing efficiency is important for profitability and water quality in intensive aquaculture as general. Investigating feeding frequency preferences could improve growth performance and survival rate of pikeperch. This study aims to determine the optimal feeding frequency for sustainable and efficient aquaculture practices in pikeperch juvenile.

Material and methods

Juvenile pikeperch [n=750; total length (TL) = 239.5 ± 16.7 mm and body weight (BW) = 106.6 ± 24.8 g] were divided into five groups (with three replications each) and subjected to different feeding regimes over a 24-hour period: 12 feeds at 2-hour intervals, 6 feeds at 4-hour intervals, 4 feeds at 6-hour intervals, 3 feeds at 8-hour intervals, and 2 feeds at 12-hour intervals. Fish in all groups were fed with an Imetronic® self-feeding system (Pessac, France) that allowed for easy adjustment of feeding frequencies, actual batched feed, and controlled daily feeding rate (DFR). The DFR was set at 1% of the total biomass of fish per tank. After 28 days, each tank was weighed and the current daily feed intake for each group was recorded. Production parameters [TL, somatic length (SL), Fulton's condition coefficient (FC), specific growth rate (SGR), feed conversion ratio (FCR), specific heterogeneity rate (SHR), survival rate (SR),] and fin condition among tested groups were assessed at the beginning and end of the experiment. The pectoral, ventral, dorsal, caudal, and anal fins, and the level of fin damage rate were determined according to Policar et al. (2016). Blood samples were collected from 6 anesthetized fish per group at the beginning and end of the feeding trial for biochemical analysis, including plasma levels of total protein (TP), albumin (ALB), globulin (GLB), amylase (AMYL), lipase (LIPA), total cholesterol (TCHOL), glucose (GLU), ammonia (NH3), and triglyceride (TAG).

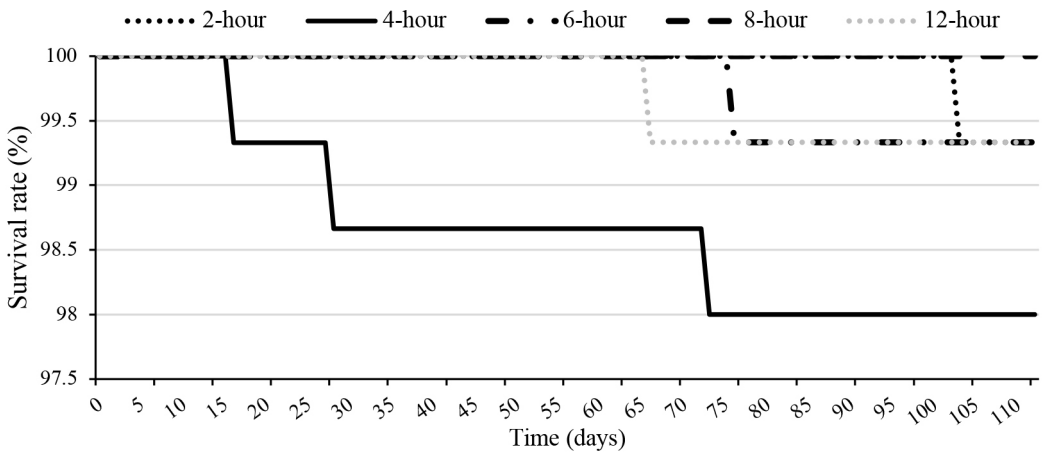

Figure 1. The cumulative survival rate course of groups with tested feeding frequencies of juvenile pikeperch (*Sander lucioperca*) during the 112-day feeding trial.

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Results

Following the 112-day feeding trial, there were no significant differences in fish biometric parameters (TL, SL, BW) among all tested groups. The group with an 8-hour feeding frequency had the highest values of FC (0.95) and SGR (0.95%.day^{-1}), while the group with a 4-hour feeding frequency had the lowest values (FC = 0.84, SGR = 0.82%.day^{-1}). No differences were observed in FCR (0.92–1.19), SHR (322.13–342.13‰.day^{-1}), and SR (98.69–100%) among all groups (Fig. 1). There was no cannibalism rate, ensuring high survival rates. Fin erosion was minimal in all groups, with juveniles with a 12-hour feeding frequency showing the most visible erosion effects. In contrast, the dorsal second and caudal fins in juveniles with an 8-hour feeding frequency were less affected (only 12%). No significant differences were found in selected biochemical parameters in all groups based on pikeperch blood plasma analysis after the 112-day feeding trial. Feeding interval 8-hour was evaluated as optimal for intensive juvenile pikeperch rearing, resulting with excellent and acceptable SGR (0.95%.d^{-1}) and FCR (0.95kg.kg^{-1}), high SR (100%) and minimal fin erosion, and well-maintained biochemical parameters in blood plasma.

Discussion

Higher feeding frequency led to smaller feed consumption, as observed in previous studies on other fish species (Gilannejad et al., 2021). However, some studies showed that juvenile carnivorous species grew better when fed to satiation (Booth et al., 2008; Enes et al., 2015). Fish size, species, and husbandry conditions may influence the optimal feeding frequency, as our study on pikeperch suggested. Nevertheless, all groups rapidly adapted to different feeding frequencies, indicating their flexible feeding behavior.

Acknowledgements

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DELTA*, A DATABASE DEVELOPED FOR INDOOR SHRIMP FARMS

Supported by real-time monitoring, comprehensive data overview and evaluation

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Background

The work presented here is part of a project funded by the "Deutsche Bundesstiftung Umwelt[1]" and performed in cooperation with the University of Veterinary Medicine Hannover Foundation. The database part of the project was developped by our company, using a sub-contract for programming tasks. The resulting database "DeltA" has been implemented at the indoor shrimp farm in Germany "Damm Aquakultur" (www.die-landgarnele.de). DeltA signifies "Datenbank für europäische land- und technikbasierte Aquakultur, in English: "database for European land- and technique-based aquaculture".

Indoor aquaculture is a growing sector of food production but still in a young and fragile stage of development that can be fostered by digital technologies, such as a specific database. Successful aquaculture is depending on rapid assessment and control of critical water quality parameters. Still, operators of aquaculture plants may rely on handwritten data protocols manually to be entered into computer-aided data processing. In case of critical developments the relevant data required for an immediate reaction is lacking in the very situation, or just coming too late. This may lead to notable decreases in production.

The solution we developed is a database for indoor aquaculture set up on the basic construction of DANA 2.0, an online database developed for public pools operated by biological water treatment, to date applied by more than 100 baths in several countries (DANA also received funding by the German DBU). The aim was to provide a tool designed for indoor shrimp aquaculture, rendering a comprehensive data overview and easy data evaluation in order to support the operator in rapid decision making and thereby substantially reducing the risks associated with aquaculture operation.

Results

DeltA is being employed at the shrimp farm "Damm Aquakultur", all functions have been accomplished and are implemented. An essential module is the feed management tool, based on an assumed zootechnical performance (e.g. survival rate), adaptable to new findings. The tool for data entry lists groups of parameters corresponding with the working routine, and each entry is traceable via a 'history' protocol. Data evaluation by graphs and summary tables is selectable as tank-related or batch-specific. Finally and most crucial: alarm messages are integrated into DeltA via PLC and sensor data can be sent via LORAWAN, independent from WIFI or Ethernet. Furthermore, consultants can receive limited or full access to DeltA, as required. In conclusion, the database offers an easy and up-to-date access to any available data, supporting a deeper understanding of the complex processes and a more efficient control of all critical parameters. Development and implementation of a higher degree of automatisation, also supported by AI, are further objectives for the near future. Basically DeltA is adaptable to the culture of any aquaculture organism, thereby opening a broad field of applications.

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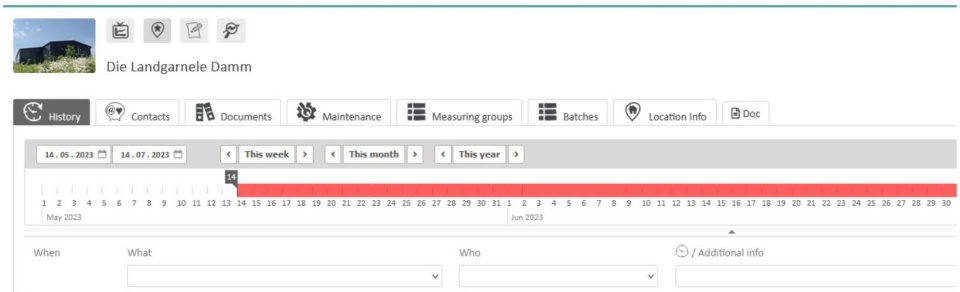
sponsored by DBU Deutsche Bundesstiftung Umwelt www.dbu.de

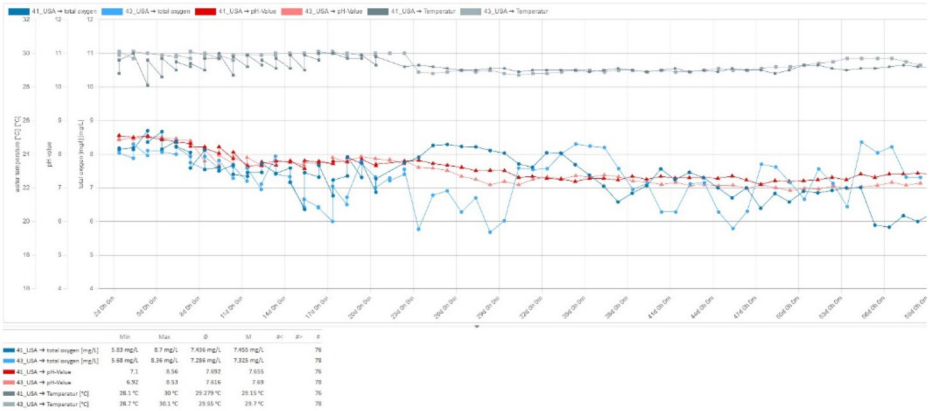
[1] DBU - German Federal Foundation for the Environment; ref. no.: 34855/01

Figures

The Indoor Shrimp Farm at Damm Aquakultur GmbH & Co. KG: www.die-landgarnele.de (‚the inland shrimp')



Main settings at location "Damm": first line icons for dashboard – settings – data entry & data evaluation, 2nd line opened under "settings", showing worksheet "history" with calendar and filter options for data entry report



Example for data evaluation per batch and Day of Culture - here for 2 cultures

DOES TRANSPORT DURATION OF SHRIMP POST LARVAE ADVERSELY AFFECT CULTURE PERFORMANCE?

L. Vannamei PL IMPORTED FROM THE USA, AUSTRIA AND GERMANY STOCKED, CULTURED & COMPARED AT AN INLAND, BIOFLOC-BASED SHRIMP FARM IN GERMANY

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Background

The work presented here is part of a project funded by the "Deutsche Bundesstiftung Umwelt[1]" and performed in cooperation with the University of Veterinary Medicine Hannover Foundation. The project was performed at the indoor shrimp farm in Germany "Damm Aquakultur" (www.die-landgarnele.de). At the project start in 11/2019 all PL were imported from the USA. Transport duration of more than 24h and poor transport conditions may impact the performance of shrimp during culture. One of the aims was to reveal a theoretical influence imposed by origin, i.e. transport duration. In 2020, despite pandemic-related shortening of PL availabilities, once three batches of *L. vannamei* (PL11-12) could be stocked in parallel. PL hatcheries were located in three countries: USA, Austria and Germany. No further parallel stocking was possible, but more cultures from different origin could be stocked during the observation period of ca. 18 months. In total we assessed: 3 batches from Germany, 4 from Austria and 7 from USA. Water quality and zootechnical data were acquired throughout the culture period. At three sampling dates during the culture period our cooperation partner examined the microbiome, its diversity and the relative abundances of bacteria phyla.

Results

In 3 simultaneously stocked batches PL from Germany (DE) grew much faster compared to PL from Austria (AT), followed by PL from USA (US) under comparable conditions of water quality and feeding regime. Harvest was highest for PL from Germany (at DOC 116), but this result was not comparable with harvest results from the other 2 batches due to much longer culture periods (>200 days of culture). Finally, comparing the average performance of 3 to 7 batches per origin growth curves showed high variability, independent of origin.

The microbiome differed at PL arrival and grew more diverse during culture, regardless of PL origin. Members of Nitrospirota increased in the biofilm on tank surface, a development observed in all sampled tanks.

In conclusion, zootechnical performance of *L. vannamei* cultured in a biofloc-based indoor shrimp farm could not be clearly related to the country of origin. The hatchery, i.e. the genetics employed, appear to be the more critical factor. More R&D in cooperation with shrimp producers seems to be needed to breed the optimum shrimp for indoor farming.

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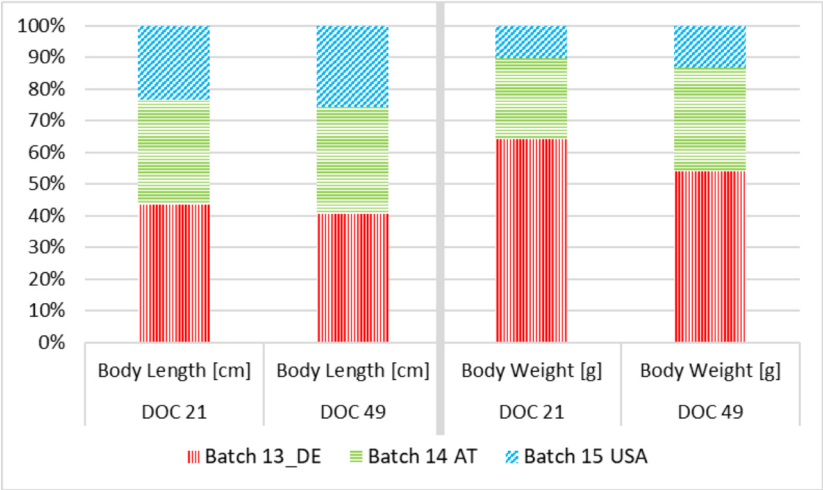
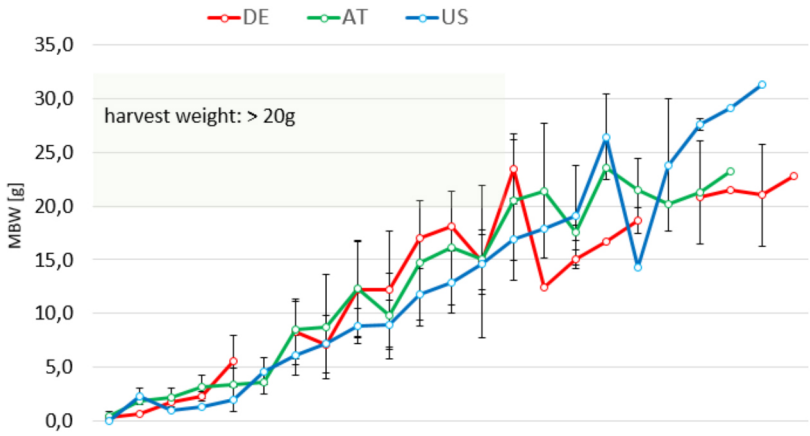
[1]DBU - German Federal Foundation for the Environment; ref. no.: 34855/01

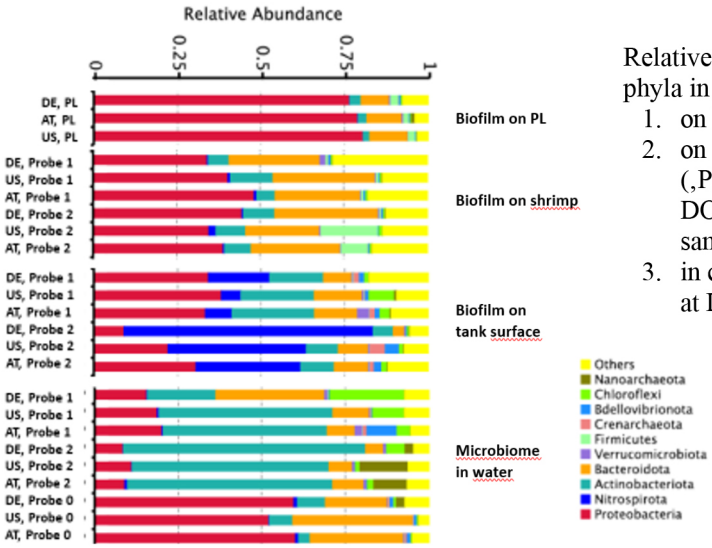
Figures



Relations in body length and body weight among PL from 3 sources, at DOC 21 and DOC 49 (DOC: Day Of Culture), DE= Germany - red; AT= Austria - green, US= USA - blue)



High variability in growth of shrimp from 3 origins (mean body weights [g] as average of several batches per origin: 3 batches DE= red; 4 batches AT= green, 7 batches US= blue)



Relative abundance of bacteria phyla in the mircobiome

1. on PL (at DOC 1)
2. on shrimp of 3 origins (‚Probe 1‘= sample 1 at DOC 50, ‘Probe 2‘= sample 2 at DOC 85), and
3. in culture water (Probe 0 at DOC 1)

OXYGEN EFFECT ON NUTRIENT SOLUBILISATION IN MARINE AQUACULTURE SLUDGE

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Introduction

In aquaculture, recirculated aquaculture systems (RAS) show potential circularity due to their capacity to reutilize water and concentrate nutrient-rich effluents (Martins et al., 2010; European Commission, 2021). While successful implementation of circular production has been demonstrated in freshwater RAS with aquaponic systems (Joyce et al., 2019), effective nutrient recycling in marine RAS is challenging. The high salinity levels in marine RAS effluents exceed crop tolerance and municipal wastewater treatment limits.

In response to these challenges, algae emerge as a promising and cost-effective solution for capturing dissolved nutrients in marine aquaculture (Neori et al., 2004; Mohsenpour et al., 2021). Their efficient uptake and assimilation of key nutrients, including nitrogen, phosphorus, and carbon, align closely with the nutrient composition of RAS effluents (Bolton et al., 2009; Villar-Navarro et al., 2021). While nitrogen's bioavailability for algae cultivation from RAS overflow water has been established (Ramli et al., 2020), the bioavailability of phosphorus, carbon and micronutrients in RAS sludge presents a significant hurdle. Although, carbon recovery from waste has been explored in diverse contexts, the recovery of dissolved phosphorus as not been fully elucidated. (Jung and Lovitt, 2011; Sarvajayakesavalu et al., 2018).

In this study, we examined the effect of oxygen on nutrient solubilization as part of a broader project aiming to the develop both a method and a biological reactor concept to enhance nutrient solubilization from marine RAS sludge.

Materials and methods

Marine sludge was taken after the denitrification tank in a commercial shrimp farm and subject to biological treatment. Treatments were conducted in duplicate within 2-liter bottles equipped with magnetic stirrers. The experimental encompassed four distinct oxygen regimens: complete aerobic, complete anaerobic, a combination of 30% aerobic followed by 70% anaerobic, and a combination of 70% aerobic and 30% anaerobic. During the study, the targeted parameters include pH, phosphorus, total phosphorus, iron, total iron, manganese, total manganese, and acetate. The outcomes of the study are currently undergoing analysis and will be showcased in the forthcoming poster presentation.

(Continued on next page)

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ANTIOXIDANT, METABOLIC AND DIGESTIVE RESPONSES OF FARMED *Spaurus aurata* AND *Diplodus sargus* BIOFORTIFIED WITH BROWN AND RED MACROALGAE

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Introduction

Aquaculture is considered a sustainable solution for the growing food demand of this century[1]. Within animal production, aquaculture is the sector that shows the fastest expansion rate, consistently increasing every year its share of the total seafood produced worldwide[2]. Despite the major technological investments made towards maximizing farmed seafood production and value, aquaculture has always had to face the double challenge of developing cost-effective aquafeeds that meet fish species (particularly, marine carnivorous ones) metabolic and nutritional requirements, while reducing as much as possible the use of meals and oils derived from wild fish, which raise strong ecological concerns and compromise oceans' sustainability[3]. During the last year, the sector was further defied by the limited availability and escalating prices of raw materials (such as cereal/vegetable meals and oils) that came along with the war in Ukrania (2022). Hence, this has recently called for the urgent discovery of new alternative sources of nutrients that suit the nutritional and energetic demands of farmed fish and, thus, can potentially be used in aquafeeds' formulations.

The inclusion of seaweeds has been pointed out as an eco-friendly strategy to naturally enrich the aquafeeds with specific nutrients that are essential, though deficient, in the human diet[4,5]. Still, the current knowledge is very limited in what concerns their nutraceutical/functional potential, as well as, the effective doses necessary to improve the welfare of farmed animals, often compromised by captivity, high rearing densities and disease outbreaks.

Hence, the present study aimed to explore the effectiveness of aquafeeds biofortified with a brown (*Laminaria digitata*) and a red (*Asparagopsis taxiformis*) macroalgae (at three inclusion percentages) in improving the antioxidant, metabolic and digestive performance of two juvenile farmed fish ecologically and commercially relevant within the Mediterranean setting - *Sparus aurata* and *Diplodus sargus*.

Material and methods

S. aurata and *D. sargus* juveniles with ~8 g of body weight (BW) were kept in recirculation aquaculture systems (RAS) under optimal development conditions (20ºC, > 7 mg/L O_2). During the feeding trials, both fish species were fed ~2% of their BW with a commercial-based diet (CTR) and the *S. aurata* specimens with three experimental functional diets biofortified with 1.5%, 3% and 6% of *L. digitata* (whole, dried, and powdered) and the *D. sargus* specimens with three experimental diets supplemented with 1.5%, 3% and 6% of *A. taxiformis* (whole, dried, and powdered).

After 30 days of trial, 6 fish from each treatment and species were randomly collected and were euthanized for dissection and collection of muscle, gut and liver tissues.

To assess the effectiveness of biofortified aquafeeds to improve animal metabolism, digestive activity and overall welfare, the following physiological endpoints were assessed: oxidative stress (total antioxidant capacity [TAC], lipid peroxidation [LPO], superoxide dismutase activity [SOD], catalase activity [CAT] and glutathione S-transferases activity [GST]), metabolic enzymes activity (lactate dehydrogenase [LDH] and citrate synthase [CS]) and digestive enzymes activity (α-amylase, pepsin, trypsin and lipase)[6]. To normalize the results of each biomarker, total protein levels were also quantified according to the Bradford assay.

(Continued on next page)

Results and Discussion

Data are still currently being analysed. Yet, preliminary results showed that aquafeeds biofortified with both studied macroalgae significantly modulated farmed *S. aurata* and *D. sargus* antioxidant, metabolic and digestive performance, especially at the highest inclusion rate (6%). Contrasting previous studies using dried macroalgae as feed additives, the present results seem very promising, evidencing that *L.digitata* and, especially, *A. taxiformis* can have a positive effect on the antioxidant and metabolic activity of farmed marine juvenile fish.

Acknowledgements

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AQUAFEEDS BIOFORTIFIED WITH RED MACROALGAE AS AN ECO-INNOVATIVE STRATEGY TO UPGRADE FARMED MARINE FISH NUTRITIONAL VALUE

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Introduction

Aquaculture is the sector that shows the fastest expansion rate within animal production, consistently increasing its share of the total seafood produced worldwide every year. It plays a crucial role on food security, being responsible for more than 50% of global seafood production and, thus, contributing to end hunger and malnutrition of the world's growing population[1]. The expansion of aquaculture and the consequent growing demand for aquafeeds has increased fish meal and fish oil prices and, although the proportion of meals and oils derived from wild fish within aquafeeds has been demonstrating a downward trend, they are still important feed components for many carnivorous fishes[2,3]. In addition, during the last year, the sector was further defied by the scarcity and escalating prices of raw materials (such as cereal/vegetable meals and oils) that came along with the war in Ukraine (2022). Therefore, it is of the utmost importance for aquaculture to find economical and sustainable alternative ingredients to be included in aquafeed formulations that not only meet fish species, especially marine carnivorous ones, metabolic and nutritional requirements, but also represent an asset for seafood consumers from the nutritional point of view[3].

The inclusion of seaweeds has been suggested as an eco-friendly strategy to naturally enrich farmed fish diets with valuable sources of bioactive compounds that are not only essential for aquafeed formulations, but also lacking in the human diet (e.g. essential minerals and polyunsaturated fatty acids) [4,5]. However, the current knowledge is very limited regarding the nutraceutical/functional potential of macroalgae, as well as, the effective doses necessary to improve the quality of the final seafood product.

Thus, the present study aimed to evaluate the potential of aquafeeds biofortified with the red macroalgae *Asparagopsis taxiformis* (at three inclusion percentages) as an eco-innovative strategy to effectively upgrade the polyunsaturated fatty acids (PUFAs) and essential minerals contents in farmed white seabream (*Diplodus sargus*) fillets, as case study.

Material and methods

D. sargus juveniles with ~8 g body weight (BW) were kept in recirculation aquaculture systems (RAS) under optimal growing conditions (20ºC, > 7 mg/L O_2) during 60 days of feeding trial. During the trial, specimens were fed ~2% of their BW with either a commercial-based diet (CTR) or with experimental functional diets biofortified with 1.5%, 3% and 6% of *A. taxiformis* (dried powder), each in triplicate tanks.

At the beginning and end of the trial, six fish were randomly collected and euthanized for biometric data registration and dissection of muscle tissue. To assess the effectiveness of biofortified aquafeeds to improve nutritional value, fish muscle fillets were freeze-dried and analysed for proximate chemical composition (moisture, ash, free fat, carbohydrates and crude protein), fatty acid profile and essential elements (potassium, sodium, magnesium, iron, copper, zinc and manganese) content. Animal condition and growth performance were also assessed throughout the trial.

(Continued on next page)

Results and Discussion

Fish muscle proximate chemical composition, mineral and fatty acids profiles were significantly affected by the type of aquafeed. Overall, fat contents tended to increase with the addition of *A. taxiformis*, while carbohydrates and protein decreased at the highest macroalgae inclusion levels. Fish fillets biofortified with *A. taxiformis* showed higher contents of K and Fe, but lower contents of Zn, Mn, Mg and Cu, most likely due to the adjustments made in aquafeeds formulation necessary to maintain their digestibility and proximate chemical composition (i.e. replacement of some ingredients by dried *A. taxiformis*). The distinct percentage of algae used in each aquafeed dictated fish fillets fatty acids profiles, with fish fortified with 6% showing higher PUFA n-6 contents, mainly due to higher levels of linoleic acid (18:2 n-6) in those fish. Fish fed with 3% and 6% also revealed the highest contents of α-linolenic acid (18:3 n-3), which is an essential fatty acid percursor of EPA (20:5 n3) and DHA (22:6 n3). Overall, results evidenced that the use of seaweeds is an interesting and efficient way to tailor farmed fish nutritional value according to animal and consumers health requirements, though refining is necessary to optimize the biofortification of specific essential nutrients.

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GRINNAQUA: GREEN INNOVATION STRATEGIES FOR ANIMAL HEALTH MANAGEMENT: TOWARDS SUSTAINABLE AQUACULTURE

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In order to strengthen CIIMAR's research strength, amplify its innovative capabilities and secure its position as a dominant force within the aquaculture industry, GRINNAQUA is strategically aligned with the goals of the Farm to Fork Strategy. The partnership with well-known institutes in European aquaculture research, with extensive knowledge in relevant fields, will boost CIIMAR's ascension to scientific preeminence and increased competitiveness. The Spanish institution INIA-CSIC is skilled at navigating vaccination procedures and has a deep understanding of the subtleties of fish acquired immunity. The University of Bergen (Norway) is highly competent in the area of animal health and the application of genetics, while the Roslin Institute at the University of Edinburgh (UEDIN, UK) has invaluable knowledge in the complex world of genetics and animal breeding.

To enhance the level of excellence of CIIMAR's staff and raise their research profile, certain actions have been thoughtfully developed. To foster a culture of ongoing learning and innovation, the collaborating partners are putting into place specialized training sessions and immersive Summer schools. Senior researchers, technicians, and administrators are specifically targeted through planned visits by CIIMAR staff to partner institutions' facilities to ensure an extensive exchange of knowledge and best practices.

A collaborative effort that makes use of the institutions' combined scientific and technical resources is at the heart of the research agenda. The goal of this coordinated effort is to address urgent problems associated with economically significant virus and parasite outbreaks in the aquaculture industry. The project intends to bring insight into the preventive potential of functional foods for shielding rainbow trout against the haemorrhagic septicaemia virus and addressing the infestation of Atlantic salmon with sea lice parasites.

Beyond the scientific domain, this proposal casts a strategic vision for enhancing stakeholder engagement and mobilization, facilitating the alignment of CIIMAR's objectives with tangible scientific accomplishments. This proactive approach reinforces CIIMAR's position as a dynamic player in market-driven research and innovation, fostering an environment that heightens its competitive edge on both national and international platforms.

In essence, the GRINNAQUA project plays a crucial part in strengthening CIIMAR's capacity for innovation, establishing the organization as a leader in bringing about the blue revolution in the aquaculture industry. This partnership is likely to be long lasting and to create synergies that significantly reinforce a more resilient and sustainable aquaculture landscape throughout Europe.

IGNITION: IMPROVING GREEN INNOVATION FOR THE BLUE REVOLUTION - NEWS TOOLS AND OPPORTUNITIES FOR A MORE SUSTAINABLE ANIMAL FARMING

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IGNITION (Improving Green Innovation for the Blue Revolution), a cutting-edge Horizon Europe project, aims to revolutionize the aquaculture sector by addressing the rising demand for high-quality animal protein while ensuring environmental sustainability and animal welfare in a changing climate. One of the main objectives of IGNITION is the development of effective antigen-based vaccines to combat major diseases, such as tenacibaculosis and infectious salmon anemia virus, which pose significant challenges to intensive aquaculture systems. These innovative vaccines will promote animal health, reduce disease susceptibility, and improve the overall sustainability of the aquaculture industry by relying less on antimicrobials. IGNITION will also focus on enhancing stress and disease resilience through the development of new feeds, formulated to provide essential nutrients and bioactive compounds that boost the immune system and mitigate stress factors in farmed aquatic animals. By bolstering stress and disease resistance, the project aims to improve animal welfare and enhance the sector's overall productivity. To further support animal welfare, IGNITION will focus on the development of non-invasive stress and health biomarkers and biosensors and on defining operational welfare indicators. All these will offer real-time insights into animal welfare and health. Based on the data obtained, IGNITION will employ machine learning techniques and disease prediction software to improve decision-making and facilitate proactive management practices.

Overall, IGNITION's visionary effort aims to transform aquaculture practices, fostering a sustainable, responsible, and thriving future for the industry by providing a wealth of knowledge and innovative solutions for the European aquaculture sector and beyond.

QUALITY OF FLESH AND LIPID DEPOSITION IN THE HEPATOCYTES OF ATLANTIC SALMON (*Salmop salar*) FED INSECT MEAL DIETS

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Introduction

Insect meals have recently gained attention because of their suitability as feed ingredients in aquafeeds. These next-generation feed ingredients are produced through bio-circular methods and can uphold aquaculture sustainability. The effect of differently processed black soldier fly (BSF, *Hermetia illucens*) meals (full-fat, de-fatted, de-chitinised) or BSF paste in the diets of Atlantic salmon parr have been tested previously by Belghit et al., 2018; Fisher et al., 2020; Weththasinghe et al., 2021a, 2021b. In addition, a few studies examined the effect of diets with BSF meal throughout the full production cycle of Atlantic salmon (Belghit et al., 2019). However, the use of yellow mealworm (MW, *Tenebrio molitor*) meal as an ingredient in salmon feed is a novel approach. Therefore, in the current trial, we aimed to study the effects of insect meals on fillet composition, flesh quality, hepatocyte morphology, and lipid deposition in post-smolt Atlantic salmon.

Material & Methods

For the feeding trial, 520 fish (143 ± 12.8g) were randomly distributed into replicate tanks of the five dietary groups (26 fish/tank). The fish were fed either a control diet (CTRL) or one of four insect meal diets, at a rate of 1.6% body weight, using automatic feeders. The CTRL diet contained 20% SPC, 14% wheat gluten and 6.23% rapeseed oil, while the four test diets were prepared with BSF meal at 5% and 10% (BS5, BS10) inclusion and MW at 15% and 30% (MW15, MW30) inclusion. The study was conducted over a period of 103 days. After 74 days of feeding, the liver of 12 fish per group (441.3± 11.7g) were examined using imaging flow cytometry to elucidate any diet-caused changes in morphology and lipid accumulation. BODIPY™ 493/503 staining revealed the accumulation of neutral lipids. At the end of the trial (550.7 ± 12.5g), 24 fish per group were analyzed for fillet proximate and fatty acid composition as well as flesh quality parameters.

Results & Discussion

The tested insect meals did not affect the fillet proximate composition. The main dietary effects were linked to fillet fatty acid composition and flesh color. Fillets from fish fed 10% BS diet contained significantly higher levels of lauric acid compared to the fish fed the other experimental diets. This is due to the high levels of lauric acid present in the tested BSF meal, which is consistent with the reports by Liland et al., 2017; 2023. Moreover, alpha linoleic acid (C18:2n-6) and total n6 fatty acids were significantly higher in the fillet of the fish fed the 30% MW feed compared to the CTRL and 5% BS groups. Such a fatty acid profile of the fillet could be attributed to the dominance (33%) of alpha linoleic acid in MW. Although fillet texture was not affected by the insect meal inclusions, Minolta color measurements revealed significant differences in the skin and flesh color. The yellowness (b^*) of the skin from the ventral region of the fish fed 30% MW meal was significantly higher compared to that of the CTRL fish. This is in line with the reports of Li et al., (2022) and Zhang et al (2022) that revealed a strong skin yellowness in mirror carp (*Cyprinus carpio*) and yellow croaker (*Larimichthys crocea*) fed MW, and the authors suggested that the pigmentation was caused by the carotenoids and riboflavin in the substrates fed to MW. A significantly lower redness (a^*) value was found in flesh from the fish fed 30% MW diet in comparison to those fed the other diets except the 5% BS. Morphological analysis of the liver, based on cell diameter, circularity, and elongatedness, indicated that insect meal inclusion did not significantly affect these parameters in hepatocytes. Concerning the BODIPY analysis, fish fed higher levels of insect meals (BS15 and MW30) had significantly higher neutral lipids in the liver compared to those in the fish fed the lower insect meal, although the values were similar to that of the CTRL group.

Conclusion

Inclusion of black soldier fly larvae meal in salmon diet at 5% and meal worm at 15% did not compromise the fillet proximate composition, fillet texture, or liver cell morphology. However, the higher inclusion levels of the two insect meals (10% BS and 30% MW) resulted in unfavorable qualities to the produced fish, in terms of skin and fillet color and fillet fatty acid composition. Therefore, further studies are needed to determine the most appropriate levels of insect meals produced on side streams with required nutrient content.

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ASSESSMENT OF *IN VITRO* POSTBIOTIC CAPABILITIES OF THE PROBIOTIC *Shewanella putrefaciens* PDP11 GROWING UNDER DIFFERENT CULTIVATION CONDITIONS CONTAINING MICROALGAE DIETARY SUPPLEMENTS WIDELY USED IN AQUACULTURE

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Introduction

Probiotics have been established as potential tools to improve host health and environmental quality in aquaculture. For example,*Shewanella putrefaciens* Pdp11 (SpPdp11), a strain described as a probiotic for use in aquaculture (reviewedby Cámara et al. 2020). Despite the proven health benefits of probiotics, recent evidence suggests that bacterial viability is not necessary to attain effects. Thus, postbiotics have emerged as potential opportunities in the field of functional foods. The International Scientific Association for Probiotics and Prebiotics (ISAPP) convened a panel that defined postbiotics as a "preparation of inanimate microorganisms and/or their components that confer a health benefit to the host". Other natural dietary supplements, including microalgae, along with their extracted compounds, have also been studied as feed additives for several fish species, and have shown beneficial effects on fish health. Some of these important and well-characterized microalgae species are *Chlorella*, *Arthrospira*, *Microchloropsis*, and *Tisochrysis*, among others. These species are commonly used in fish diets and are known to improve health (Ansari et al., 2021).

Recent studies conducted by our group demonstrated how postbiotic production can be affected by different cultivation conditions of bacteria, especially the culture media (Domínguez-Maqueda et al., 2022). Information on this type of postbiotic activity is very scarce, especially in the case of aquaculture (Mora-Sánchez et al., 2020), and evaluation of the nutraceutical use of postbiotics to improve health management in fish and other cultivated aquatic organisms is an emerging area of research in aquaculture.

The present work evaluates the potential postbiotic, as extracellular products (ECPs), of SpPdp11 grown undern different cultivation conditions. These included different culture media composed of a blend of microalgae, to observe a possible synergistic effect. The ECPs obtained were evaluated for their cytotoxicity against different fish cell lines, enzymatic and antibacterial activity, and their effect on biofilm formation by several fish pathogens

Material and methods

SpPdp11 was grown on tryptic soy agar supplemented with NaCl (1.5 %) (TSAs) at 23º C for 24 h. Then, one to two colonies were transferred to 50 mL of tryptic soy broth supplemented with NaCl (1.5%) (TSBs) at 23ºC for 36h (10^9 CFU mL^{-1}, start of the stationary phase) with shaking at 80 rpm. Then, 1 mL of each culture was spread over sterile cellophane sheets placed on TSAs plates (T medium). Another 1 mL was spread on sterile cellophane sheets placed on plates containing an experimental aquafeed (160 g/L) agar (1.5 %) (F media), partial replacement of aquafeed with 25% of a blend of microalgae (*Chlorella fusca, Tisochrysis galbana, Microchloropsis gaditana* and *Arthrospira platensis*) (160g/L) and agar (1.5 %) (medium FM) and total blend of microalgae (50 g/L) (medium M). The plates were incubated at 23 ºC and 15 ºC for 24 h and 48 h. Samples were collected by adding 2 ml sterile saline phosphate buffer (PBS). The obtained suspension was centrifuged (10,000 x g, 20 min, 4ºC) and the supernatant was filtered through membranes (0.22 µm, pore diameter), and kept at -80ºC until use. Internal controls (ICs) and culture media without bacterial inoculation, were obtained under the same conditions.

The cytotoxicity of ECPs on european sea bass brain (DLB-1), hepatocellular carcinoma of *Poeciliopsis lucida* (PLHC-1), *Fundulus heteroclitus* brain (FuB-1) and fibroblast cell line of *gilthead seabream* (SAF-1), cells was verified by exposing the cells to different doses of ECPs. After exposure, cell viability was determined using the MTT assay. Then, using the agar-well diffusion assay, the ECPs were screened for nutritional (protease, collagenase, lipase and amylase) and

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anti-nutritional (phytase, tannase and cellulose) activities. As virulence factors, hemolytic and DNAse activities was also determined. The antibacterial activities of the ECPs against pathogenic bacterial strains *Aeromonas hydrophila*, *Vibrio anguillarum* and *Tenacibaculum maritimum* were also evaluated. In all cases, 50 µL of ECPs was inoculated into 6 mm-diameter wells made in the plates and incubated at 23ºC for 24-48h. Plates were observed in the presence of a clear zone around the wells. Finally, the effect by ECPs on biofilm formation of *A. hydrophila, V. anguillarum* and *T. maritimum* was evaluated according crystal violet (CV) technique.

Results and discussion

Only four ECP conditions were not cytotoxic at the protein concentration tested in any case against the different fish cell lines assayed, T2324, FM2324, FM1548 and M2324. In addition, three of these four ECP conditions are included in the best six that are capable of hydrolyzing more than three nutritional compounds (casein, lipids and gelatin), but none of the ECP conditions hydrolyze antinutritional compounds in any case. Although no antibacterial activity wasobserved on ECPs, the biofilms of *V. anguillarum* and *T. maritimum* were significantly reduced by several conditions, especially those from FM media (FM2324 and FM1548).

The results obtained demonstrate the influence of culture conditions on the production of postbiotics, which has been widely reported (Aggarwal et al., 2022), suggesting that the activity, quantity and type of these derived products are mainly related to the type of bacterial strain and culture medium. Because of the wide target points of postbiotics, their application could be very extensive for use in many industries, such as food, healthcare products, cosmetics, and nutraceuticals (Sudhakaran et al., 2022). In terms of the aquaculture industry, optimized growth conditions can allow us to obtain promising postbiotics, which may be of interest for future *in vivo* experiments as supplemental additives for improving fish health.

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PROTEOMIC PROFILE OF *Shewanella putrefaciens* PDP11 ON DIVERSE CULTURE CONDITIONS SUPLEMENTED WITH ALGAE MIX

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Introduction

Probiotics have been established as valuable tools for improving fish health and environmental quality in aquaculture such as *Shewanella putrefaciens* Pdp11 (SpPdp11) [1], which was extensively studied by our group. These probiotics have demonstrated important capacities for farmed fish species such as *Solea senegalensis* and *Sparus aurata* [2]. Among various natural dietary supplements, microalgae represent an effective and natural source [3]. We focused on four important and well-characterized microalgal species: *Chlorella*, *Spirulina*, *Nannochloropsis*, and *Isochrysis*. These species are commonly used in fish diets and are known to contribute to improved growth performance (in terms of size and weight gain), as well as immune system stimulation in fish.

The objective of this study was to evaluate the proteomic profile of potential postbiotics derived from extracellular products (ECPs) obtained from the bacterial strain SpPdp11 under different cultivation conditions of temperature (15 or 23°) and time (24 or 48h). For this, we formulated four different culture media: commercial media (T); aquafeed media (F); aquafeed media supplemented with a 25 % of blend of microalgae (FM); and a mixture of 25% of blend of microalgae (M). Based on their pathogenic and hydrolytic activities FM2324, FM1548 and M2324 were selected for proteomic analysis.

Materials and Methods

Each ECP condition was analyzed in the Proteomics Unit of the Central Research Support Services (SCAI) at University of Malaga. These samples were analyzed in triplicates, as the control condition (T2324) through tandem mass spectromety using a quadrupole-orbitrap nano HPLC-ESI-MS/MS system. Mass spectrometry is based on the differential behavior of ionized molecules passing through the electric and magnetic fields. This technique allows ion separation based on the mass/charge ratio (m/z), which is subsequently detected. Mass spectrometry (MS)-based proteomics is the technology of choice for large-scale protein identification and quantification, interactions and post-translational modifications. The MS/MS2 spectra were obtained from the Uniprot protein database of *Shewanella baltica* BA175. Raw data were analyzed usingthe Proteome Discoverer 2.2 platform (Thermo Fisher Scientific) with the Sequest HT search using mass tolerances of 10 ppm and 0.02 Da for the precursor and fragment ions, respectively. Oxidation was established a as variable modification of methionine and N-terminal acetylation, whereas carbamidomethylation of cysteine residues wasestablished a fixed modification. The false positive rate (FDR) for consecutive peptide and protein assignments was determined using the Percolator software package, based on a target-decoy approach that uses an inverted protein database a decoy with a strict limit of 1% FDR. The results were filtered to accept only those proteins master (highest ranking protein of a group) with at least two peptide sequences. The protein COG annotation and interactions were developed by STRING database.

Results and Discussion

The comparative proteomic analysis revealed the frequency of proteins for each selected ECP condition (FM2324, FM1548 and M2324) compared the control (T2324), expressed as the log2 abundance ratio. At the same time, the principal coordinate analysis (PCoA) scores showed a higher similarity related to the temperature and growth phase, at 23 ºC and 24 h, than the media FM or M. Based on log abundance ratio with threshold >=6, Venn diagram shows only three common proteins between FM1548 and M2324, followed by FM2324 and M2324 with 16 common proteins. The FM2324 and FM1548 share 7 proteins transporter and structural proteins, such as ABC-type transporter, DNA-binding protein, efflux transporter or proton channel, among others. Although M2324 also overexpressed these type of proteins, other interesting proteins related to bacterial tolerance and survival were present. Similarly, we observed proteins that were unique to each ECP condition.

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The interception among different culture conditions were analysed in order to deepen in proteomic composition and their variations. For score interaction value > 0.74, the FM2324 and FM1548 condition included two different relevant interaction among COG2804 and COG2165, COG4974 and COG0583. The COG2804 and COG2165 has been implicated in type II secretory system or pilus assembly pathway ATPase. By other hand, COG4974 and COG0583 were associated to site-specific recombinase XerD and DNA-binding transcriptional regulator (LysR family), respectively. The transcriptional regulation protein binding to specific DNA region were repressor or inductor of genes expression [4]. The XerD was a tyrosine recombinase which change the dimers to monomers in the end region of chromosome [5]. In the same score threshold, FM2324 and M2324 included the COG2863, COG2010 and COG0526 proteins with close interaction between them and which function was cytochrome c553, cytochrome c, mono- and diheme variants and thiol-disulfide isomerase or thioredoxin. The higher value of cytochrome protein on FM was release to increase the oxidative activity [6].

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HEALTH STATUS OF MUSSELS AND OYSTERS FROM TWO AQUACULTURE SITES IN THE EASTERN ADRIATIC, CROATIA: RELATION TO NATURAL AND ANTHROPOGENIC STRESSORS

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Introduction

Preservation of marine environment quality is of paramount importance for bivalve aquaculture. When it comes to the eastern Adriatic coast, recent drastic intensification of human activities, mainly urbanisation, touristification and marine transport, have raised concerns on intensification of anthropogenic pollutants' input. Concomitantly, seawater surface temperature increase related to global warming has been considered as the most influential environmental stressor negatively affecting the health of bivalves. Consequently, negative impact of both anthropogenic and natural stressors on farmed bivalves and on seafood production success have been anticipated. Thus, the aim of the present study was to evaluate the performance of Mediterranean mussel *Mytilus galloprovincialis* and European flat oyster *Ostrea edulis* from two eastern Adriatic bivalve aquaculture sites. A set of well-established early warning biochemical biomarkers of sub-lethal stress were analysed, taking into account the spatio -temporal variations of seawater temperature, reproductive status and body burden of potentially toxic chemical pollutants.

Materials and methods

This work was carried out in two distinct and geographically distant ecosystems of the Eastern Adriatic, namely, Lim Bay (LB) and Mali Ston Bay (MSB), long been known for bivalve and fish farming, and both under legal protection as protected marine reserve. Sampling was carried out bimonthly from June 2020 to May 2021. Condition index (CI) of mussels and oysters as well as biochemical biomarkers of stress, namely, activities of acetylcholinesterase (AChE; neurotoxicity indicator) and glutathion *S*-transferase (GST; oxidative stress challenge indicator) in the gills, concentration of thiobarbituric acid reactive substances in the gills (TBARS; lipid membrane peroxidation indicator) and concentration of metallothioneins in the digestive gland (MTs; metal detoxification proteins) were determined by widely applied protocols (Davenport and Chen, 1987; Perić and Burić, 2019). Biomarker data were interpreted taking into account the seawater temperature, CI and concentrations of chemical pollutants (metals, PAHs) in the tissues. The latter were determined in accordance to previously reported protocols (Perić et al, 2017).

Results

Season dependent variations of all measured biochemical parameters were observed, but a link to temperature oscillations was not detected. While AChE activity annual trend was inconsistent, seasonality of GST activity was found for both species. Significant difference between sites occurred only sporadically for AChE activity, but almost regularly for GST activity of both species, albeit displaying an inconclusive pattern. Significantly higher MTs and TBARS concentrations were recorded in MSB oysters and mussels, respectively, for almost the whole sampling period, but seasonality of these parameters was less evident. For mussels, significant positive correlation was found between MTs and in particular TBARS concentration to the burden of potentially toxic metals (Cd, As, Hg) in the tissues (r=0.58-0.86). For oysters, association was detected between MTs concentration and As tissue concentration (r=0.63), while TBARS concentration was found to significantly correlate with condition index (r=0.82).

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Discussion and Conclusion

This study presents the first data on variability of biochemical performance of farmed bivalves across seasons and sampling sites as well as on susceptibility to different stressors within local farming environment. Mussel and oyster seem to be affected mostly by pro-oxidant conditions related to higher bioavailability of toxic metals and reproductive effort, respectively. The former may be more pronounced in MSB mussels, considering a consistently higher TBARS levels with respect to their LB counterparts. In conclusion, data reported here represent a valuable baseline information for future studies related to biochemical stress in farmed bivalves posed by natural and anthropogenic pressure along the coastline. Despite the lack of association with biochemical parameters measured in this study, temperature remains an important factor affecting the performance of bivalves in aquaculture due to its tight link to reproductive cycle dynamics, and in particular in the light of steady seawater warming trend recorded in the Adriatic over the last decades. Finally, the present study illustrates the utility of biomarker approach for assessment of farming habitats' quality crucial for sustainability of eastern Adriatic bivalve aquaculture.

Acknowledgements

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SUSTAINABILITY ASSESSMENT OF A MULTI CIRCULAR MODEL CONSIDERING POLYCHAETES AS AN EFFECTIVE FISHMEAL ALTERNATIVE FOR AQUACULTURE AND EXPLOITATION OF AQUACULTURE SIDESTREAMS TO OBTAIN VALUABLE PRODUCTS SUCH AS ASTAXANTHIN, VIA BIOCONVERSION PROCESS

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Introduction

As the aquaculture sector continues to expand rapidly, there is a growing emphasis on the implementation of sustainable methods in fishmeal preparation and waste management practices. In recent years, the pursuit of sustainable aquaculture practices has focused on alternatives to fisheries-derived ingredients, in order to reduce economic and environmental burdens. Low-trophic organisms, such as polychaetes (that may be fed on aquaculture side streams), could be a suitable partial alternative to fishmeal due to their high protein content. Furthermore, aquaculture side streams may be better exploited for enabling bioconversion processes yielding valuable bio-based chemicals, including astaxanthin as pigment with antioxidant properties, which is widely used in various industries, including food and pharmaceuticals. The demand for astaxanthin has led to the development of multiple production methods, including algal, bacterial, and synthetic approaches. In this work, we perform the environmental sustainability assessment of a multi-circular model. The analysis includes polychaete-based fishmeal (PM) production and seabass feeding trials compared with conventional best fish meal (FM) formulations, and a comparative analysis between two alternative bio-conversion methods to obtain astaxanthin: the bacterial bio-conversion process employing aquaculture side streams and the algal bio-conversion process. Both polychaetes-based diets and side streams fed bacteria bio-conversion process prove to be more sustainable alternatives with respect to current solutions relying on linear economy model, thus enabling a multi-circular value chain.

Materials, methods and results.

To address the sustainability issues associated with fishmeal, various alternatives have been investigated. One of these alternatives is polychaete meals (PM) that offer a well-balanced nutritional profile, high protein content, and omega-3 fatty acids.

In order to assess the environmental sustainability of using polychaete meal (PM) as a substitute for fishmeal (FM) in diets for European seabass, the Life Cycle Assessment (LCA) analysis has been performed for the production of 50kg of FM, considering four experimental diets, which were formulated with varying levels of PM, replacing 0%, 10%, 20%, and 40% of standard fishmeal, referred to as FM, PM2.5, PM5, PM10, respectively.
Environmental impacts have been normalised based on the result of the growth trials, which have been conducted by CIIMAR [1]. The assessment was performed referred to the diet functional key performance indicator represented by the Protein Efficiency Ratio defined as: (PER) = (W_f − Wi) / (total protein intake (g)), where W_i and W_f are the initial and final weights.

According to the trendline in Figure 1, which represents the average impacts per FU as a function of the PM rate (PM=0, corresponds to pure FM) indicates that an increasing inclusion of PM dramatically reduces the diet environmental impact profile. The feed trials through the addressed KPI prove that proposed PM diets are technically feasible, thus reinforcing the sustainability claim of the PM circular solution.

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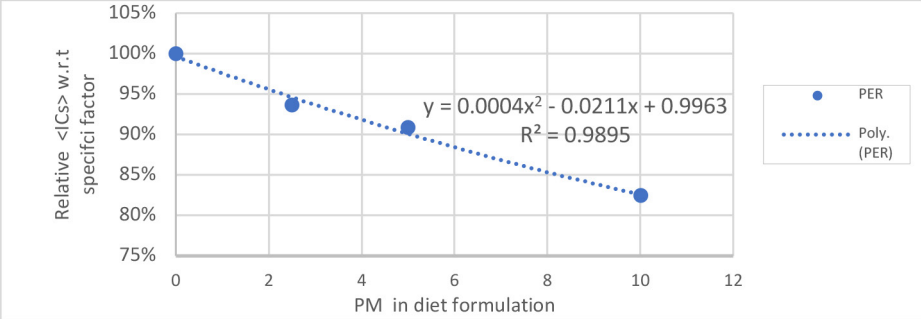

Figure-1: TU vs. FU of 4 feed formulations improvements trends

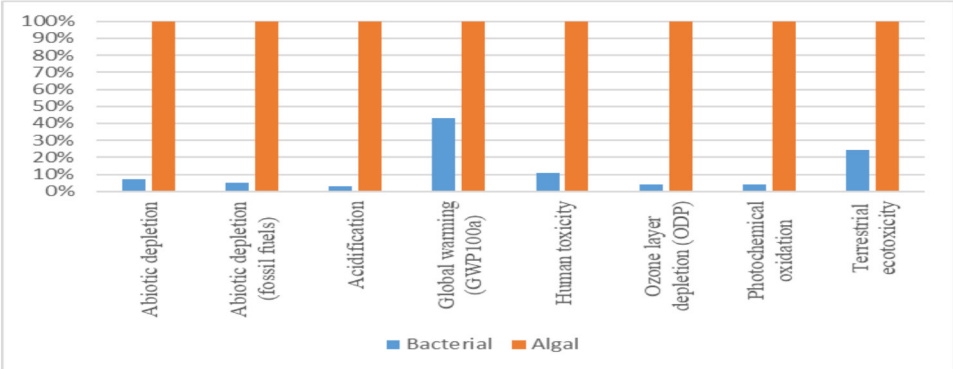

Figure-2: Comparative assessment between the two bacteria and algae-based bioconversion processes to produce astaxanthin.

Considering the significant increase in the consumption of aquatic foods, it is imperative, from environmental perspectives, to efficiently utilize by-products generated in the aquaculture sectors. Starting from this principle Schmitt [2] suggested an innovative bacterial astaxanthin production process by C.glutamicum employing aquaculture side streams. Our dedicated LCA provided the comparative assessment between the two main sources for producing natural astaxanthin: bacteria and algae bioconversion processes. Figure 2 shows that the bacterial astaxanthin production using renewable energy and aquaculture side stream looks to be a promising solution to enable multi-circular blue-economy schemes. This approach shows a dramatic decrease of environmental impacts on all represented categories compared to the algal production method, with the exception of the aquatic toxicity and eutrophication potentials, in which algae solution display negative values. Furthermore, this circularity framework for producing astaxanthin incorporates aquaculture side streams as a sustainable nutrient source, therefore the burden of waste management and its associated environmental consequences can be avoided, while supporting the astaxanthin production process within the circularity framework also may turns costs into economic exploitable valuable.

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HEAT-PRIMING AS AN EFFECTIVE TOOL TO IMPROVE TOLERANCE TO MARINE HEATWAVES IN THE MANILA CLAM

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Introduction:

Bivalve aquaculture is currently considered among the most suitable activities to meet the ever-increasing protein demand of our population. Bivalves are not only a protein source, but provide also important ecosystem services. However, bivalve aquaculture is threatened by climate extreme events such as Heat Waves (HWs). In fact, it is predicted that by 2090 suitable areas for mollusc aquaculture will decrease globally by ~10%. These extreme events are globally increasing their frequency, intensity and duration and may result in massive mortality of benthic organisms. However, we recently showed how HWs may also trigger sublethal consequences in a bivalve mollusc, such as the onset of dysbiosis in the digestive gland microbiota, a significant reduction in energy reserves, impairment of the reproduction.

In shellfish farming the scope for contrasting the negative impact of HWs is limited, however a possible solution may lie in heat-priming, a process by which a plastic response of the phenotype is triggered (following sublethal stress) to mitigate the impact of a subsequent lethal stress. Our aim was to test the efficacy of heat-priming to improve the tolerance of Manila clam to HW.

Material and Methods:

To test the efficacy of thermal priming, we obtained a hatchery-produced population of Manila clam, *Ruditapes philippinarum,* from SATMAR, one of the biggest hatcheries in Europe, and acclimated them to summer conditions (25 ˚C) in the lab for 2 weeks. Then, half population was subjected to thermal priming ("P") by exposing animals for 7 days to a constant temperature of 30 ˚C, while the remaining half was kept at 25 ˚C as non-primed control ("NP"). After this treatment, all animals were let recover for 2 weeks at 25 ˚C. Finally, each group (P and NP) was divided in two sub-groups: the first half was subject to HW conditions (i.e. daily thermal stress from 31 ˚C to 35 ˚C; sub-groups "P/HW"; "NP/HW") while the remaining animals were kept at 25 ˚C as controls (sub-groups "P/noHW"; "NP/noHW").

A large array of traits was recorded during the HW (and over the following 15 days). Burying behavior (percentage of burying animals, speed of burying) was tested and recorded at the end of heat-priming and after the HW. Physiological parameters (e.g. condition index, hepato-somatic index, enzymatic activity of SOD, GPx and Catalase, amount of lipid peroxidation) and functional genomic profiles (gene expression via RNA-seq and chromatin accessibility via ATAC-seq) were assessed 15 days after the exposure to HW, in order to reveal the effects of heat-priming on the physiology of the species. Mortality was recorded from all groups.

Results:

Overall, we observed a significant increase in the survival rate of "P/HW" clams in comparison to "NP/HW" clams. Further, our results indicated that, following priming, while the proportion of clams that successfully buried was equal among "P" and "NP" groups, "P" clams took longer to bury in contrast with "NP" clams. On the other hand, following HW, a significantly higher number of "P/HW" clams buried in contrast with "NP/HW" clams, suggesting that priming had an effect of the behavior of animals. Finally, condition index and hepato-somatic index were both decreased in "P/HW" clams compared to "NP/HW" clams, suggesting that priming is energetically demanding. However, the higher energetic cost imposed by priming is counterbalanced by the higher survival rate of primed animals following a lethal stress, suggesting that priming may be an effective tool to increase resistance of clams to extreme heat events.

PRODUCTION OF CAROTENOIDS FROM AQUACULTURE SIDESTREAMS AS FEEDSTOCK USING THE BACTERIUM *Corynebacterium glutamicum*

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Introduction

Carotenoids are valuable secondary metabolites with important physiological functions as for instance being precursors in vitamin biosynthesis and high antioxidant potential. Especially astaxanthin is a high value fish feed supplement, already used in salmonid farming, mainly in chemical but also in natural form. Aquaculture is one of the fastest-growing food production systems, enabling to meet the rising consumer demand for healthy seafood, thus the expansion of the demand for natural astaxanthin alternatives is expected. To establish a flexible feedstock concept using aquaculture sidestreams (Wendisch *et al*. 2022; Fig. 1), the GRAS bacterium *Corynebacterium glutamicum* that naturally synthesizes the yellow C50 carotenoid decaprenoxanthin was metabolically engineered to produce a variety of different carotenoids including astaxanthin (Cankar et al. 2023; Henke et al. 2022). Preprocessed aquaculture sidestream (AQ) from a Norwegian aquaculture facility was tested for growth and production of these carotenoids. In a step towards a circular economy in aquaculture, the applicability of astaxanthin produced by *C. glutamicum* was demonstrated in a first trout feeding trial (Zeytin et al. 2022).

Material and Methods

The construction of metabolically engineered *C. glutamicum* was described elsewhere (Cankar et al. 2023; Henke et al. 2022). The aquaculture sidestream was collected from the sump of a post-smolt RAS for salmon operated by Lumarine AS (Tjeldbergodden, Norway) outside of Trondheim (Norway). Preprocessing of the liquid aquaculture sidestream in order to use it as a growth medium component was implemented by centrifugation and subsequent sterile filtration of the supernatant. The resulting AQ was supplemented in different amounts to a defined minimal salt medium and used in growth experiments performed with the different *C. glutamicum* strains at 30°C and 180 rpm in shake flasks. Fermentation of the astaxanthin producing *C. glutamicum* strain was performed in either 2 L glass or 19 L steel bioreactors using the same medium.

Preparation of astaxanthin containing biomass for the trout feed trial was performed as shown in Fig. 2.

Results and Discussion

Astaxanthin production by an engineered *C. glutamicum* producer strain was notably enhanced by addition of 20 % AQ in a mineral salts medium (Schmitt *et al.* 2023). The use of AQ as medium supplement was shown to be transferable to production of ß-carotene, lycopene as well as zeaxanthin, canthaxanthin, sarcinaxanthin, BABR and C.P.450. Astaxanthin production was scaled up to the 2 L bioreactors. Fermentation of the astaxanthin producing *C. glutamicum* strain yielded 5.3 g biomass, containing 1 g natural glycosylated astaxanthin. This biomass was formulated into fish feed and this feed was compared in a trout feed trial to commercially available synthetic and natural astaxanthin sources resulting in a very promising pigmentation although lower growth of the trouts.

Here we could show for the first time a fully circular bioeconomy concept in aquaculture.

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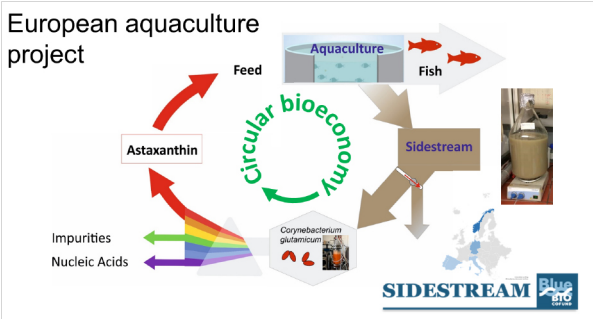

Figure 1. Example of a circular economy in aquaculture using Astaxanthin producing bacterial biomass which is cultivated on preprocessed aquaculture sidestream (AQ).

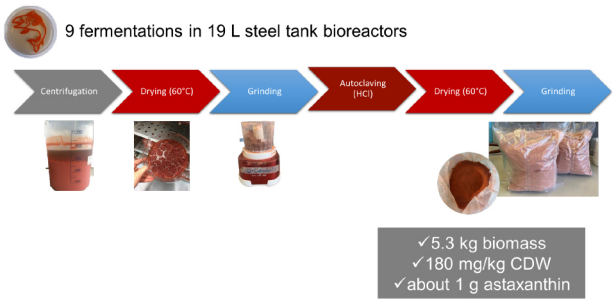

Figure 2. Example of a circular economy in aquaculture using Astaxanthin producing bacterial biomass which is cultivated on preprocessed aquaculture sidestream (AQ).

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THE EFFECT OF LIGHT COLOUR AND INTENSITY ON THE STRESS OF THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei* IN VIDEO TRACKING EXPERIMENTAL DESIGN

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Introduction

Litopenaeus vannamei is one of the most bred crustaceans worldwide. In farms feed is, by far, the main expenditure. Thus, the objective of feed and premix manufacturers is to find affordable but attractive formula. Currently the methods for measuring the attractivity of a feed are poorly precise and reproductible. One option is to conceive a device and a procedure that will be able to track shrimp during a feeding process and evaluate their behaviour in relation with pellet attractivity. Light is an important environmental factor for animals living in water. It has significant effects on behaviour, feed intake and growth of aquatic animals (Giri et al., 2002). Light can also be a source of stress that can bias behavioural experiments. Thus, to determine which colour and intensity of light cause less stress for this species, three experiments were conducted. The first experiment aimed to find the less stressful colour. The second one aimed to find the less stressful light intensity. The last experiment aimed to determine which colours *L. vannamei* is able to perceive.

General protocol

For these three experiments, *L. vannamei* shrimp were purchased from a local Bulgarian farm and brought to the station Halieutica (France) during a 24h journey.

Experiment 1 – colour preferences. Protocol

Thirty PL20 *L. vannamei* shrimp were acclimatised for four weeks. After acclimatisation, six waiting tanks of 10 L with five shrimp were prepared.

Shrimp were tested in a random order in a closed structure and were enlightened and filmed once with each following colour: red, orange, yellow, green, blue, or purple for 15 min. Following Takahashi (2021), four motion behaviours were recorded to assess stress: swimming, freezing, time spent along the walls and time spent in the centre of the tank. In addition, the number of loops, tail flips, and eye beats were counted, as they are considered as stress markers in shrimp (Bardera et al., 2019; Takahashi, 2021)however to date there has been limited focus in this area. The Pacific white shrimp (Litopenaeus vannamei. Median speed was also analysed. We hypothesise that non-stressed shrimp will slowly explore their environment, leading to a low speed.

Experiment 1 – colour preferences. Results

Median speed and the number of loops and tail flips were significantly lower for red and orange light ($p<0.05$) suggesting that these colours are less stressful. We decided to choose orange for Experiment 2.

Experiment 2 – light intensity preferences. Protocol

Thirty PL20 *L. vannamei* shrimp were acclimatised for twelve weeks. Procedure and tested behaviour were the same as in Experiment 1.

For this experiment, shrimp were enlightened and filmed once with every following orange light intensity: 17, 64, 170 or 430 Lux for 15 min.

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Experiment 2 – light intensity preferences. Results

No effect of the light intensity was shown on any stress markers, meaning that orange is not stressful for shrimp, whatever the light intensity. One possible explanation is that shrimp do not perceive this colour. Thus, we studied *L. vannamei*'s colour vision in a third experiment.

Experiment 3 – *L. vannamei*'s colour vision. Protocol

Ten PL20 *L. vannamei* shrimp were acclimatised for twenty-two weeks.
Shrimp were individually tested in an optomotor device. This device consists of a rotating cylinder (6 rpm), which inside walls were covered with a filter made of vertical stripes (6,28 cm) of grey, and one of the following colours: red, orange, yellow, green, blue, purple, and white (positive control). We also used a negative control (uniform grey). The shrimp was placed on a still platform in the middle of the cylinder. Each shrimp was tested once for each colour. The device turned 1min clockwise, and 1min counter clockwise, the percentage of time the shrimp spent turning in the same direction and at the same speed as the device was measured, this is called the optomotor response. We considered that the shrimp perceived the colour (and hence discriminated the stripes) when the percentages of rotation in the same direction as the cylinder was at least superior to 50%.

Experiment 3 – *L. vannamei*'s colour vision. Results

Shrimp showed an optomotor response to the green, blue, purple striped filters and to the positive control. Thus, *L. vannamei* might perceive green, blue, and purple, and not red, orange, and yellow.

Discussion

This study underlines that the less stressful colours for *L. vannamei* are red and orange, probably because they cannot perceive these colours. These results are in accordance with other studies held in crustaceans (crabs and crayfish mainly) (Goldsmith and Fernandez, 1968; Marshall et al., 2003)Palaemonetes paludosus (Everglades prawn. High intensity orange light can thus be used in a tracking device, as it will not stress shrimp and allow a good recognition of the animals by the tracking software. More generally, these colour and light intensity can be used for any experiment led in this shrimp species.

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EFFECTS OF ACUTE AND CHRONIC STRESS ON CARP

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Introduction

Different stressors can have diverse effects on fish. For fish in aquaculture, acute as well as chronic stressors are relevant. Distress interferes with the appetite and well-being of an animal, while eustress, e.g. a feeding event, also leads to increased locomotory activity and should be categorized as a stressor as well. It is assumed that fish are capable of distinguishing between different stressors to ensure that they react appropriately to their stressful environment. In our studies, the acute and chronic effects of stress on common carp (*Cyprinus carpio*) were investigated.

Material and methods

For the experiment investigating responses to acute stress, the fish were treated with air exposure, chasing or confinement for 1 min, were rewarded with a feed or left unfed for comparison. Control groups were reared in parallel and left untreated during the entire experiment. For the chronic experiment, the fish were treated with one 6 different stressors every day for 7 and 28 days in order to avoid habituation of the fish to the stressors, and were challenged on the sampling days with air exposure as an additional stressor. Control groups were reared in parallel and left untreated during the entire experiment or challenged with air exposure only on the sampling days. Physiological parameters were measured in each fish and gene expression and neurotransmitter analyses were used to analyze stress responses at the brain level.

Results and discussion

The mRNA expression patterns of genes belonging to the stress axis revealed that negative stress caused by exposure to air had broad-ranging effects on the gene regulation patterns in the fish brain, even if the fish were only treated for 1 min. This parallels the effects that have been observed on blood cortisol and glucose. In contrast, a limited number of genes allows discrimination of eustress and distress which indicates that further research is needed in the future. Furthermore, it is known that a complex brain network including different components of the stress axis interacts with mechanisms involved in the regulation of appetite and feed intake in fish. Our study indicated that social isolation, feed rewarding and distress are perceived differently in carp and differently affect the appetite regulation at the brain level. Finally, the chronic exposure to stress affected among other parameters the brain neurotransmitter levels and skin coloration of the fish.

Acknowledgement

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A NOVEL APPROACH TO ESTIMATE WATER QUALITY OF INLAND AQUACULTURE PONDS USING SATELLITE DATA AND WQI MODEL

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Assessment of the water quality in aquaculture ponds on land is essential for maintaining the wellbeing and productivity of aquatic ecosystems. A popular tool for thoroughly assessing the quality of surface water is the Water Quality Index (WQI) model. In order to assess water quality parameters in inland aquaculture ponds, this work introduces a novel method that integrates satellite data and the WQI model, improving the accuracy and effectiveness of water quality monitoring. The proposed approach integrates multispectral satellite imagery and machine learning techniques to predict key water quality parameters. The WQI model is used concurrently to combine various parameter predictions into a single index, offering a comprehensive evaluation of water quality. This hybrid methodology provides a solid framework for overcoming classic WQI models' drawbacks, such as their region-specific applicability.

An extensive dataset of in-situ water quality measurements gathered from several aquaculture ponds is used to validate the methodology. Strong relationships between the predicted models for the individual parameters and the field measurements are shown. A machine learning model was explored to predict the water quality parameters and the outcomes are measured against ground truth values. This validation emphasises how accurate the suggested method is for calculating and assessing water quality conditions. In conclusion, the WQI model and the integration of satellite data offer an innovative approach for determining and assessing the water quality in inland aquaculture ponds. This methodology creates new opportunities for resource management, environmental preservation, and sustainable aquaculture practices by overcoming the constraints of conventional methods. The hybrid approach provided in this study has potential for redefining water quality evaluation across multiple water bodies and geographical contexts as remote sensing and modelling techniques continue to progress.
Keywords: Satellite data, WQI, Shrimp farming

EFFECT OF THE USE OF FISH PROTEIN ISOLATED FROM SIDE STREAMS ON THE QUALITY AND STORAGE STABILITY OF AMBERJACK *(Seriola dumerili)* FISH BALLS

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Introduction

The amberjack (*Seriola dumerili*) is an important commercial fish species in Europe. The farming of the amberjack has attracted great interest in the Mediterranean region and is now considered one of the most important species for the diversification of commercial fish production in Mediterranean countries (Corriero et al., 2021). In the fish processing sector, the further utilization of side streams and waste left after industrial production can contribute to the sustainability of raw materials, access to added value and higher profitability, and environmental protection by reducing the amount of by-products (Šilovs, 2018). Although fish by-products have a high potential for reuse and recycling, they are not yet sufficiently recovered to obtain high value-added products (Galanakis et al., 2015). In addition, fish by-products are an excellent source of bioactive compounds such as amino acids, proteins, peptides, enzymes, gelatin, collagen, long-chain omega-3 polyunsaturated fatty acids, chitin, and vitamins (Vázquez et al., 2019) which can be used both as raw materials and as functional ingredients for the production of value-added products (Suresh et al., 2018). The demand in the global market is shifting toward higher value-added processed seafood that features convenience, ease of preparation, and high nutritional value, and that can also satisfy the sensory-hedonic aspect. Fish protein hydrolysates (FPH) have great potential for use in food formulations because they possess several functional properties, including solubility, water-holding capacity, emulsifying, and foam-forming ability (Kristinsson and Rasco, 2000). Processing can strongly influence the nutritional value of fish products, with fatty acids being the most vulnerable nutrients from fish , as they can easily be subjected to oxidation reactions resulting in quality degradation. For the development of added value, ready-to-cook fish products , it is therefore important to evaluate modifications possibly occurring during processing and storage, and to carefully check whether innovative processing affects the quality and nutritional value of the final product. The aim of the present study was to evaluate the effect of the addition of a protein hydrolysate from fish side streams on the quality and stability of fish balls made from mechanically separated amberjack meat during cold storage.

Materials and methods

In this study, the minced meat of amberjack was obtained by mechanical separation from the by-products of fish filleting. Fish balls were prepared with amberjack meat and fish protein hydrolysate (FPH) was added to the base formulation at a concentration of 1.5% by replacing the same amount of amberjack meat; all other ingredients remained the same. The conventional product (control) was prepared using the basic formulation without fish protein hydrolysate. The fish balls were packed in polypropylene trays (PP) sealed with a high barrier film and packed in a modified atmosphere (MA)

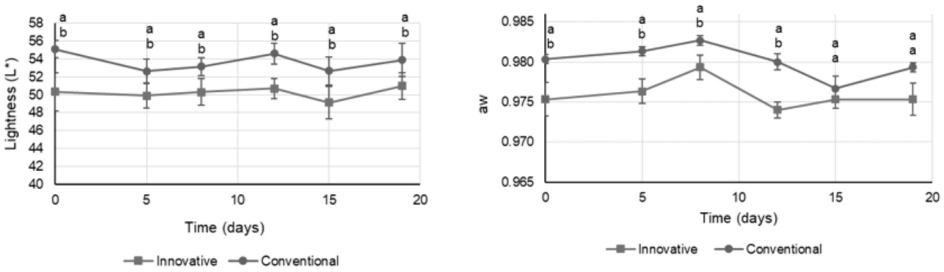

Fig. 1 Changes in Lightness (L*) and a_w values of conventional and innovative fish balls during storage at 4°C. Different letters indicate significant differences between samples at the same storage time ($P < 0.05$).

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with a gas mixture of 80% N2 and 20% CO2. After packaging, the control and the innovative products were stored at 4°C for a total of 20 days. Analytical determinations were carried out on the two groups of samples, the conventional product (control) and the innovative product, by taking samples for physicochemical analysis and microbiological analysis during storage. Experimental data were subjected to one-way analysis of variance (ANOVA) to determine the significant differences between samples. Tukey HSD (Honestly Significant Difference) multiple range test, with a significance level of $p < 0.05$ was applied.

Results

The results of this study showed that the presence of protein hydrolysate in fish balls significantly improved the microbiological shelf-life of the product (less than 12 days instead of less than 8 days). Moreover, the FPH delayed the accumulation of histamine by keeping its concentration below the EU limits throughout the shelf-life (12 days), contrarily to the conventional product where the limit was already exceeded after 8 days. During the shelf life, the innovative fish balls showed lower water activity than the conventional fish balls until the 12th day of storage (Fig. 1); this may have contributed to improved microbiological stability of the samples. Lightness (L*) was lower in the innovative samples than in the conventional samples, but during the storage period, L* values decreased in the conventional samples, while they remained almost constant in the innovative samples (Fig.1). The addition of 1.5% of protein hydrolysate showed no effect in counteracting the phenomenon of lipid oxidation in the fish balls. The amberjack fish balls produced in this study can be considered as an interesting new fish product obtained through amberjack by-product (fish components remaining after filleting) valorization. At the same time, to extend fish balls shelf-life and make them safer, more stringent handling procedures must be implemented during by-products production since the starting material presented a high microbial load.

Acknowledgements

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THE USE OF DIFFERENT NITRATE SOURCES FOR GROWTH OF THE HALOPHYTE *Plantago coronopus* IN A DECOUPLED AQUAPONICS SYSTEM

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Introduction

Aquaponics is an innovative and sustainable production alternative. It combines aquaculture with hydroponics to use water and nutrients to promote the growth of aquatic organisms and plants in an integrated manner. The application of biofloc technology in aquaponic systems is relatively new. Recent studies demonstrate that, when compared to recirculating aquaculture systems (RAS), BFT can increase the plant and fish/shrimp yields and also improve the use of nutrients (e.g. nitrogen) in the culture system.

However, to apply this system to marine culture, it is necessary to use plants that have commercial value and are salinity tolerant so that they can develop in saline effluents. The species *Plantago coronopus* (known as Hirschhornwegerich) is a halophyte native to Europe that it grows in sand dunes, saline grasslands, and salt marshes. It is an edible plant with nutraceutical and antioxidant properties, and it has potential for a cash crop. In this context, this study aimed to assess the growth of *P. coronopus* when irrigated with three different nitrate sources: commercial fertilizer and water from marine shrimp culture (*Penaeus vannamei*) in RAS and BFT systems.

Material and methods

The study was conducted at the Center for Aquaculture Research from the Alfred Wegener Institute in Bremerhaven, Germany. The experiment was established in a tent inside of a glasshouse, with ventilation and air conditioning to keep the temperature in the tent around 20ºC.

The experimental units were based on the deep water system, where the roots are constantly in contact with the water. They consisted of nine plastic boxes with lids and a useful volume of 10 L (40 x 30 x 12 cm, 0.12 m² of useful area), which were distributed on a 3-floor shelf (three boxes per floor). To evaluate nitrate sources (fertilizer, RAS, and BFT), three replicates per treatment were randomly assigned to each floor. The light intensity was 180 µmols photons.$m^{-2}.s^{-1}$, and the photoperiod was adjusted to 12h during the 4-week trial.

Table 1: Water quality variables of the different nitrate sources used to grow *Plantago coronopus* during four weeks. Mean ± standard deviation. Different letters in the same line indicate a significant difference by Tukey post-hoc test.

Parameter	Treatment		
	BFT	RAS	Fertilizer
Salinity (ppt)	25.7 ± 2.5	25.5 ± 3.2	23.5 ± 3.1
NO_3-N (mg.L^{-1})	44.6 ± 7.9[c]	26.1 ± 7.5[b]	17.4 ± 6.1[a]
pH	7.92 ± 0.03[b]	8.08 ± 0.04[c]	7.79 ± 0.08[a]

Variables	Treatment			*p*-value
	BFT	RAS	Fertilizer	
Shoot weight (g)	3.2 ± 2.0	3.9 ± 1.8	4.6 ± 2.7	0.248
Shoot length (cm)	9.8 ± 1.7	10.7 ± 1.7	11.0 ± 1.9	0.124
Root length (cm)	17.7 ± 5.1	19.2 ± 3.7	19.4 ± 5.7	0.551
Shoot biomass gain (g)	2.5 ± 2.0	3.4 ± 1.6	3.9 ± 1.7	0.216
Yield (g/m²)	92.4 ± 56.9	111.4 ± 52.0	130.8 ± 78.1	0.248

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The day before the experiment, the boxes were filled with water and 10 mL of garden fertilizer was added to 10 L of saline water for the Fertilizer treatment. In the RAS and BFT treatments, 10 L of water from each shrimp culture system was used, from the biofilter of a recirculation system and a biofloc tank, respectively. Once a week, half of the volume was replaced with new water, and freshwater was added to correct the salinity when necessary. Salinity, pH, and nitrate concentration of the water in the boxes were monitored once a week.

The seedlings were prepared four weeks prior to the start of the experiment. Seeds of *P. coronopus* were planted in a tray of organic medium (coconut fiber, Eazyplug) and irrigated with freshwater for two weeks. Then, they were slowly acclimatized with seawater for two weeks, until reaching the desired salinity in the experiment (around 25 ppt). To start the experiment, six seedlings (initial weight with substrate = 13.9 ± 1.7 g) were placed in each box, with a density of 50 plants/m². The seedlings were inserted into plastic baskets with 4cm diameter and 5cm height, which fit into 4cm holes on the box lids. At the end of the experiment, all plants were individually weighed and measured to calculate the growth indexes.

Results and discussion

The salinity remained within the range determined for the treatment ($p > 0.05$). The pH was significantly different among treatments ($p < 0.001$). Since the water in the boxes was replenished every week, the pH remained stable, as observed in the different water sources used in the experiment. The nitrate concentration was higher in the BFT treatment, followed by RAS and Fertilizer ($p < 0.001$). The RAS used as a water source in the experiment may not have had a fully established bacterial community since nitrate concentration did not accumulate as expected (Ray et al., 2017). In the Fertilizer treatment, the dose recommended by the manufacturer was used. However, the nitrogenous fraction of the fertilizer is composed of 2% urea, 3.7% ammonia and only 2.3% nitrate. This could explain the lower concentration of nitrate in this treatment (Table 1).

Reduced plant growth is one of the first and most general responses to stress. Plant growth was the same regardless of the nitrate source used (Table 2). The constant flooding of *P. coronopus* roots with saltwater, despite their ability to tolerate salinity up to 25 ppt as a halophyte, might have affected their growth in the used system (Al Hassan et al., 2016). In addition, a shoot growth inhibition was observed in all treatments, but the plant roots expressively increased in length. Under saline conditions, plants can have a rapid root elongation. Tolerant plants exhibit a larger and more extensive root system, enabling them to access deeper layers of soil for water and nutrients (Ltaeif et al., 2021).

Table 2: Production indexes of *Plantago coronopus* cultivated during four weeks under different nitrate sources. Mean ± standard deviation. The *p*-value is from one-way ANOVA.

Conclusion

The results of this experiment suggest that, despite the low growth rate, the water from the cultivation of *Penaeus vannamei* can be used as a source of nitrate for the cultivation of *P. coronopus*, regardless of whether it comes from the RAS or the biofloc systems.

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BODY SIZE OF MALE ATLANTIC SALMON INTERACTS WITH CHANGES IN PHOTOPERIOD UNDER AQUACULTURE CONDITIONS TO INFLUENCE THE DECISION TO MATURE OR SMOLTIFY

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Introduction

Early maturation of male Atlantic salmon postsmolts represents an economic and welfare challenge for aquaculture producers, and occurs as a result of intensive manipulation of factors like temperature, photoperiod or diet among others (Good and Davidson, 2016). Light is often maintained constant to increase growth rate, but interrupted by a period of reduced daylength ("winter signal") to induce smoltification (Björnsson et al., 2011). However, this light regime has also shown to synchronize early maturation (Pino Martinez et al., 2023b). The body size attained before the winter signal could affect the decision to mature, as larger fish may have more energy reserves and be driven to initiate maturation in response to photoperiod cues (Fraser et al., 2019)we report on an observation of out-of-season male Atlantic salmon initiating puberty as pre-smolts (jacks. This study assessed if a winter signal introduced at different sizes would impact the incidence of early maturation and the development of smoltification.

Materials and methods

900 parr (41.3 ± 3.7 g) were transferred to eight freshwater tanks (112/tank) in our flow-through facilities (Bergen). Temperature was always 12.5°C. Four photoperiod treatments (Fig. 1A) were established in duplicate: constant light (LL), a winter signal (5-week LD12:12) at 70.0 ± 8.4 g (70WS), a similar winter signal at 114 ± 9.9 g (110WS), and at 182.9 ± 35.7 g (180WS). After the winter signals, light returned to constant and the groups spent 7 weeks in freshwater to complete smoltification, followed by 2 weeks in seawater (35 ppt) (Fig. 1, left). A baseline and twelve main samplings (n ≥ 4 males per tank and sampling) were performed (N total = 396 males), measuring weight and length, and collecting plasma for cortisol analysis. Testes were excised and weighed to calculate *GSI (%) = Gonad W (g) × 100/Body W (g)*, using this index to assess maturity status (Pino Martinez et al., 2023b, 2023a). Condition factor (K) was calculated as *K = Body W(g) × 100/ Length 3 (cm)*. Plasma cortisol was analyzed with a commercial ELISA kit (Demeditec Diagnostics GmbH). Differences in maturation among treatments were explored with Fisher's exact test for count data. Differences in K and cortisol between treatments and over time were assessed with Linear Mixed Models. Statistical analyses were performed in Rstudio (α = 0.05).

Results

Maturing males were only observed after some weeks in constant light following the winter signal, and their proportion was higher in 180WS (6 males of 58) than in LL (2 of 142), 110WS (1 of 100) and 70WS (0) ($p < 0.005$). Condition factor in groups 70WS and 110WS decreased after the winter signal (both $p < 0.001$), but remained stable in 180WS and LL. Plasma cortisol (Fig. 1, right) peaked in the three groups that received winter signal some weeks after returning to constant light, but this peak was more remarkable in 180WS. After 2 weeks in seawater, plasma cortisol remained stable in 70WS and 110WS, while in contrast, displayed a large increase in 180WS and LL (both $p < 0.001$).

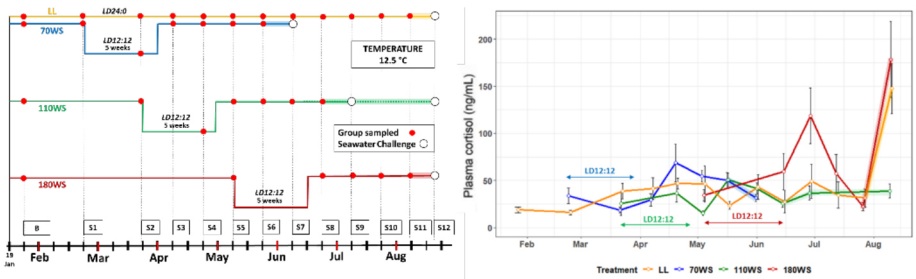

Figure 1. Experimental set up (left) and plasma cortisol in the four treatments (right). The experiment took place from 19 January to late August 2021. Red dots are samplings in freshwater, and white dots in seawater. Shadowed areas in both graphs with the same color of the treatment show the period in seawater. The double-ended arrows on the right graph show the duration of the winter signal in each treatment. Plasma cortisol is displayed as mean ± SE.

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Discussion and conclusions

Body size of male salmon at introduction of a winter signal influenced their decision to undergo maturation or smoltification. An early winter signal (70WS and 110WS) induced better signs of smoltification (reduced condition factor and less stress in seawater) and no tendency to mature early. In contrast, a late or no winter signal (180WS and LL) induced a strong stress response in seawater, poor morphological signs of smolting, and a higher tendency to mature early. The larger incidence of maturation in 180WS suggests that salmon of approximately 180 g may have accrued sufficient energy resources for early maturation, prioritizing this life strategy instead of smoltification in response to the photoperiod cue. In the LL group, however, the lack of light cue probably prevented more individuals to initiate the process despite being energetically ready, and only those highly determined initiated maturation driven by internal signals. These results evidence that increasing daylength when salmon is large enough to have accrued sufficient energy resources may pose high risk that a proportion of them prioritize sexual maturation over smoltification. Producers may take this into consideration when optimizing protocols for postsmolt production.

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A TALE OF BRAIN AND NOSE: DOES EMBRYONIC TEMPERATURE RESHAPE THE IMMUNE RESPONSES TO YERSINIA INFECTION IN ATLANTIC SALMON PARR?

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Introduction

The nasopharynx-associated lymphoid tissue (NALT) in the olfactory organ of teleost fish represents an ancient arm of mucosal immunity that has protected them from waterborne stimuli for millions of years. Many neurotrophic pathogens exploit the olfactory route to access the central nervous system. Despite the significant advances in understanding the neuro-immune interaction in fish in recent years, there is a considerable knowledge gap, especially of how this interaction is influenced by external factors such as temperature.

Atlantic salmon (*Salmo salar*) is one of the most economically important farmed fish species in the world. However, the industry is facing high mortality rates of up to 20%, largely caused by infectious diseases. In recent years, a significant body of evidence indicates that disease resistance, robustness and performance later in life could be influenced by stimuli, like temperature and oxygen, during the crucial stages of early development (Krasnov et al. 2021; Mateus et al. 2017). However, our understanding of how host immunity is shaped by exposure to these stimuli during early development is limited.

The present study explored the molecular and structural consequences in the brain and olfactory organ of *Yersinia ruckeri* infection in Atlantic salmon parr. We compared two groups of fish with different embryonic temperature histories to investigate if temperature stress during early development could reshape the responses to *Y. ruckeri* infection. Previous studies in rainbow trout (*Oncorhynchus mykiss*) revealed that *Y. ruckeri* is a neurotropic pathogen.

Materials and methods

Eggs were exposed to two different temperatures – 4ºC and 8ºC, from fertilization until the eyed stage (~320-day degrees). The latter was considered the commonly employed temperature in the industry. Thereafter, the fish were reared at 8ºC until they reached the parr stage. Fish (around 12 cm in length) were then exposed to *Y. ruckeri* serotype *O1* by bath exposure. Disease development was followed for 2 weeks, and organs were sampled the day before infection and 1, 2, 3, 7, and 14 days after infection. Brain and olfactory organs were collected for qPCR, histopathology, and immunohistochemistry.

Results and Discussion

Infected fish showed the classic signs of *Y. ruckeri* infection, including exophthalmos, skin darkening and splenomegaly (Figure 1). The pathogen was detected in the brain and olfactory organs of infected fish by qPCR. This was further verified by the identification of the pathogen in the optic tectum in the brain and in the basal lamina of the olfactory lamella by immunohistochemistry (Figure 2).

Gene expression analysis in the brain showed an increase in the transcript level of microglia cell markers (e.g., *aif1*) in infected groups compared to the control group. The expression of microglia cell markers in infected brain increased over time, and there was a temporal delay in immune response tendency in the 8ºC group. The expression of *cd4* also increased in infected groups, and a significant difference was observed between the two temperature groups at several time points, suggesting that embryonic temperature history affected the CD4-mediated response to infection.

In the olfactory organ, the expression of antimicrobial peptides (e.g., *cathelicidin*), was elevated in infected groups compared to the control group. This elevation increased over time in both temperature groups. *Interleukin 1β* (*il1β*), an important pro-inflammatory cytokine, increased around 3 days after infection.

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Figure 1: Infected fish with splenomegaly

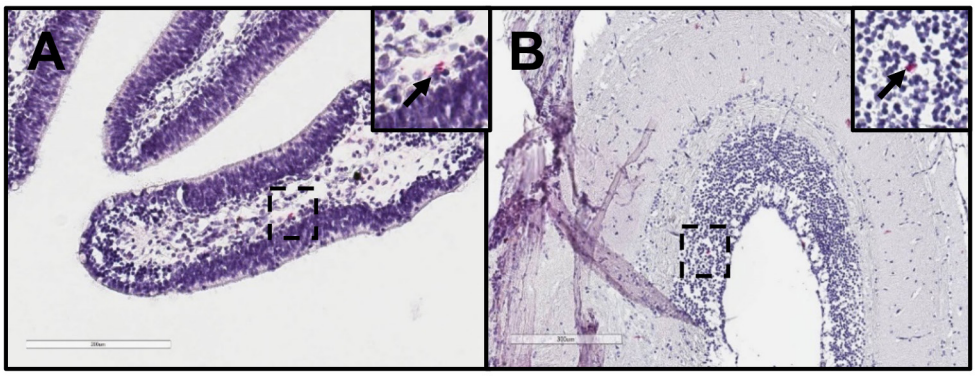

Figure 2: Immunohistochemistry of detection of *Y. ruckeri* in olfactory rosette (A) and in the optic tectum in the brain (B). Arrows point to *Y. ruckeri.*

Preliminary histopathology analysis shows mild signs of infection in both tissues. Significant differences were not found in the nasal morphometries, such as the thickness of mucosa and lamina propria of the olfactory lamella and the number of mucus cells present in the nasal wall and on the tip of the olfactory lamella.

Conclusions

The study confirms the neurotrophic nature of *Y. ruckeri* and further supports earlier evidence suggesting that it is taking the olfactory route to gain entry to the brain. The results indicate that the olfactory organ and brain could mount immune responses to the pathogenic infection. Both temperature groups were able to respond to infection, however, embryonic temperature history modulated the expression of some immune genes.

Acknowledgements

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DAILY RHYTHMS IN THE BEHAVIOURAL STRESS RESPONSE OF TENCH *Tinca tinca*, A NOCTURNAL FISH FARMED SPECIES

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Introduction

In nature, animals must face challenges and adverse conditions that vary with different likelihood throughout the day, such as food availability, social competition or even human disturbances. These factors usually act as stressors for fish, resulting in physiological consequences that endanger their welfare and survival. Tench (*Tinca tinca*) is a freshwater species naturally distributed in most parts of Europe which has been identified as a promising species for diversifying the freshwater aquaculture industry (Celada et al., 2009). However, although this species can adapt and tolerate a wide range of rearing conditions, it is very sensitive to handling practices related to intensive aquaculture and its growth performance and productivity may remarkably depend on its welfare condition (Pula et al., 2018). Considering that tench has been described as a strictly nocturnal species, this study aimed to determine whether the time of day at which fish are exposed to stressors could affect their stress response.

Methodology

A total of 72 adult fish were reared for 1 month in community tanks subjected to a 12:12h light:dark (LD) cycle and fed once daily, during night-time, at 2% body-weight. After this acclimatisation phase, 12 independent fish were individually exposed to the novel tank test (NTt) every 4h during a 24h cycle (at ZT 2, 6, 10, 14, 18 and 22; n=12 fish/ZT). The NTt consisted of exposing fish to a novel environment (i.e., a 3L glass tank) for 10 minutes while fish anxiety-like behaviours (i.e., bottom-dwelling, freezing and angular velocity) and activity parameters (i.e., distance travelled) were recorded and quantified by the EthoVision XT software ®. The experimental arena was illuminated from above with a white LED strip which was only on during daytime (ZT0-12), and from its background with an infrared lighting panel. To evaluate the presence of daily rhythms on tench behaviour, Cosinor analyses were performed ($p<0.05$). Besides, one-way analyses of variance (ANOVA I, $p<0.05$) were conducted to determine the effect of the time of the day on each behaviour. When ANOVA I was significant, differences between ZTs were studied by the Tukey HSD test.

Results

All analysed behaviours exhibited significant daily rhythms (Cosinor $p<0.05$) along with remarkable day and night differences. The bottom-dwelling response indicated that tench spent significantly more time at the bottom of the arena during the day in comparison to the night (Fig. 1A; ANOVA I, $p<0.01$), showing maximum bottom-dwelling values around the middle of the light phase (time in bottom acrophase: ZT= 6.2). Besides, freezing behaviour showed significantly higher values during the day than at night (Fig. 1B; ANOVA I, $p<0.01$) with an acrophase close to the middle of the day (ZT= 6.64). In addition, angular velocity behaviour showed that tench movements were significantly more erratic during the day compared to the night (Fig. 1C; ANOVA I, $p<0.01$), with an acrophase found closer to the middle of the light phase (ZT= 7.03). Finally, as expected for a nocturnal species, tench exhibited a nocturnal activity pattern characterised by more distance travelled during the night (Fig. 1D; ANOVA, $p<0.01$) and an acrophase close to the middle of the dark phase (ZT 18.25).

Discussion and conclusion

This study showed that tench anxiety-like behaviours significantly varied throughout the day, suggesting that the time of day affects the behavioural stress response in this species. In the NTt, the innate aversion of the fish to explore new and potentially threatening areas is assessed. In our results, we observed that fish spent more time at the bottom of the tank and more time freezing during the day in comparison to the night. Considering both anxiety-like behaviours as the most used in the NTt paradigm, this result suggests the impact of the stressor was stronger during the resting phase of the species (day) than during its active phase (night). Consequently, tench displayed higher angular velocity values during the day, a parameter linked to erratic movements and pronounced anxiety-like states in fish. To conclude, this research provides relevant information on daily rhythms of stress response in fish, which might be useful to improve their welfare in captivity. Hence, our results suggest that fish handling during routine aquaculture procedures might be scheduled at those times of the day when the impact of stressors is reduced.

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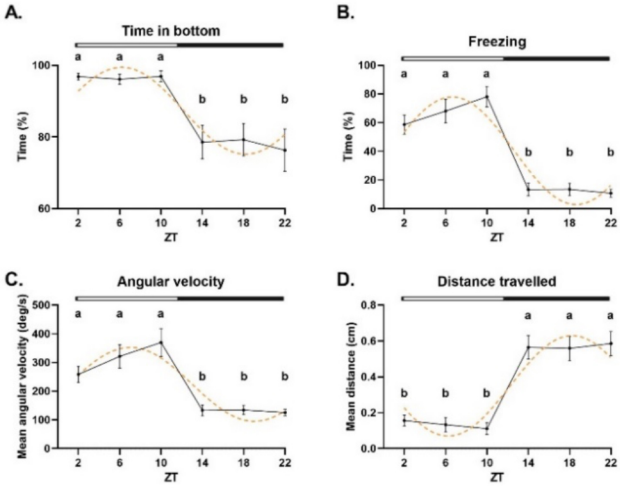

Figure 1. Daily variations in tench behaviour when exposed to the novel tank test. **A.** Time in bottom **B.** Freezing. **C.** Angular velocity. **D.** Distance travelled behaviour. Dotted orange lines indicate significant daily rhythms of best-fitting models calculated by Cosinor analysis. Data are presented as mean ± SEM and different letters indicate statistical differences by means of Tukey *HSD* test.

Acknowledgements

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STRATEGIES FOR THE INTRODUCTION OF THE SEAWEED *Ulva lactuca* IN THE MULTI-TROPHIC SYSTEM WITH BIOFLOC

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Introduction

During production in a biofloc system (Biofloc Technology - BFT) there is an accumulation of total suspended solids and an accumulation of nutrients (Krummenauer et al., 2011). Nitrate is accumulated in the system from the presence of chemoautotrophic bacteria that transform ammonia into nitrite and then into nitrate (Ferreira et al., 2021). Phosphorus, on the other hand, is accumulated due to feed degradation. According to Silva et al. (2003), only 22% of the nitrogen input is converted into shrimp biomass, 14% remains deposited in the sediment and 57% is discarded in the environment, suggesting little efficiency in the use of available nitrogen. To take advantage of these accumulated nutrients, macroalgae can be integrated into the crop, as a way of absorbing nitrogen to form biomass. This work aims to determine the best strategy for introducing macroalgae into the IMTA system, along with other cultivated organisms or after harvesting them.
Materials and methods

The first treatment consisted of integrated cultivation with the shrimp *Litopenaeus vannamei*, the fish *Oreochromis niloticus* and the macroalgae *U. lactuca* (All together - AT). This treatment was carried out in a greenhouse during 56 days of cultivation. Consisting of three systems, each system consisted of a 4 m3 tank with shrimp (350 shrimp m-2), where the water circulated by gravity to a 0.7 m3 tank with fish (7 fish m-3) and a pump submerged water circulated to a 300 L tank with macroalgae (100g of macroalgae m-3, relative to the total volume of the system), totaling 5 m3 each system.

The second treatment consists of treating the effluent from an integrated cultivation, with the introduction of algae after this period (After harvest - AH). At the end of 56 days of integrated shrimp and tilapia culture, the animals were removed from the system and the water stored. The macroalgae cultivation was carried out at a density of 100g of macroalgae m^{-3} (relative to the total volume of the system) in the effluent from the integrated cultivation.

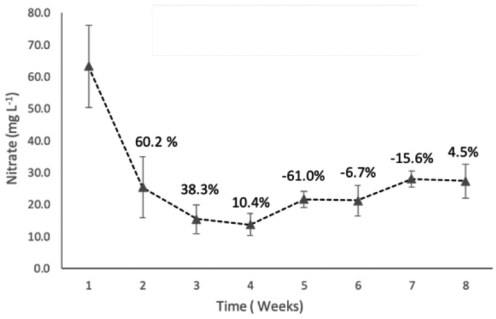

Figure 1. Average nitrate concentration over the weeks of cultivation in the treatment AT (All together). Percent values on the curve represent the nitrate removal rate for each week.

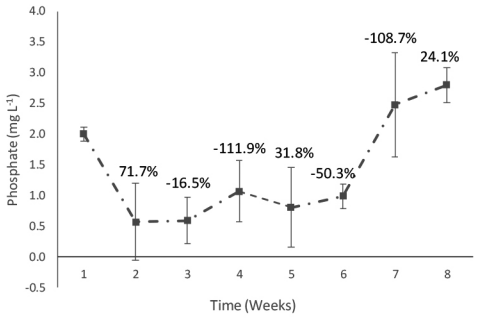

Figure 2. Average phosphate concentration over the weeks of cultivation in the AT treatment. Percent values on the curve represent the phosphate removal rate for each week.

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The analysis of nutrients in the AT treatment was carried out twice a week, and the concentration of ammonia, nitrite, nitrate and phosphate was determined. And for the second experiment (AH) the nutrient analysis was daily. The nutrient removal rate was performed using the following formula: NRR (%): 100 × [(nutrient concentration at the initial time (mg L-1) - nutrient concentration at the final time (mg L-1)) / concentration of nutrients in the initial time (mg L-1)]. Data normality and homoscedasticity were verified using the Shapiro-Wilk and Levene tests, respectively. Once the assumptions were met, a t-test was performed to verify the difference between treatments. A minimum significance level of 5% ($p<0.05$) was applied in all analyses.

Partial results

The AT treatment showed a mean removal rate of 56.48 ± 4.84% of nitrate, with a significant decrease up to the fourth week and in the last week of the experiment (Figure 1). For phosphate, there was an increase of 40.0 ± 36.1% in the final concentration of phosphate in relation to the initial one, with a significant decrease only in the first week and an increase in the last weeks (Figure 2).

Even with the constant production of nutrients in the AT treatment, the nitrate was still removed by the macroalgae. However, macroalgae did not show phosphorus removal values at the end of the experiment. Solid production is also constant in a biofloc culture (Gaona et al., 2017), which can also interfere with greater nutrient absorption. It is expected that in the AH system, as there will be no production of nutrients and solids, the macroalgae will maximize the absorption of nutrients.

Acknowledgments

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EFFECT OF PARTIAL HARVESTING ON THE GROWTH OF THE MACROALGA *Ulva lactuca* INTEGRATED WITH SHRIMP AND FISH

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Introduction

The integrated multitrophic culture allows the insertion of species of different trophic levels for the utilization of waste and increase the productivity of the system (Chopin, 2015). The availability of nitrate and phosphate in the integrated culture in biofloc system allows the cultivation of inorganic consumers, such as macroalgae, which absorb nutrients from the system. Alencar et al., (2010) showed that initial densities of 1g L^{-1} allow for better macroalgae growth and nutrient uptake compared to higher densities of 2 and 3g L^{-1}. However, with the limited area and long-term cultivation, the increase in the biomass of macroalgae throughout the cultivation may interfere with their performance. Therefore, this study aimed to evaluate the effect of partial harvests on the final biomass gain of the macroalgae *Ulva lactuca* cultivated in biofloc system with the shrimp *Litopenaeus vannamei* and tilapia *Oreochromis niloticus*.

Materials and methods

The two experiments were conducted in the autumn period in a greenhouse, with 22ºC, and constant aeration. Experiment 1 (EXP01) was 70 days of culture, with a recirculation of water in the shrimp tank at a density of 400 shrimp m^{-2} (16 m^3 of usable volume), to the fish tank at a density of 35 fish m^{-3} (4 m^3 of usable volume), and to the macroalgae tank of 4 m^3 of usable volume at a density of 0.1 kg m^{-3}, considering 24 m^3 of the entire system, with an initial average weight of 2.40 ± 1.64 kg.

The experiment 02 (EXP02) consisting of three systems, each system consisted of a 4 m^3 tank with shrimp (350 shrimp m^{-2}), where water circulated by gravity to a 0.7 m^3 tank with fish (7 fish m^{-3}) and a submerged pump circulated the water to a 300 L tank with macroalgae (0.1 kg m^{-3}, considering 5 m^3 of the entire system), with an initial average weight of 0,502 ± 0,05 kg.

Both experiments received a biofloc inoculum from an grow-out shrimp fattening culture. The macroalgae were maintained in structures made of polyethylene netting and PVC pipes, and the macroalgae were weighed every two weeks. For EXP01, the weighed macroalgae were returned to the culture structure. For EXP02, the macroalgae were weighed, and the biomass exceeding the initial weight of 0.502 kg was counted and removed, and the same weight was maintained at each weight measurement. The specific growth rate was calculated as follows: SGR (% d−1): 100 × [ln (current weight (g)/ previous weight (g))/(final time − initial time)].

Table 1. Mean and standard deviation of biomass gain at the end of each experiment.

	EXP 01	EXP 02
Biomass gain (kg)	0.26 ± 0.27 [b]	0.82 ± 0.20 [a]

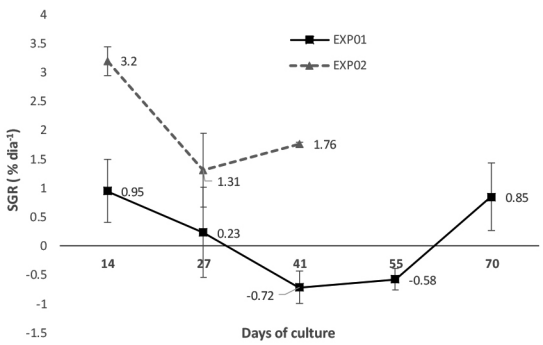

Figure 1. Specific growth rate (SGR) of EXP01 (without partial harvest of macroalgae), and EXP02 (with partial harvest of macroalgae) over the days of culture.

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Data normality and homoscedasticity were verified using the Shapiro-Wilk and Levene tests, respectively. Once the assumptions were met, a t test was performed to verify the difference between treatments. A minimum significance level of 5% ($p<0.05$) was applied in all analyses.

Results

As a result, the biomass gains at the end of cultivation showed significant differences between the experiments. EXP02 with the use of partial harvest showed a higher biomass gain compared to EXP01 (Table 1).

Figure 1 shows the specific growth rate (SGR) in each experiment. The highest values were found in EXP02 (partial harvest), with a maximum of 3.2 ± 0.25 % day^{-1}, compared to the maximum value of 0.95 ± 0.54 % day^{-1} found in EXP01.

Discussion

The increase in biomass during the culture cycle may allow for a maximization of nutrient uptake, however it may also interfere with macroalgae growth if it exceeds the load limit of the structure. The biomass loss seen between days 41 and 55 of EXP01 may be relative to the increase in biomass in the first two weeks. Viaroli et al., (1996), found that the increase in biomass caused the macroalgae to overlap and the stalks decompositio, which may have happened in EXP01. The use of partial harvesting in EXP02 showed a higher biomass production, being advantageous for the productivity of the integrated system.

Conclusion

The use of partial harvesting of macroalgae is feasible for production in closed system with integrated culture, generating higher biomass production and culture productivity.

Acknowledgments

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QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO FLAVOBACTERIUM PSYCHROPHILUM IDENTIFIED USING HIGH-DENSITY GENOTYPING IN RAINBOW TROUT FRENCH SELECTED LINES

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Introduction

Bacterial cold-water disease (BCWD) and rainbow trout fry syndrome, caused by *Flavobacterium psychrophilum* (Fp), are among the most problematic diseases in rainbow trout (*Oncorhynchus mykiss*) farming. Trout farming often suffers substantial economic loss due to problems associated with BCWD outbreaks, including high mortality, increased susceptibility to other diseases and skeletal deformities resulting in quality reduction in recovering fish. The management of this disease is a significant cause of antibiotic use in rainbow trout farming which raises environmental concerns and issues about the emergence of antibiotic resistance. Therefore, there is a crucial need for other methods to control the disease. Selective breeding of resistance to BCWD is a promising approach to reducing the frequency and severity of BCWD outbreaks. Several Quantitative Trait Loci (QTLs) for BCWD resistance have been identified in previous studies (e.g., Fraslin et al., 2018, Vallejo et al., 2022). With the development of a new high-density Axiom™ Trout HD genotyping array (Bernard et al., 2022), the detection and mapping of QTLs can now be more precise. This work refines the description of genetic architecture of BCWD resistance in rainbow trout using waterborne experimental infection model and genome-wide association studies (GWAS) with high-density genotyping and imputation.

Materials and Methods

Fish used in this experiment were from two French commercial breeding programs (pop. A and pop. B) and derived from partial factorial mating design plans from approx. 100 dams and 100 sires for each line. "Eyed" eggs were disinfected before entering the INRAE-IERP experimental unit (Jouy-en-Josas, France). After 4 months of breeding in flow water at 10°C, at an average weight of 3.9 g for pop. A and 3.3 g for pop. B, 1200 fish for each population were challenged with the virulent strain FRGDSA 1882/11 isolated in France from diseased rainbow trout. Briefly, bacteria were maintained in contact with fish at a concentration of 10^6 CFU mL^{-1} by stopping the water flow for 24 h. Water was maintained at 10°C with vigorous aeration and physical parameters (NH4+, O_2, ...) were monitored. Over a 29-d period, mortality was monitored twice a day, and live and dead fish were fin-clipped for DNA extraction and genotyping.

Challenged offspring and their sires were genotyped with the 57K Axiom™ Trout Genotyping array (Palti et al., 2015) at the INRAE genotyping Platform Gentyane. Their dams (96 for pop. A and 90 for pop. B) were genotyped with the high-density 675K Axiom™ Trout HD array. Offspring's 57K genotypes were imputed to HD thanks to the dams reference HD genotypes using FIMPUTE3 software (Sargolzaei et al., 2014). After quality controls, 1148 individuals genotyped for 403,863 SNPs and 1038 individuals genotyped for 402,750 SNPs were kept for analysis in pop. A and pop. B, respectively. Variance components analysis was performed for each line considering a binary survival trait (dead or alive at 29 days) in a threshold mixed animal model using genomic relationship matrices with THRGIBBS1F90 from the BLUPF90 family of programs (Misztal et al., 2014). GWAS was then performed using the Bayesian sparse linear mixed model (BSLMM) implemented in the GEMMA software (Zhou et al., 2013). This model allows partitioning the genetic variance into two components: a polygenic small effect for all SNPs and a QTL large effect for a limited set of SNPs. GWAS have been performed either on each line or the two of them combined using phenotypes corrected for the line effect to capture shared QTL. The Bayes factor (BF) was calculated to quantify the degree of association between a SNP and the phenotype. Evidence for a QTL was provided by a value $2\ln(BF) \geq 14$ at a SNP position. A credibility interval for the QTL was computed around the peak SNP including all SNPs for which $2\ln(BF) \geq 6$ in a sliding window of 250 kb.

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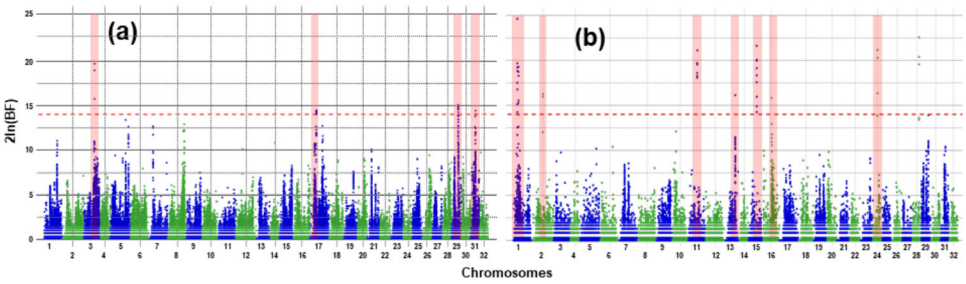

Figure 1. Manhattan plot of QTL detected for Fp resistance trait (A) in pop A and (B) in pop B. The red line corresponds to the QTL evidence threshold (2ln(BF) ≥14).

Results and Discussion

Survival rate post immersion challenge was 49% and 29% and heritability of Fp resistance was estimated to be 0.57 (CI: 0.47-0.68) and 0.39 (CI: 0.25-0.53) for the pop. A and B, respectively, which is consistent with estimates in the literature. Overall, for the two lines, only 10 to 20 SNPs were jointly selected in the BSLMM model, but they explained ~60% and ~75% of the genetic variance of Fp resistance in pop. A and B, respectively. Such results suggest that Fp resistance is a polygenic trait but with a few genes having very large effects. In pop. A, we found 4 QTLs located in chromosomes (chr) 3, 17, 29 and 31 (Figure 1A), while 10 QTLs on chr 1, 2, 11, 13, 15, 16 and 24 were identified in pop. B (Figure 1B). The GWAS combining the two lines revealed only a few shared QTLs, suggesting distinct molecular pathways underlying host resistance. Some of the detected QTL have been reported in Troutlodge, Inc., May-spawning line (Vallejo et al., 2022) or French INRAE lines (Fraslin et al., 2018). Investigations are in progress to identify candidate genes.

Acknowledgements

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IMPACT OF EARLY COLD TEMPERATURE ON RESPONSE TO ACUTE CONFINEMENT AND TEMPERATURE CHALLENGES IN JUVENILE RAINBOW TROUT DIVERGENT LINES FOR MUSCLE FAT CONTENT

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Introduction

To expand the availability of marketable eyed-eggs, a common practice in rainbow trout aquaculture is to store eyed-eggs at low temperature (2-4°C) for periods of up to 2-3 weeks. Recent studies have shown that early exposure to environmental stimuli (such as hypoxia or temperature) could impact fish physiology, growth, metabolism and nutrition at mid-or long-term questioning about the potential effects of such breeder practice. This study aimed to test the impact of incubating rainbow trout eyed-eggs at low temperature (3°C instead of 11°C) for 15 days on resistance to later stresses (acute temperature and confinement). Two experimental lines divergent for muscle lipid content (Quillet et al. 2005) that have been shown to utilise feed differently and to possess a well-differentiated intermediary and energetic metabolism (Kolditz et al., 2008) have been used. Our hypothesis is that they will react differently to the cold exposure during incubation.

Materials and methods

At 17 days post fertilization (dpf), eyed-eggs from two experimental lines selected for high or low muscle lipid content, fat line (FL) and lean line (LL), were either incubated at standard temperature (11°C) or incubated at 3°C for 15 days in 12 tanks (2 lines x 2 incubation temperatures x 3 tanks). From hatching to the beginning of the experiments, consisting of confinement and temperature challenges, fish were kept at 11°C.

Confinement challenges were performed at 217-219 dpf (12 tests in total, one per tank), by increasing fish density to 200 kg/m^3 for 4 minutes with no water renewal or oxygen supplementation. Four fish per tank were sampled for blood before the confinement stress (control condition) and 4 other fish per tank 1h after the confinement stress (stressed condition). Blood was taken from the caudal vein using heparinized syringes and then centrifuged. Plasma samples were stored at -20°C prior to analysis. Plasma cortisol was determined using the Neogen cortisol in saliva ELISA kit. Plasma glucose and lactate levels were assessed using Accu-Chek® Mobile (Roche) and The Edge™ Analyzer (Apexbio) systems, respectively. Chloride ions levels were assayed using a colorimetric method (Biolabo SAS). Statistical analyses were performed with linear mixed models using nlme package in R, with treatment (control or stressed), line and temperature as fixed effects and weight as a covariate when it significantly affected the response variable while tank was included as a random effect.

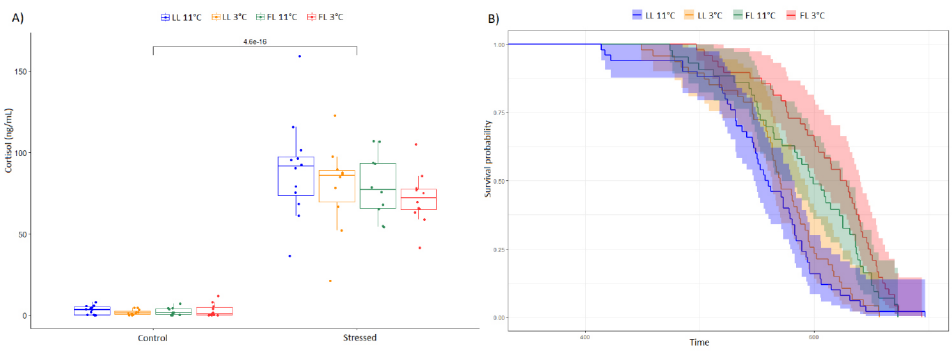

Figure 1. A) Plasma cortisol concentrations before (control) and 1H after (stressed) confinement challenges. B) Kaplan-Meier curves for acute temperature challenges.

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Acute temperature challenges were performed at 224-226 dpf. About one month before the challenges, fish were individually PIT-tagged and grouped into 3 separate tanks containing each 200 fish, i.e. 50 fish per line and per incubation temperature. The three temperature challenges (one per tank) were performed on three successive days. Fish were transferred into the challenge tank the day before the challenge for acclimation. Water temperature was increased progressively, about 0.7°C every 10 minutes until 22°C, then 0.1°C every 15 minutes. Water was oxygenated to keep oxygen levels near saturation. Fish were removed from the tank after the loss of equilibrium, weighed and their tag recorded alongside the time at loss of equilibrium. Kaplan-Meier curves and survival analysis were performed using the survival package in R, while differences between lines and temperature were assessed using linear mixed models fitted using nlme package, with line and temperature as fixed effects and weight as a covariate while tank was included as a random effect.

Results

As expected, there was a significant increase of plasma cortisol levels after confinement challenges ($p<0.001$); however, there was no significant effect of the genetic line or incubation temperature (Figure 1A). For plasma glucose and lactate levels, there was a significant effect of the confinement challenge ($p<0.001$); the genetic line had a significant effect only for glucose ($p=0.015$). There were no significant differences in chloride ion levels.

For acute temperature challenges, Kaplan-Meier curves are shown in Fig. 1B. There was a significant effect of the genetic line ($p<0.001$) and incubation temperature ($p= 0.036$), with FL line and fish incubated at 3°C resisting longer.

Discussion

Using two divergent lines for muscle fat content allowed for testing the impact of the genetic background on the observed responses. As expected, the two lines differently responded to the confinement and temperature challenges. Preliminary results suggest that the incubation temperature did not impair the responses of the two genetic lines to confinement and temperature challenges performed on 7-month-old juveniles. Based on this information, the practice of cold storage of eyed eggs does not appear to be detrimental to subsequent rearing. This study will contribute to the use of early programming as a lever to improve the long-term performances of animals.

Acknowledgements

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WILD VERSUS F1 BROODSTOCK: COMPARISON OF SEMEN QUALITY, INCUBATION RATES, LARVAL REARING AND SETTLEMENT SUCCESS IN PURPLE SEA URCHIN (*Paracentrotus lividus*) PRODUCTION

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Introduction

The gonads (also known as "roe") of the sea urchin *Paracetrotus lividus* are a lucrative fishing resource since they are highly valued as a delicacy in Europe, where local populations have been intensively fished. In the past 20 years, interest in echinoid aquaculture has grown. There is now a considerable effort to develop and improve the different production techniques to optimize production and profitability. The quality of the broodstock is one issue that needs to be addressed in the aquaculture production of any marine species since the success of the many stages of development, from hatching to adulthood, will be influenced by the quality of the eggs. In the production of many marine species, using wild spawners to gather eggs is a standard practice, and in certain cases it is required due to the difficulties in locating natural spawners. The direct benefit of not disrupting natural populations may be a direct benefit of using captive broodstock. But another compelling argument in favor of using F1 broodstock is the potential for greater spawn quality and better acclimatization to life in captivity. In this work the quality of semen, eggs and larvae of sea urchins produced by wild broodstock and F1 generation captive-born broodstock was compared.

Materials and Methods

Wild sea urchin adults (*Paracentrotus lividus*) were collected in a rocky area on the south coast of Portugal. Captive-born animals aged about 4 years were used as F1 broodstock. The two groups of broodstock (wild and F1) were kept for 1 month under equal captive conditions: rectangular fiberglass tanks, 70 liters in volume, with open water circuit and aeration. All broodstock were fed ad libitum with macroalgae *(Ulva spp.*). Spawning inductions were performed by injecting a 0.5M KCl saline solution through the peristomial membrane into the coelom in order to extract male and female gametes. Cell viability and motility metrics were used to compare the sperm quality of wild and F1 generations. About 30,000 pre-fertilized eggs were placed in 2-liter glass beakers to test the hatching rate of sea urchin eggs. After 48h the percentage of hatched larvae was estimated by microscopic counting. After assessing the hatching rate, about 16,500 larvae were placed in 6-liter glass flasks. Three replicates were used for each treatment (wild and F1). Partial water renewals (30%) of each glass flask were performed every 2 days, using reverse filtration with a mesh size of 50 microns. After each renewal, a microalgae mixture composed of *Phaeodactylum crostaum*, *Isochrysis galbana* and *Nannochloropsis oculata* was provided. Survival of each replicate was monitored for 22 days and periodic observations were made to assess larval development by counting larvae at each developmental stage (4, 6 and 8 arms). At the end of the larval stage, a group of competent larvae was taken to test the success of settlement for both groups (wild-type and F1 offspring). To analyze the settlement rate, 6 petri dishes with agar substrate inoculated with microalgae *Phaeodactylum tricornotum* were prepared in advance, where 50 ml of sterilized seawater was added. In each petri dish 20 sea urchin larvae were placed, with 3 petri dishes for each treatment. Observations were made daily to count sea urchins' post-larvae settled on the substrate.

Results and Discussion

It was possible to confirm that there were no significant changes in the cell viability of sperm produced by the two types of breeders (wild and F1) by examining the semen quality. However, it was found that F1 sperm had higher motility both at 5 and 10 min after activation (wild: 6.8 ± 6.0 % [5 min] and 0.8 ± 0.6 % [10 min]; F1: 33.2 ± 17.1 % [5 min] and 40.4 ± 16.2 % [10 min]. Regarding egg quality, there was a significantly higher hatch rate for eggs produced by F1 breeders compared to wild ones (58.3 ± 5.6 and 30.4 ± 3.4 % respectively) (P=0.008). The larval stage was monitored for 22 days, until a considerable presence of larvae in the pre-settlement and metamorphosis stages was verified. After 4 days of rearing, a higher mortality of larvae descended from F1 broodstock was observed. At the end of the larval stage, there was a higher mean survival rate of larvae from wild progenitors than from F1 (38.1 ± 18.7 and 23.4 ± 18.9), however the differences were not considered statistically significant (P=0.395). Regarding larval development, it was found that larvae descended from F1 broodstock showed faster development than larvae from wild type, this difference being more marked after 18 DAH. At the end of the trial 71.0 % of the larvae from the F1 treatment were at the 8-arm stage. For larvae hatched

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from wild broodstock, this percentage was 54.5%. The settlement experiment began on DAH 24 in petri dishes with agar substrate inoculated with microalgae. Settlement started in individuals descended from the F1 generation on day 26 DAH. On that day, a settlement rate of 31.7± 19.2% was recorded. This value grew to 44.4 ± 13.7% on the last day of the test. On this day, 2.8 ± 2.7% of sea-urchins descended from wild progeny were likewise settled, representing the ultimate value for both treatments.

Conclusions

Through this study, sea urchin (*Paracentrotus lividus)* production stages were analyzed, from the quality of male gametes to the settlement stage, with the aim of comparing the use of wild and captive-born broodstock (F1 generation). In general, it was found that production of this species is feasible and potentially better when using captive-born broodstock. This may be due to a genetic advantage of F1 individuals that allows them to better adapt to the captive environment and consequently a better quality of their offspring. In future work, a genetic study would be interesting to try to understand this phenomenon.

Acknowledgment: Project DIVERSIAQUA II (MAR2020.P02020101.FEAMP0175) and OURIÇAQUA (FA__05_2017_009 – OURIÇAQUA – Production of sea urchin (*Paracentrotus lividus):* reproduction, larval culture and fattening of juveniles.)

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EPPO: A PORTUGUESE RESEARCH FACILITY FOR THE DEVELOPMENT OF AQUACULTURE

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The Portuguese Institute for the Ocean and Atmosphere (IPMA, I.P.) is a public research institute and act as a counselor to the national authorities on the sea and atmosphere. IPMA, I.P., possesses a strong cluster of competences for the ocean and marine resources related to research, carried out by different groups, particularly dedicated to aquaculture and fisheries.

The Aquaculture Research Station of Olhão (EPPO, figure 1) stands out for the unique experimental conditions on aquaculture at the national and international levels. This marine core facility is equipped to carry out production studies at every scale from bench-top laboratory work to a much larger semi-industrial level. EPPO has an area of about 7ha with more than 250 tanks, including an hatchery fully equipped for research and experimental production with different rearing circuits (for broodstock, larvae, juvenile production, research with live animals and recirculation systems), a support building (with rooms for trophic chain production, daily routines and biological sampling), three RAS systems for research purposes, several analytical laboratories (biochemical, histological, molecular, microbiological and fish pathology), an unit for seafood packing, an area for pre-fattening (for earthen ponds and sea cages production) and 17 earthen ponds. It holds breeders of several marine fish species (e.g. meagre, gilthead seabream, seabass, Senegalese sole and sardine among others), microalgae and invertebrates as well as the know-how on the production of these species.

Production of new species, nutrition, welfare, environmentally friendly production systems and assessment of onshore and offshore and production systems for fish grow-out are some of research lines developed at EPPO (figure 2).

Acknowledgments: The research lines are partially funded by AQUARAS (Mar-02.05.01-FEAMP-0223).

Figure 1 - Aerial view and RAS system on EPPO

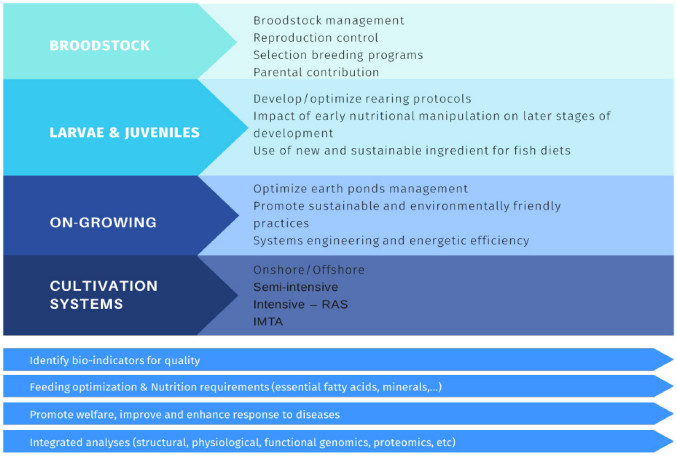

Figure 2 - On going research lines at EPPO

A RAPID MICRORADIOGRAPHIC METHOD TO DETECT SKELETAL ANOMALIES IN EARLY JUVENILE FISH

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Introduction

Skeletal anomalies affect fish growth, welfare and health, increase mortality and decrease product marketability (Boglione et al., 2013). Skeletal anomalies of early juveniles can be identified through an *in toto* double staining procedure, whose execution takes several days thus delaying diagnoses. Recently, a rapid microradiographic method to identify skeletal anomalies in early sea bream *Sparus aurata* juveniles (total length ≥ 25 mm) was proposed (Pousis et al., 2022). The aim of the present study was to assess the use of microradiography to identify skeletal anomalies in gilthead sea bream of younger age than previously reported and to extend the use of this method to the European sea bass *Dicentrarchus labrax*, the common carp *Cyprinus carpio* and the silver carp *Hypophthalmichthys molitrix*.

Material and Methods

Gilthead sea bream (N = 92; total length, TL, 18-31 mm), European sea bass (N = 145; TL 16-21 mm), common carp (N= 51; TL 18-31mm) and silver carp (N= 87; TL 17-23 mm) were sampled in fish farms operating in Italy and in Albania, anaesthetized, fixed in 10% buffered formalin and then stored in 50% ethanol. The fish were then micro-radiographed in latero-lateral projection. Since the high-resolution film is sensitive to water, a transparent polyethylene film was placed between the photographic film and the specimens. X-ray exposure was set up at 12 kV and 18 mA. The specimens <20 mm TL were subjected to 6500 X-ray shots, while the specimens ≥20 mm TL underwent 7500 shots. Films were then developed with Kodak HC-110, fixed in Kodak UNIFIX, and dried at room temperature. After microradiography, all the samples were stained with a routine *in toto* double staining procedure for bone and cartilage (Boglione et al., 2013).

Results and Discussion

In gilthead sea bream, the majority of the analyzed individuals (82.6%) showed at least one skeletal anomaly and most of them showed more than one anomaly (average: 3.3 ± 2.4). A total of 248 anomalies were recorded of which 21.4% were several anomalies such as haemal lordosis, vertebral body shape anomalies, vertebral body fusion and cephalic deformities. In European sea bass specimens, a total of 533 anomalies were recorded of which 10.3% were several anomalies such as cephalic deformities, kyphosis, lordosis and vertebral body fusion. The frequency of individuals with at least one (severe or slight) anomaly was found to be 93.1% while the average of anomalies per individual were 3.9 ± 3.1. In silver and common carp, a total of 1119 and 131 anomalies were respectively recorded. In silver carp 93.1% of the analyzed specimens showed almost one anomaly (average: 13.8 ± 15.4) while in common carp the frequency of deformed specimens was 78.4% (average: 3.5 ± 3.0). The most common severe anomalies in both species were vertebral body shape anomalies and vertebral body fusion, while no cephalic deformities were found. The frequency of specimens with almost one severe anomaly was 57.5% and 41.2%, respectively. In all the analyzed specimens the most common slight anomalies were deformed neural and haemal arches. All the skeletal anomalies observed after double staining method were also identified in the microradiographic plates (Fig. 1).

The present study suggests microradiography as a useful tool for the rapid identification of skeletal anomalies in early juveniles of different species, provided that X-ray exposure conditions appropriate to the size of the examined samples are set up.

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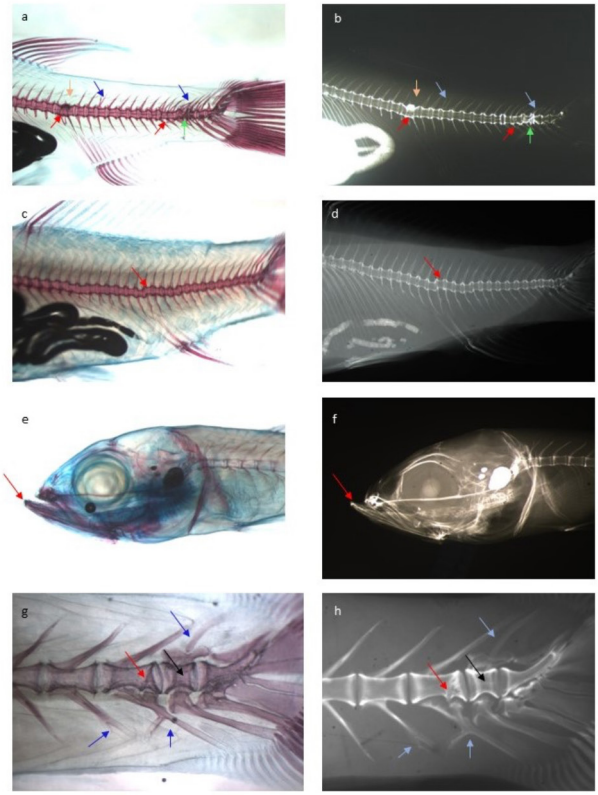

Fig. 1. Skeletal anomalies observed in double stained samples (left) and in microradiographic plates (right). a) and b), silver carp. Orange arrow: absence of neural arches of haemal vertebra; red arrow: fused haemal vertebra; blue arrows: deformed neural arches; green arrow: deformed bodies of haemal and caudal vertebrae. c) and d), common carp. Red arrow: kyphosis and deformed vertebrae between the 21st and the 24th vertebrae. e) and f), sea bass. Red arrow: anomalous dentary. g) and h), gilthead sea bream. Red arrow: partial fusion of two caudal vertebrae; black arrow: deformed body of a caudal vertebra; blue arrows anomalous neural and haemal arches.

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MICROBIOTA ASSOCIATED TO VIBRIO INFECTIONS IN BIVALVE HATCHERY: CO-OCCURRENCE OF ANTIBIOTIC-PRODUCING BACTERIA (*Pseudoalteromonas citrea-aurantia* AND/OR *Phaeobacter inhibens-gallaeciensis*)

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Introduction

In 2022 *V. pectenicida* emerged as a serious pathogens, affecting the survival of larval cultures of the different species of bivalves cultured in CCM, in Galicia (NW Spain) [AqEu 2022]. The presence of the pathogen continues this year, compromising cultures of *Venerupis corrugata*, *Ensis magnus* and *Ruditapes decussatus*. *Vibrio ostreicida*, a close relative of *V. pectenicida*, also pathogenic to bivalve larvae has appeared this year, infecting currently cultured *Ruditapes decussatus* larvae.

The global analysis of samples from cultures affected by *V. pectenicida* and *V. ostreicida* showed an interesting fact: the co-occurence of two marine bacterial taxa, *Pseudoalteromonas citrea-aurantia* (Pca) and/or *Phaeobacter inhibens-gallaeciensis*.

Materials and methods

The Centro de Cultivos Mariños-CIMA (Ribadeo. Xunta de Galicia) specialises in the development of bivalve molluscs from spawning to the appropriate size for outdoor cultivation. They have been diversifying the bivalve species, so that the facility is active throughout the year, combining clam and solenid cultures.

The USC-CIMA group has been developing microbiological control protocols for the facility, which include the transversal compartments (seawater circuit and microalgae cultures), as well as the larval, post-larval and spat cultures. The controls of the larval cultures are of particular relevance, and are mainly focused on detecting possible pathogens that may influence their development.

Results and Discussion

Recently, two pathogens, news to the installation, have been identified as responsible for mortalities in the cultures: *Vibrio pectenicida* and *V. ostreicida*. The review of the samples from a global point of view allowed us to observe the recurrent presence of two taxa in these episodes. The identification by 16S sequencing of the pigmented isolates showed that these bacteria are related to *Pseudoalteromonas citrea-aurantia* (***Pca***) and *Phaeobacter inhibens-gallaeciensis*.

Pca has been detected in most of the cultures suffering mortalities caused by *V. pectenicida-V. ostreicida*. Its presence were linked to the pathogens, over the year and independently of the bivalve species.

Phaeobacter was also associated to problems in the larval cultures. It was found together with ***Pca***, but also alone. As with ***Pca***, its proliferation does not seem to follow a seasonal pattern and is not related to bivalve species.

Phaeobacter inhibens-gallaeciensis has been proposed as probiotic for marine aquaculture. In fact, we have demonstrated the wide antibacterial spectrum of *Phaeobacter* PP-154, including aquaculture pathogens, especially members of the genus *Vibrio*, in solid medium as well as in seawater. *In vivo* studies in laboratory confirmed its ability to control the growth of vibrios (including *V. ostreicida*) in the water of larval cultures and in microalgal cultures. *Phaeobacter* PP-154 was also effective to combat bacterial infections, naturally produced or induced, with the condition of its use as preventive agent. However, its behaviour in scale-experiments in hatchery was not as expected. In addition, members of these species has been repeatedly isolated from specimens with problems, even caused by vibrios.

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Seyedsayamdost *et al*. (2011) have proposed two distinct phases in the *Phaeobacter gallaeciensis-Emiliania huxleyi* interaction. In the first phase, the bacteria contribute antibiotics that protect the algal host from bacterial pathogens, and substances that promotes algal growth. In the alternative phase, signals of high algal density and/or algal senescence led *P. gallaeciensis* to switches its metabolism to the production of algicide, converting the bacteria into an opportunistic pathogen. The findings presented here suggest that some similar could occur in *Phaeobacter*-larvae interaction, which would explain the risk of failure in scale-up experiments *in vivo*.

Pseudoalteromonas citrea and *P. aurantia* are known to produce antibiotic substances (Gauthier 1977, Gauthier and Breittmayer 1979, Holmström et al., 2002), with a broad range of antibacterial activity. However, there is scarce knowledge about their role in marine aquaculture systems and specific interactions with vibrios and/or bivalve larvae.

In summary, both pigmented bacteria share a high antibacterial activity, but in our observations their presence was related to larvae infected by *Vibrio pectenicida-V. ostreicida*, and they proliferated under these conditions without avoiding mortalities. These facts should be taken into account in their potential use as probiotics in aquaculture. Possible chemical signals in degraded larvae, that could trigger pathogenic behaviour in these bacteria, should be studied.

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IS CLIMATE CHAGE DRIVING THE RE-EMERGENCE OF THE PATHOGEN *Vibrio ostreicida* IN SEAWATER? RISK OF ITS ENTRY IN AQUACULTURE SYSTEMS

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Introduction

Vibrio ostreicida is a pathogen for bivalve larvae isolated in a Galician hatchery (Prado, 2006, Prado et al., 2005) and posteriorly defined as a new species in genus Vibrio (Prado et al. 2014). In the continuous studies carried out in Galician hatcheries since its original detection, over the last two decades, *Vibrio ostreicida* had not been isolated.

Recently, this pathogen has suddenly emerged in different installations in Galicia, located in different environments, pointing to the seawater as source. Its entry has caused problems in the larval cultures and it seems able to remain in the installation.

Materials and methods

USC and Xunta de Galicia are presently collaborating in the monitoring of the microbiota in two different facilities, belonging to Consellería do Mar (Xunta de Galicia): Centro de Cultivos Mariños-CIMA de Ribadeo and Mini-hatchery at IGAFA.

Microbiological protocols have been implemented for both facilities, taking into account the particularities of each one. Routine samples include seawater and phytoplancton (food) circuits, as well as bivalve cultures. Initially, the bacteriological medium Thiosulphate-Citrate-Bile-Sucrose (TCBS medium) was mainly used as it is selective for vibrios, the main pathogens known in bivalve aquaculture. Further, Marine Agar (MA) was incorporated to obtain a broader view of the marine microbiota present.

Results and Discussion

The first episode of *Vibrio ostreicida* was recorded in 2022, at the Mini-hatchery in Ría de Arousa (W Galicia, NW Spain). It is a small facility with a light greenhouse-type structure for the cultivation of molluscs seed of commercial interest in Galicia. Routine microbiological controls of the seawater revealed its entry with the seawater in May and its circulation through the circuit of distribution.

It was recorded again in August, in an experience of pre-fattening of pulled carpet clam (*Venerupis corrugata*) at different densities, in cylindrical lanterns in a "batea" (raft) at Ría de Arousa. After one month at sea, mortalities recorded in the lantern with the highest density pointed to be related to *V. ostreicida*. Its presence was favoured by density and its pathogenicity by depth.

In 2023, *V. ostreicida* was detected in the seawater circuit of CIMA, in the Ría de Ribadeo (N Galicia, NW Spain), at least in two successive controls in May. Unfortunately, the pathogen reached the currently cultured larvae, of *Ruditapes decussatus*. Since the first detection, *V. ostreicida* has been repeatedly isolated in larval cultures.

This species was originally isolated from a Galician hatchery, with continuous mortalities of young spat of *Ostrea edulis* (<500 µm in size) (Prado, 2007). Microbiological samples were taken from spat, seawater and tank surfaces. Pathogenicity tests demonstrated that PP-203, isolated from the surfaces of nursery culture containers, caused severe mortalities within 24-48 h. In more recent experiments, we demonstrated the ability of PP-203 to form a biofilm on culture tanks surfaces under hatchery conditions (Prado et al. 2019).

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Regarding its source, in both installations seems to be linked to seawater from outside. So far, this pathogen has been found in summer, when the temperatures are rising. Hence, its recent reappearance, after so much years without detection, could be related to the environmental temperature, because in both places a worrying increase of this parameter is being recorded, regardless of their marked differences in their characteristics. Arousa, the biggest "ría" in Galicia, has a high density of population and it is the main aquaculture area, while Ribadeo is a small "ría", with low human impact.

Vibrio ostreicida belongs to the same clade as *V. pectenicida*, within genus *Vibrio*. In fact, they share some special characteristics, such as their poor ability to grow in TCBS medium. Thus, although *V. ostreicida* grows in TCBS, it was mainly isolated from samples in MA medium, as observed with *V. pectenicida*. Both species, once they enter the installation, showed a high capacity of remain "under the radar", causing continuous problems in the cultures. The demonstrated biofilm formation of *V. ostreicida* on the surface of the tanks under hatchery conditions clearly points to this persistence strategy.

In summary, the appearance of this pathogen in the seawater of Galicia coinciding with the observed increase in environmental temperatures, suggests that this could be a consequence of the climate change. Moreover, its persistence in the installations once it enters, causing mortalities in larval cultures of bivalves, could led to severe problems if it is not properly controlled.

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Acknowledgements

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EFFECT OF DIETARY STARCH AND NON-STARCH POLYSACCHARIDE (NSP) LEVELS ON NUTRIENT DIGESTIBILITY, WASTE PRODUCTION AND FAECAL CHARACTERISTICS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Waste management is a critical issue in aquaculture. Waste accumulating in the recirculating aquaculture system (RAS) is a function of amount of waste produced and their efficiency of removal. Since most solid waste is of faecal origin, manipulating the dietary factors may result in attributes desirable for their easy and efficient removal. In earlier studies, increasing the starch level in diet improved the faecal removal efficiency in Nile tilapia, *Oreochromis niloticus* (Amirkolaie et al., 2006), but reduced it in yellowtail kingfish, *Seriola lalandii* (Horstmann et al., 2023, under review), and African catfish, *Clarias gariepinus* (Phan et al., 2022). Similarly, NSP level in diet was shown to improve the faecal quality in Rainbow trout, *Oncorhynchus mykiss* (Meriac et al., 2014). Hence, in this study, we investigated the effect of starch and NSP levels in the diet and their interaction on nutrient digestibility, faecal waste production (digestibility), and faecal characteristics of rainbow trout.

Materials and Methods

Four diets were formulated according to a 2 × 2 factorial design. The first factor, starch was tested by including 0% gelatinized wheat flour (LS- low starch) or 20% gelatinized wheat flour (HS- high starch) in a plant based basal diet. The analysed starch content of the LS- and HS-diets was 40 and 240 g/kg respectively. The second factor, NSP was tested by adding 0% soya hull (LNSP- low NSP) or 10% soya hull (HNSP- high NSP). Tanks (experimental units) were stocked with 25 rainbow trout (initial weight, 81 grams) and the experiment was run for 6 weeks. Fish were fed restrictively (12 g/kg$^{0.8}$/d, expected FCR 0.9) twice a day. For each tank, faecal waste production and faecal removal efficiency was determined by sedimentation. To get insight into the faeces stability, part of the collected faeces (non-stressed) were exposed to mechanical stress and the PSD was measured.

Results

The amount of faecal waste produced was influenced by the interaction effect of starch and NSP supplementation ($P<0.001$; Figure 1). At low NSP levels, starch inclusion did not alter faecal waste production. Inclusion of NSP increased waste production, being the highest at the HS-HNSP diet ($P<0.001$). For most other faecal waste characteristics (faecal removal efficiency; PSD of non-stressed faeces; amount of non-removed faeces) the interactions effect was not significant. Increasing dietary starch content, reduced faecal removal efficiency by 11.3% ($P<0.001$). NSP inclusion in the diets increased the removal efficiency by 4.4% ($P<0.05$), which fully compensated for the increased faecal waste produced. The amount of non-removed faeces was unaffected by NSP inclusion ($P>0.05$), whereas dietary starch inclusion resulted in a doubling of the non-removed faeces ($P<0.001$). Both starch and NSP inclusion significantly altered PSD of non-stressed faeces ($P<0.05$). Starch inclusion reduced the size of faecal particles, while NSP inclusion increased it (Image 1). Exposing faeces to mechanical stress reduced the particle size, but this reduction seemed to be affected by an interaction effect between starch and NSP inclusion. After stress exposure, the amount of large faecal pellets (>1600 µm) was lower at the HS-diets, but the difference between LS and HS-diets was largest at the diets without NSP inclusion ($P<0.05$).

Conclusion

Non-removed faeces by settling is determined by dietary starch content and not by NSP. Dietary starch negatively affects faecal removal efficiency while NSP improves it. Dietary starch content reduces the faeces stability but this effect is dependent on the NSP content.

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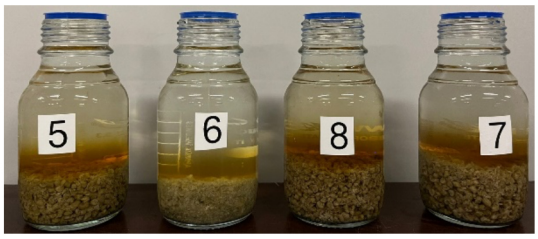

Image 1. *Collected faeces of rainbow trout fed with LS-LNSP, HS-LNSP, LS-HNSP and HS-HNSP diets (left to right) restrictively for 42 days. Note the dominance of small size faecal particles at HS-LNSP diet.*

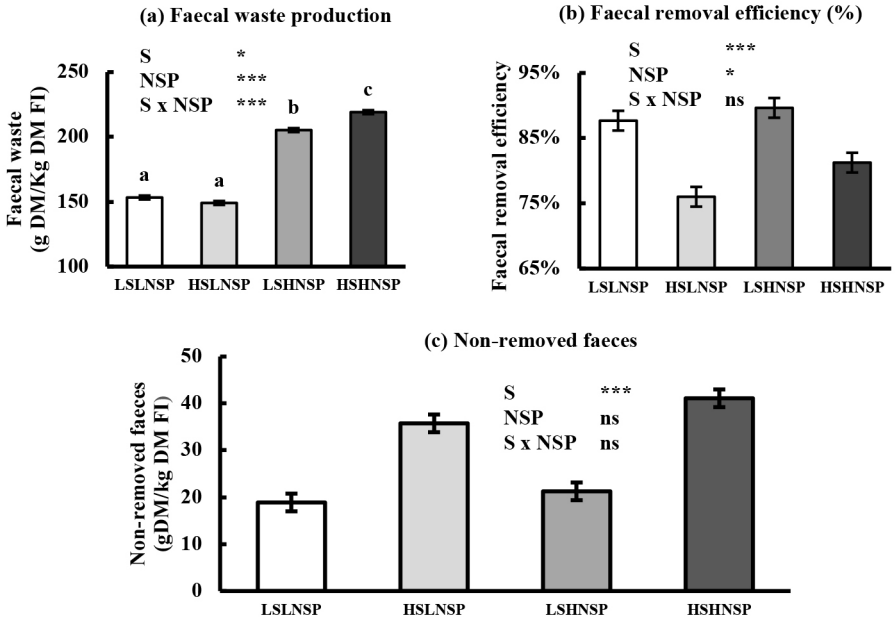

Figure 1. (a) Faecal waste production (g DM/kg DM FI), (b) Faecal removal efficiency by settling (%) and (c) Non-recovered faeces (g DM/kg DM FI) of rainbow trout fed the experimental diets restrictively (3 replicates) for 42 days; DM – dry matter; FI – feed intake; LS-LNSP – low starch low NSP; HS-LNSP – high starch low NSP; LS-HNSP – low starch high NSP; HS-HNSP – high starch high NSP; bars are means; error bars indicate standard error of means; bars not sharing a common letter are significantly different ($P < 0.05$); ns - not significant; * - $P < 0.05$; *** - $P < 0.001$.

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EVALUATION OF THE CHRONIC EFFECT OF NITRATE AND RECOVERY THROUGH COMPENSATORY GROWTH IN THE PACIFIC WHITE SHRIMP (*Penaeus vannamei*) IN BIOFLOC TECHNOLOGY SYSTEM

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Introduction

The development of new technologies for aquaculture systems is essential to reach economic, social and environmental sustainability and allow the continuously growth of aquaculture production. In this scenario, the Biofloc Technology System (BFT) have been considered one of the possible solutions and opportunities to further develop aquaculture. However, one of the concerns in the BFT systems is the nitrate accumulation during the culture period, due to zero or low water exchange during the culture period and the constant reuse of water. Therefore, the present work aimed to evaluate the chronic stress of nitrate in *L. vannamei* and the possible recovery of growth rates when nitrate low levels are re-established through compensatory growth, which is defined as a physiological process where the organism goes through a rapid phase of growth after a restricted period of development.

Material and methods

The present work was divided in two experiments. Firstly, a Lethal Concentration 50 (LC50- 96h) was conducted in clear water to define the nitrate safe level for *L.vannamei* in salinity 25 mg., calculated using the Trimmed Spearman-Karber Method. Therefore, Nine concentrations of nitrate were chosen to the lethal test: zero (control), 1000, 1500, 2000, 2500, 3000, 3500, 4000 and 4500 mg.of N- NO_3.

From the result obtained from the lethal test, the chronic experiment was conducted in BFT. Thus, *L. vannamei* juveniles were stocked with an initial weight of 0.82 g (± 0.25 g) at a stocking density of 300 shrimps/m³.The experiment was performed using a 3 × 2 experimental design (two nitrate concentrations and three exposure time), plus the control (which the nitrate level was maintained lower than 20% of the safe level),totaling seven treatments (in triplicate) and lasted 58 days. The experiment was divided into two phases: (1) Stress and (2) Recovery. Two nitrate concentrations were chosen (safe level determined in the first experiment and half-safe level - 278.91 and 139.45, respectively) and three exposure times were established for each nitrate concentration (10, 20 and 30 days). After the stress period, which varied according to the treatment, the experimental units were exposed to optimum conditions until the experiment complete 58 days.

The treatments exposed to half of the safe level and the groups that were submitted to the nitrate safe level limit during 10, 20 and 30 days were named as 0.5*SL - 10, 0.5*SL - 20 and 0.5*SL - 30, 1.0*SL -10, 1.0*SL - 20 and 1.0*SL – 30, respectively.

Results and discussion

From the lethal test, the estimated LC50-96h was 2789.11, and safe level was determined through Sprague Factor (0.1 of the LC50-96h). Regarding the chronic experiment, survival did not present any difference among treatments over the experimental period. At the end of the stresses phases (day 10, 20 and 30), the weight, Weekly Growth Rate (WGR) and Specific Growth Rate (SGR), of the treatments submitted to 0.5 and 1.0 times of the nitrate safe level during 10 and 20 days (0.5*SL – 10, 0.5*SL - 20, 1.0*SL -10, 1.0*SL – 20) did not statistically differ from the control group. However, after 10 days (day 30) of recovery, in the treatments submitted to 20 days of stress (0.5*SL – 20, 1.0*SL – 20), a statistical difference was found between both treatments and the control. Regarding the treatment exposed to 0.5 times the safe level for 30 days (0.5*SL – 30), it did not present differences compared to the control treatment on day 30, while the treatment exposed to the safe limit level during 30 days (1.0*SL – 30) presented a lower weight compared to the control group. At day 40, the control statistically differ from the treatments stressed during 30 days (0.5*SL – 30, 1.0*SL – 30), which did not differ between them. The Apparent Feed Converstion Rate (AFCR) from the stressed groups during 10 and 20 days did not differ from the control group. Despite that, both 0.5*SL – 30 and 1.0*SL – 30 treatments presented higher AFCR compared to the control group.

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After the end of the recovery phase (day 58), no significant differences were found in the body mass among treatments. In addition, no differences were found in the WGR and SGR among treatments previous stressed during 10 and 20 days and the control group. The treatment 1.0*SL – 30 presented higher WGR compared to the control treatment and did not differ from the 0.5*SL – 30. SGR also had an also rate in the 1.0*SL – 30 treatment, followed the 0.5*SL – 30 group, while the control presented the lower SGR. AFCR did not present statistical differences among control and both groups exposed to high nitrate levels during 10 days. Regarding to treatments previous stressed during 20 and 30 days, the control group presented higher AFCE compared to the 0.5*SL – 20 and 0.5*SL-30 treatments, and did not differ from 1.0*SL – 20 and 1.0*SL – 30 groups.

Despite the depressed growth after the nitrate exposure in the treatments mentioned above, the animals were able to recovery the growth rates when exposed to lower levels of nitrate, characterizing total compensatory growth in all treatments previously affected by the high nitrate concentration.

CG is usually evaluated through the improvement of SGR and the feed conversion rate in the stressed treatments compared to the control after the recovery phase, which are both considered an adequate parameter to conclude the occurrence of CG. In the present study, the SGR at the end of the experiment of the 1*SL – 30 and 0.5*SL – 30 treatments, previous affected by the chronic exposition at day 30, was higher than the control, even though the feed conversion rate did not differ from the control. Therefore, since no survivor losses was identified and the final weight did not differ among treatments, it is possible to conclude the treatments previous affected by the nitrate toxicity reached the control treatment weight through CG.

Conclusion

Even though *L. vannamei* presents a great resistance to nitrate exposure, long expositions (more than 20 days) in high nitrate concentrations can negatively affect the growth rates. However, when nitrate lower values are re-established, the animals are capable to regrow in high rates and compensate the weight through compensatory growth.

GENETIC PARAMETERS OF FLESH COLOUR TRAITS IN MARKET SIZE AMUR MIRROR CARP AND THEIR RELATION TO PRODUCTION TRAITS

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Introduction

Common carp is one of the most important freshwater fish species for world aquaculture, and its annual production is continuously increasing. Carp meat from pond aquaculture is a healthy product due to the highly nutritious protein, fat, minerals, and vitamins provided by natural food (Mráz et al., 2012). However, to reach sufficient productivity, carp pond aquaculture has to be supported by supplemental feeding, mainly by wheat grain, which might lead to decreased flesh quality in terms of fatty acids and potentially to a change in flesh colour (Mráz et al., 2012; Prchal et al., 2018). Colour and odour of raw fish flesh are important traits for consumers. Despite its affordability and high nutritional value, sometimes carp flesh is ignored at the market because of its unattractive look. In salmonids, it was shown that consumers prefer flesh products with richer red colour (Skonberg et al., 1998). Salmonids species present a moderate genetic variability of flesh colour which enables selective breeding for this trait (Norris and Cunningham, 2004; Blay et al., 2021). The aim of this study was to estimate genetic parameters of raw flesh colour traits in market size common carp and their relation to other economically important production traits (growth-related traits, muscle fat content, survival) to evaluate the possibility of improving carp flesh colour by selective breeding.

Materials and methods

The experimental stock was established by a partial factorial design of 20 dams and 40 sires of broodstock of Amur mirror carp (AMC). The stock was reared communally until the market size of an average of 1,910 g body weight under semi-intensive pond conditions ,and 1,277 progenies were phenotyped and assigned to their parents using 12 microsatellites. The flesh colour traits were measured with a Minolta CR400 Chroma Meter and expressed in the L*, a* b* CIE system (Robertson, 1977) to obtain the mean value of the lightness, redness, and yellowness, respectively. Likewise, C* polar coordinate of the chromaticity (relative saturation) was also calculated as . Fish were also phenotyped for survival (Surv) during the last (third) growing season, body weight (BW), Fulton's condition factor (FC), muscle fat content (%Fat) and slaughter yields (headless carcass: Res_Carss; fillets: Res_Fill). Heritability and genetic and phenotypic correlations were estimated with DMU software using an animal model with a fixed effect of sex.

Results

The mean values for flesh colour traits were typically low for "white-fleshed fish" – L* = 42.4, a* = 1.19, b* = 1.50, C* = 1.74 compared to salmonids. Heritability estimates differed significantly from zero and were low to moderate for flesh colour traits (0.16 – 0.39) and low to high (0.15 – 0.97) for production traits. A negative genetic correlation was observed between FC and a* (-0.44), FC and C* (-0.34) and headless carcass (Res_Carss) and a* (-0.33). Oppositely, a positive genetic correlation was estimated between %Fat and b* (0.50), %Fat and C* (0.47) and Res_Carss and L* (0.41). Moreover, a* was positively genetically correlated to b* (r_g = 0.36) and L* was negatively correlated to a* (r_g = -0.64).

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Discussion and conclusion

Heritability estimates for flesh colour traits in common carp were higher than in a previous study using the same CIE colour space in Atlantic salmon (Norris and Cunningham, 2004) but similar to flesh colour parameters in rainbow trout (Blay et al., 2021). It suggests that although the carp flesh colour is very different from that of salmonids, selective breeding might change these traits. FC, a trait related to body shape in AMC, was negatively genetically correlated to a* and C*. Thus, selecting fish for an elongated body shape might lead to more redness and generally more colourful flesh. We may hypothesize that in our study fish with an elongated shape, closer to that of the Amur wild carp, utilized more pond natural food (copepods, cladocerans and small zooplankton) rich in carotenoids with red pigmentation (Schneider et al., 2016). Conversely, selection for improved Res_Carss would indirectly lead to less redness and more lightness of flesh but also to higher fat content of muscle. Accordingly, muscle fat content was positively genetically associated with b* and C*, which could be explained by higher consumption of supplemental feed. Nevertheless, it might lead to a change in flesh colour and lower flesh quality in terms composition of fatty acids (Prchal et al., 2018). If this was the case, it would question how carp improved for flesh colour would valorise supplemental feed, and the impact on yield. However, further genetic correlations linked to other important performance traits recorded from fry to the market size might bring a better genetic picture of flesh colour traits in common carp and their relation to other traits.

Acknowledgements

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FORCE MEASUREMENT ANALYSIS OF A FULL SCALE OFFSHORE SEAWEED CULTIVATION SYSTEM

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Introduction

As part of the Interreg Wier en Wind project, a full-scale offshore seaweed cultivation system was installed in the Dutch part of the North Sea, 12 km off the coast of Scheveningen. The system was installed in February 2022 and the seaweeds were harvested in June 2022. During this period, a storm of 5.01 m significant wave height was recorded at the location. This study focuses on the impact of environmental conditions of wave, current and tidal variation, as well as biomass, to the measured load in the full-scale seaweed cultivation system.

Materials and methods

The cultivation system consists of a 50 m x 2.1 m net structure on which the seaweeds grown. A 58-meter polyethylene (PE) pipe is utilized as a floatation device to provide buoyancy to the cultivation line. The distance between the two anchors is 230 m at a water depth of 18 m. A main rope is used to connect the net structure to a shackle that connects to the mooring chain. The main rope is connected to the PE tube using loops that are flexible to move along the pipe. As such, the PE tube does not act as a load bearing component. The general arrangement (GA) of the system is visualized in Fig 1. A force gauge with a sampling frequency of 0.2 Hz was fitted to measure the tension experienced by the main rope.

Results and discussions

During the measurement campaign, the highest peak tension was recorded during the harvesting activities. This value is 44 % higher compared to the highest peak load during the system's operational condition. The measured load compared to the water level variations, changes in current magnitudes and significant wave heights (Rijkswaterstaat 2023) is presented in Fig 3. The recorded highest and second highest tensions during operational condition occurred during ebb current and flood current, respectively. This indicates that the tidal variations have dominant influence to the measured peak tension. There are two time windows where the significant wave height is higher than 4.5 m. In those cases, the measured peak load is only up to 66 % of the highest peak tension during operational condition (i.e., excluding harvesting). To analyse the influence of biomass, two time windows are compared to observe the mean measured force, presented in Fig 2. In the first time window (21/02/2022 – 25/02/2022) shown in blue dots, the net structure had just been installed, therefore, no biomass increase is present. This is the opposite for the second time window where the biomass has increased more than 1 month after it was installed. From Fig 2, no clear trend can be observed from the influence of the biomass on the mean measured force.

Conclusion

This analysis focuses only on the low frequency load because the influence of waves with a period shorter than 10 s cannot be clearly identified due to aliasing (Nyquist frequency of 0.1 Hz). A power spectral density (PSD) of the measured load reveals that the peak energy is found to be at the frequency of 2.24E-5 Hz or a period of 12.4 hours, which correlates to the cycle of a tidal current. The tidal variations found to be the biggest attribution to the measured peak tension, albeit high frequency forces were not clearly identified. During this measurement campaign, the increase in biomass does not have a significant influence to the measured low frequency load, albeit little biomass was found throughout this campaign.

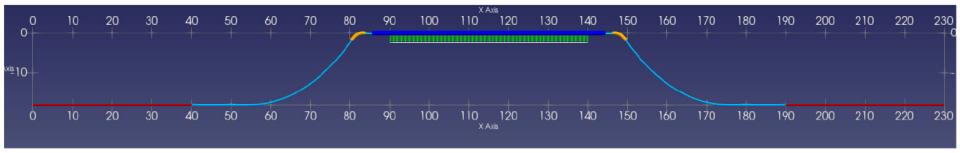

Fig 1. GA visualization of the full-scale offshore seaweed cultivation system

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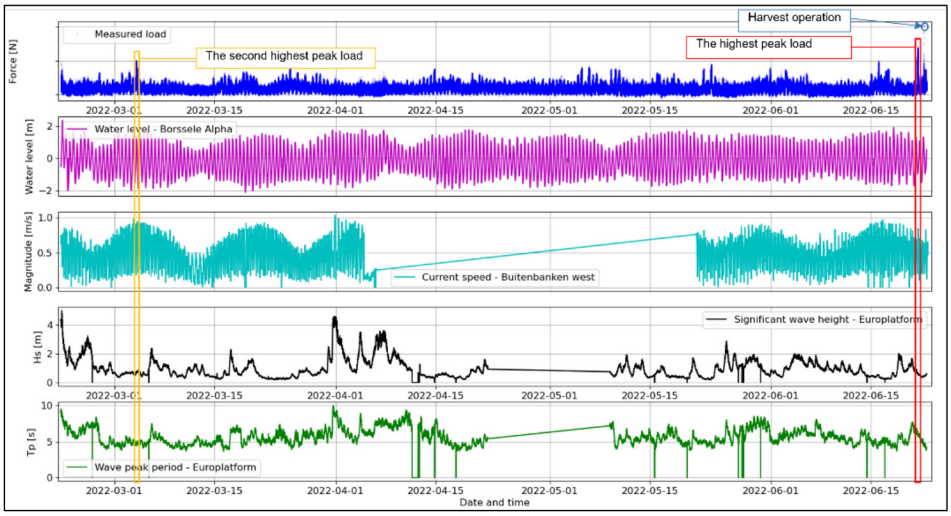

Fig 3. Measured load compared to the wave and tidal conditions at nearby locations (Rijkswaterstaat 2023)

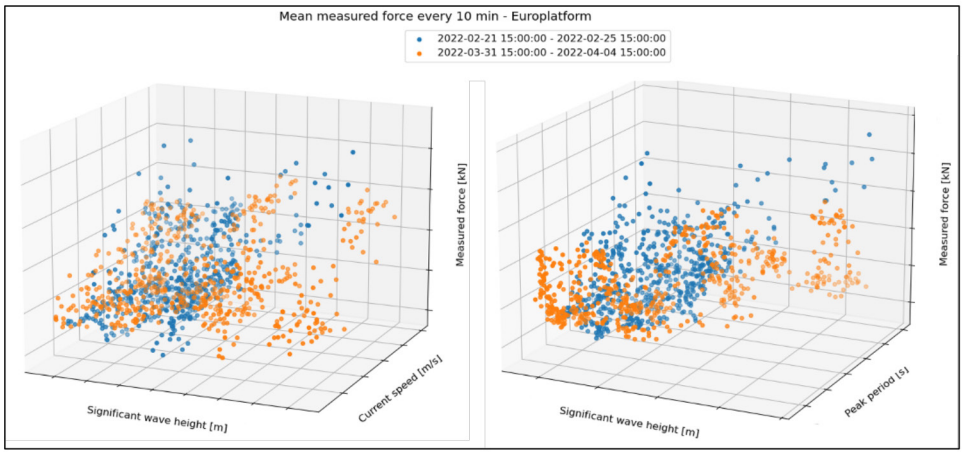

Fig 2. Mean measured force comparison of two different time windows

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EFFECT OF PEPTIDE DIETS ON SEA BASS (*Dicentrarchus labrax*) SKELETAL DEVELOPMENT

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Introduction

Fish skeletal development is under the continuous influence of biotic and abiotic parameters (1). Being a valuable welfare index (2), skeletal abnormalities appearance undermines the quality of the experimental results and inevitably, the marketability of the aquaculture products. One of the most critical factors towards normal fish skeletal development is appropriate larval nutrition (3). Protein fraction, being the major component of fishmeal, has been proven advantageous for larval growth, survival and antioxidative response in the form of various sources and hydrolysis levels (4). Simultaneously, an effect of dietary peptides in harmonious skeletal development has been observed (5). Despite the well characterized beneficial effect of small dietary protein products on the normal development of fish skeletal elements, the molecular basis of these observations remains scare.

The purpose of the present study is to evaluate the effect of three experimental diets with partial peptide incorporation on a) the skeletal development of sea bass larvae and b) the resistance of their vertebral column against swimming induced lordosis. A possible effect of the diets on gene markers of larval maturation (digestion, ossification, and mineralization) was also examined.

Materials and methods

Three isonitrogenous experimental diets were formulated by partial substitution of the total protein source with 0% (C, Control group), 6% (P6) and 12% (P12) shrimp di and tri-peptides. After a short Artemia inclusion (6-20dph), experimental diets were provided exclusively throughout the trials. Study of larval and juvenile quality was performed through means of whole mount staining, histology analysis and micro-ct scanning. To evaluate bones' integrity against haemal lordosis induction, early juveniles with normal skeletal development were subjected to swimming challenge test (SCT, 6). Gene expression analysis on selected gene of interest was performed by means of rt-qPCR.

Results and Discussion

Results of the early skeletal development revealed an advantageous peptide-enhanced ossification in the case of P12 individuals (Fig. 1). Similarly, P12 had a beneficial effect in the frequency of exercise-induced lordosis after the SCT reducing the deformities rate in only 15.8% (Fig. 2a). RNA analyses confirmed a peptide-enhanced total larval maturation, in terms of their digestive function, bone mineralization and myogenesis in P12. After the exercise, a differential regulation of the genes in the affected haemal area confirmed the scenario about an early peptide nutritional programming of the post-larval musculoskeletal responses.

With the present project, we highlight the beneficial effect of the early peptide diets enhancing the total larval maturation in terms of digestive function and ossification in sea bass larvae. The limitations concerning the free amino acid participation should be taken into consideration. Potential incorporation of these small di- and tripeptides in inert larval but also post-larval diets could enhance larval and post-larval skeletal development.

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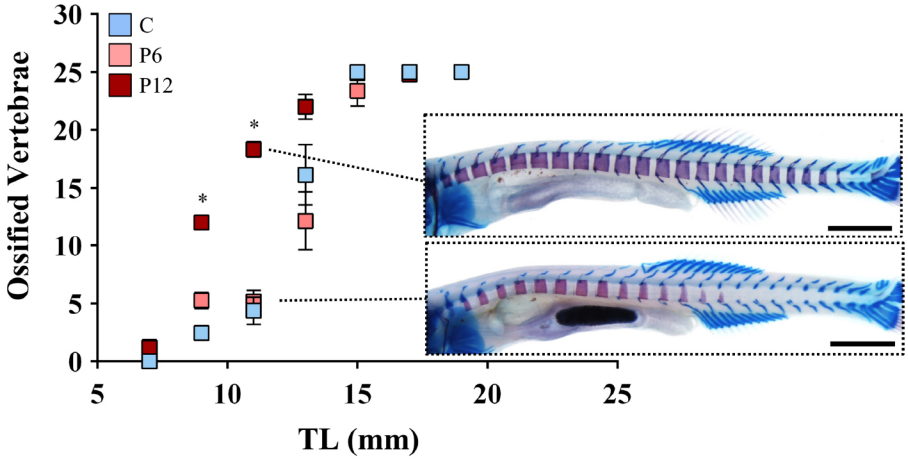

Figure 1. Effect of the experimental diets (C, P6, P12) on the ossification rate of the vertebrae centra in sea bass larvae. The asterisk (*) indicates significant differences. Scale bars equal to 1mm. TL, Total Length.

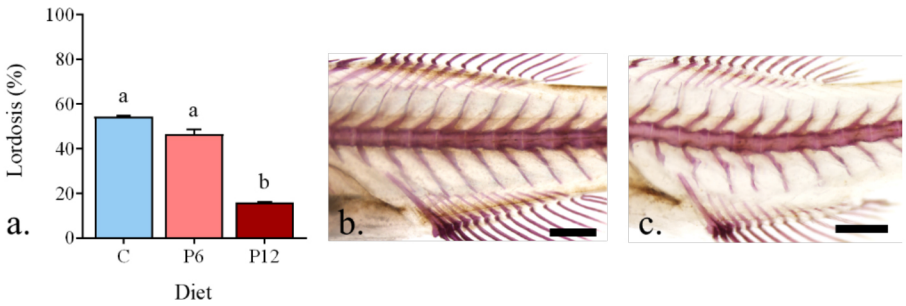

Figure 2. Effect of the peptide diets (C, P6, P12) on the swimming-induced lordosis (a). (b, c) Characteristic images of a normal haemal vertebral column and a lordotic one respectively. Lack of a common letter indicates statistically significant differences.

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AQUAPONICS OPTIMIZATION: ASSESSING SYSTEMS OF SUSTAINABLE PRODUCTION AND CONSUMPTION GLOBALLY

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Introduction

Sustainable food production depends on the recovery of water, energy, and nutrients from waste streams within existing supply chains. Aquaponics (AP) is a system composed of both hydroponic systems (HYP) and recirculating aquaculture systems (RAS) with biofilters in between where bacteria convert organic matter from RAS wastes into nutrients to fertilize HYP edible crops. Such systems are gaining the attention of entrepreneurs and policymakers globally because of their ability to meet local consumer demand for fresh produce from vegetable/fruit crops and high-quality protein from fish. The widespread adoption of aquaponics as a 'green' sustainable food production system needs further research and innovation to optimize system performance through enhancing biofilters, reducing reliance on fossil fuels, and assisting producers in transitioning to energy-efficient AP designs that fit their local human and natural resources while supporting them in management, marketing, and distribution decisions.

Globally, AP systems' feasibility and environmental footprint depend on climate, technology adoption levels, and cultural contexts. To address these needs, "Aquaponics Optimization" leverages socioeconomic expertise and stakeholder knowledge to create a genuinely transdisciplinary team on five continents with core research partners in Sweden, Germany, Turkey, Taiwan, South Africa, Brazil, and the United States.

The partners aim to advance AP technology and implementation that supports regional environmental, economic, and social sustainability objectives. The project aims to enhance a climate-change-resilient food production system that:
1. Reduces, reuses, and recycles inputs while increasing nutrient and water efficiencies.
2. Reutilizes industrial and agricultural wastes to turn them into food, energy, and profit.
3. Provides ways to produce food that adapt to local climate change conditions.
4. Builds community capacity through skills development and knowledge exchange.

Methodology

In this study/ poster, the Aquaponics Optimization team will share their overall interdisciplinary research design of connecting scientific advancement and optimization of the RAS and HYP system and required infrastructure while at the same time using social science methods to assess stakeholders' best practices. The team will primarily collect data through semi-structured interviews.

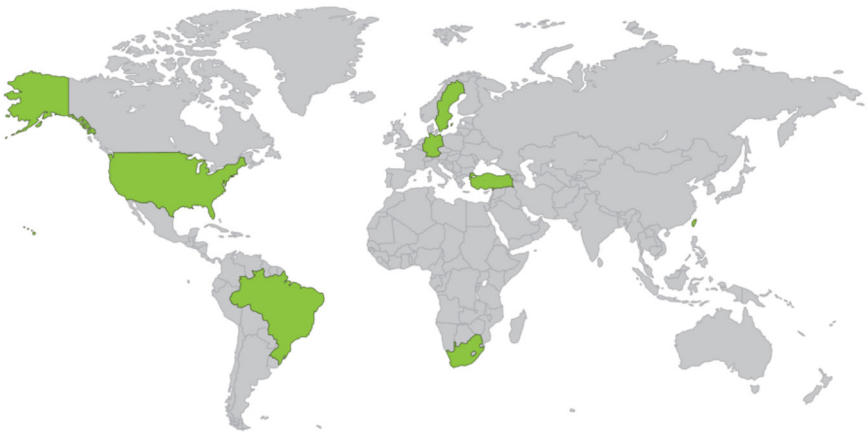

Figure 1: Global network of core collaborators.

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As a first step, Aquaponics Optimization aims to identify active, commercially operating aquaponics farms (in addition to their database of over 600 aquaponics farms) globally and solicits self-registration through a form (docs. Google) and the snowball principle following takeaways from earlier AP surveys (Love et al. 2014; Pattillo et al. 2022).

Based on the directory, the team will identify a representative sample of operating aquaponics farms to conduct semi-structured interviews, following the methodology established in previous interdisciplinary research (Horn et al. 2023). As part of this study/ poster, the team will share its framework for the questions and interview guide for its semi-structured interviews. The main topics that Aquaponics Optimization will address in stakeholder interviews include regional market analysis, consumer preferences, marketing strategies, supply chain issues, social benefits, food security advancement, and regulatory frameworks in selective cultural contexts across the world.

Commercial aquaponics producers from the global database of active commercial aquaponic farms will be recruited for semi-structured interviews. The interview guide will undergo an Institutional Review Board (IRB) review. Once the interviews have been conducted, their transcripts will be qualitatively content coded utilizing the established framework structure within the qualitative data analysis software Atlas.ti. The dissemination of the findings will be based on a collaborative analysis and review process of the interviews, combined with literature and policy data.

Results

The data collection about the emerging aquaponics industry globally will generate the following results: The directory entries will be consolidated and analyzed using Tableau Prep and Tableau by the Aquaponics Optimization team at the University of Washington. Results will indicate the current scale of aquaponic farming worldwide. Additionally, the team will use the data to create a searchable Global Aquaponic Directory for public use.

The results of the semi-structured interviews will be published in peer-reviewed journal articles. Five core questions will be addressed: (a) market analysis of AP operations around the world in different cultural contexts; (b) roadmap to increase marketability of AP; (c) assessment of social benefits and empowerment through AP; (d) opportunity map for AP to address food insecurity; and (e) regulatory barriers for the advancement of AP identified by stakeholders.

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AQUAPONICS FOR FRONTLINE COMMUNITIES: SYNERGIES BETWEEN BENEFITS OF DIFFERENT OPERATION TYPES

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Introduction
Aquaponics (AP) has received much praise for its manifold environmental benefits as a system that recovers and reuses water, nutrients, and energy; economic potential for creating local healthy and safe food and jobs in the green economy; and potential to generate social community and educational benefits. This wide range of benefits is partly possible through the different types of AP that are typical in and around cities, such as large commercial production farms (Type 1), urban, commercial mixed-income AP (Type 2), community and educational operations (Type 3 a and b), and domestic AP (Type 5) (Proksch et al. 2022). While the benefits and types are well understood, more research needs to be conducted on how the different farm types could be integrated into specific neighborhoods. How would a mix of AP operations support a just transition towards a circular community?

This study investigates how the urban integration of AP operations helps to establish circular city principles in the frontline communities of South Park and Georgetown in Seattle, WA.

Methodology
Based on the speculative integration of different AP types in the same urban area, this investigation first establishes the (1) urban capacity of the two frontline neighborhoods for the integration of AP by identifying suitable sites and warehouse roofs for AP operations (Figure 1). It then assesses the (2) resources and infrastructure needed to operate the most common five types. An operating case study example for each type of AP has been identified (Figure 2), and their needs have been adjusted to the climate, geographic, and socioeconomic conditions in Seattle, WA. In the third step, (3) a representative available site is matched with an enclosure type most appropriate for each operation type. For example, Type 1, the large commercial production farm, will be assumed to occupy an on-grade greenhouse (Figure 3). Type 2, the urban, commercial mixed-income AP operation, will be co-located with an existing (food) business in a rooftop greenhouse. In the final step, the inputs, outputs, and benefits of the five types of operations will be compared. The inputs include data on start-up funding, potential funding sources, resources needed for operation, and the environmental footprint. As part of the outputs, yields, jobs, training opportunities, and non-monetary benefits are considered.

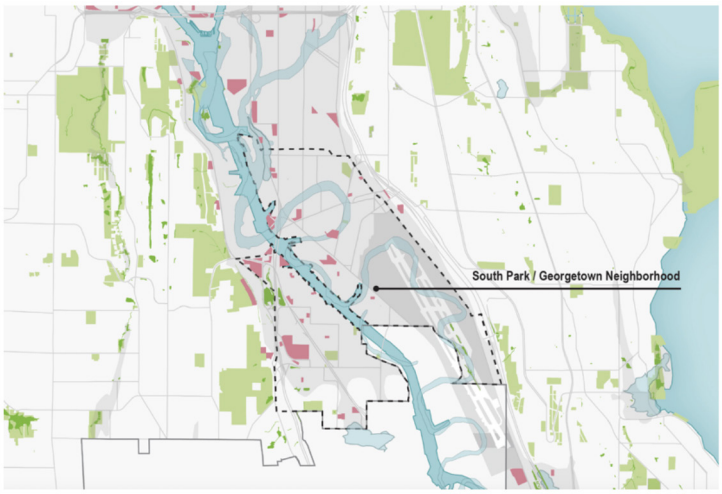

Figure 1: Suitable sites for AP operations in two frontline communities in Seattle, WA.

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Figure 2: Farm on Ogden in Chicago, IL, case study for Type 3.

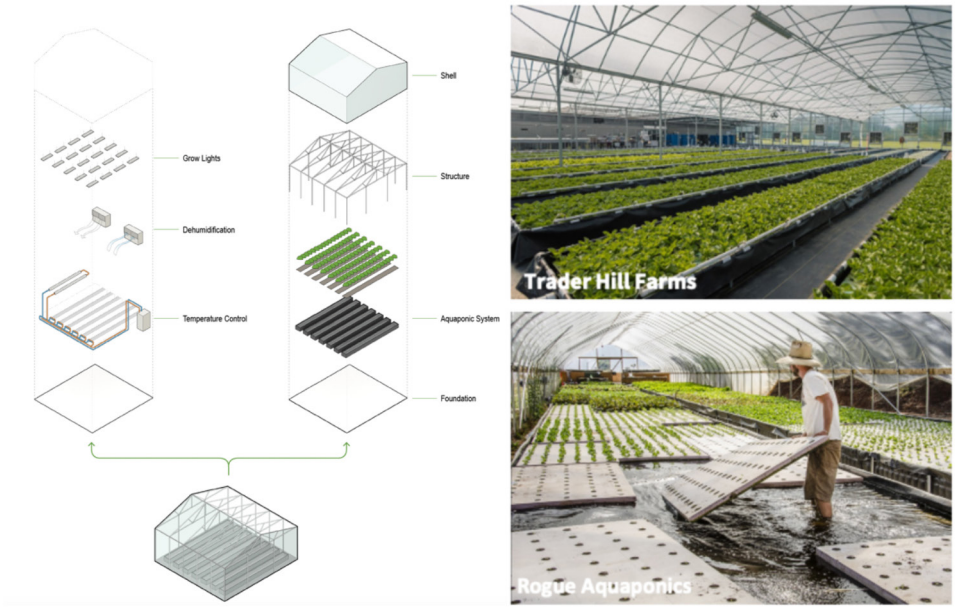

Figure 3: On-Grade greenhouse and possible operating case studies.

Results

The assumptions and data collected for the five AP types will be compared in a graphic format. The main assessment criteria are the return on investment measured in different community benefits and the environmental footprint. The investigation concludes with a recommendation that a mix of aquaponics operations would make the most significant impact in transforming Seattle's frontline communities, South Park and Georgetown, into a circular community.

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CO-LOCATING AQUAPONICS GREENHOUSES AND BREWERIES: COMBINING FISH, HOPS, AND BEER PRODUCTION TO MITIGATE EFFECTS OF CLIMATE CHANGE

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Introduction

The growing interest in aquaponics (AP) reflects its positioning as a technology able to help advance the implementation of circular city principles (Proksch 2023) and support the sustainable development of food systems (Horn et al. 2023). The co-location of AP operations with other (food) industries further increases resource efficiency (Baganz et al. 2020). One promising co-location partner is breweries (Horn and Proksch 2020); a related one that has not received as much examination yet is hops production. Globally, hop production has decreased due to abnormally hot and dry growing seasons. Hops are grown in temperate climates; about 80 percent are produced in Germany and the United States. In Washington State, hops are commercially grown in the Yakima Valley, located in the eastern part of the state. This speculative investigation co-locates an AP production greenhouse with one of Washington State's largest breweries and indoor hop production in Georgetown, an industrial neighborhood in South Seattle.

Methodology

Potential sites for AP facilities are identified adjacent to the five existing breweries in Georgetown. Locating vacant sites and warehouses reveal proximities and potentials for co-location. Each brewery facility is studied for greenhouses sites, and Elysian Brewery is identified as the primary case study.

The Elysian Brewing complex already offers an existing bar and restaurant and can be expanded with a rooftop greenhouse, outdoor growing, indoor growing, and a fish farm (Figure 2). This program accommodates community space and education facilities. With this co-location, the waste heat and spent grain from the brewery can be used as resources for the AP facility to produce nutritious food.

Results

The combination of all three production facilities for fish, hops, and beer in one complex (Figure 3) fosters resource and energy-efficient production of all three products, generates an urban, commercial mixed-income (Type 2) facility (Proksch et al. 2022), and helps to mitigate effects of climate change.

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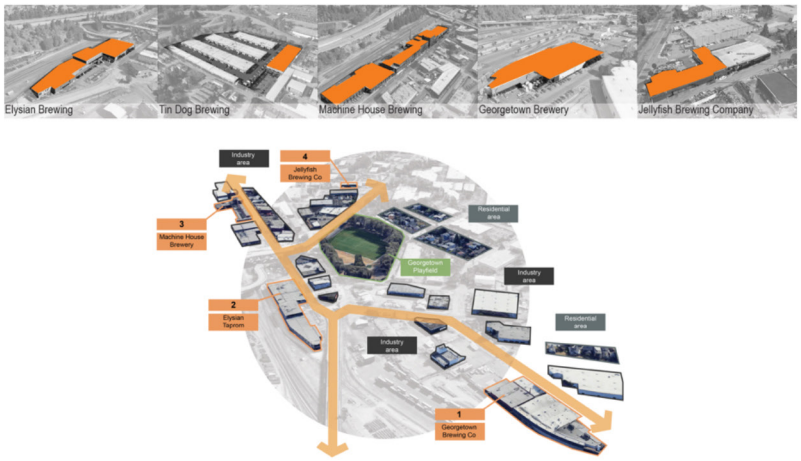

Figure 1: Sites of breweries in Georgetown in Seattle, WA.

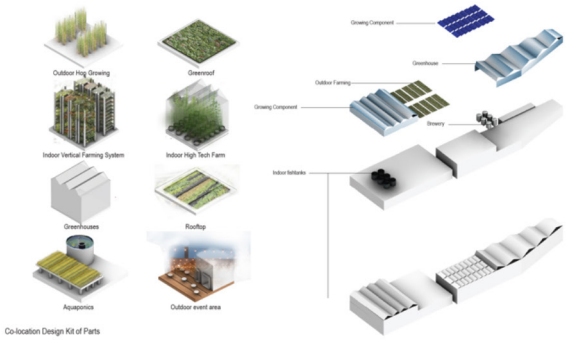

Figure 2: Kit of parts for the integration of an AP facility in the Elysian Brewing facility.

Figure 3: Co-location of an AP rooftop greenhouse and hop growing with an industrial brewery.

DIETARY SUPPLEMENTATION WITH VARIOUS YEAST PRODUCTS AND EXTRACTS ON GROWTH PERFORMANCE, IMMUNOLOGY AND HISTOMORPHOLOGY OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Yeasts products have been proved to be as functional nutritional supplements in fish diets with beneficial effects on growth, immune response and gut health (Agboola et al. 2021). This is mainly due to the high concentrations of mannan-oligosaccharides (MOS), β-glucan and chitin in their cell walls. Despite the importance of gilthead seabream (*Sparus aurata*) in aquaculture, there is limited relevant information and thus this study aimed at evaluating the effects of dietary supplementation with various yeast products on growth, immunology and histomorphology of the species.

Materials and Methods

S. aurata juveniles of 3.36 g initial mean weight were stocked at 12 tanks (125L) in a closed seawater recirculation system. Fish in triplicate groups (22 fish/tank, 3 tanks/dietary group) were fed to satiety, twice a day for 75 days, each of the six isonitrogenous (50%), isoenergetic (21.5 MJ/Kg) and isolipidic (15%) diets: a Control, three diets supplemented with 2% of either grain distillers dried yeast (GDY), brewery yeast (BY) or concentrated autolyzed yeast (AY), a diet (MOS) supplemented with 0.05% of a commercial premium yeast fraction from *Saccharomyces cerevisiae* containing mainly MOS, and a diet (LY) supplemented with 0.25% of a commercial live yeast product. At the end, fish were weighted to measure growth and feed utilization parameters. Blood samples from 18 fish per group were used for immunological analyses, while liver and foregut samples from 15 fish per group were used for histology.

Results and Discussion

The 2% supplementation with yeast products (GDY, BY, AY) significantly increased the feed intake, but that did not improve fish growth, while also decreased feed efficiency (Table 1). In fact, BY led to impaired ($P<0.05$) growth compared to the control group. MOS and LY did not exert any significant influence on fish growth and feed utilization compared to the control group. The histological analysis of seabream fed the experimental diets revealed a normal and similar histomorphological structure of liver and foregut among all dietary groups, but all yeast-based diets increased the intestinal epithilial brush border width with the highest values found in MOS and LY groups (results are not shown). GDY and BY increased the antibacterial activity against *E.coli,* but BY was slower to assemble the complement complexe. BY also tended to increase the trypsin inhibition and to decrease the ceruloplasmin activity, while LY tended to increase the alkaline phosphatase activity. MOS increased significantly the trypsin inhibition but reduced the antibacterial activity of the complement. The autolysed yeast did not show any significant effect on the immune system of the tested fish.

These results indicate that distiller grain yeast, brewery yeast and live yeast should be further studied as they may improve the immune system of *S. aurata*, despite the negative effect of the first two on feed efficiency. MOS may also be an efficient supplement showing some conflicting results with increased protection against pathogen evasion but decreased antibacterial activity, without affecting seabream's growth performance. Several studies suggested that substantial dietary levels of yeast products did not have an adverse effect on growth and feed efficiency of *S. aurata* (Fronte et al. 2019, Reis et al. 2021, Nazzaro et al. 2021). Some gut morphological changes have been observed in the studies of Dimitroglou et al. (2010) and Fronte et al. (2019). Ortuño et al. (2002) reported that 1% *S. cerevisiae* yeast in the diet at 1% did not enhance the serum complement titres but did enhance the cellular innate immune response of *S. aurata*. Rodriguez et al. (2003) reported decreased serum peroxidases and complement activity and an increased lysozyme activity with 1% *S. cerevisiae* yeast dietary supplementation. When Reis at al. (2021) used a diet with 0.06% β-glucan dietary concentration extracted from yeast found no changes in plasma innate immune status. Further studies are needed to enlighten the effects of yeast products on *S. aurata* nutrition and immunology.

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Table 1. Growth, feed utilization and immunological parameters of *S. aurata* fed with the experimental diets.

Parameters / dietary groups	Control	GDY	BY	AY	MOS	LY
Growth performance and feed utilization						
Survival (%)	97.0±2.6	93.2±3.2	93.2±3.2	92.4±2.6	90.9±5.8	90.9±5.8
FI (% BW/day)	3.0±0.1^{c}	3.7±0.1^{a}	3.8±0.3^{a}	3.6±0.1^{a}	3.1±0.2^{bc}	3.5±0.1^{ab}
FW (g/fish)	40.1±1.9^{a}	36.2±2.2^{ab}	34.4±0.6^{b}	35.4±1.0^{ab}	37.9±2.5^{ab}	37.2±1.5^{ab}
SGR (%/day)	3.32±0.06^{a}	3.18±0.08^{ab}	3.12±0.02^{b}	3.15±0.06^{ab}	3.25±0.09^{ab}	3.22±0.06^{ab}
FCR	1.01±0.02^{b}	1.22±0.04^{a}	1.19±0.05^{a}	1.21±0.04^{a}	0.98±0.05^{b}	1.01±0.11^{b}
PER	1.82±0.04^{ab}	1.65±0.06^{b}	1.69±0.08^{b}	1.67±0.05^{b}	2.01±0.12^{a}	1.88±0.19^{ab}
Immunological parameters						
E.coli GI (%)	64.43±3.99^{ab}	66.92±5.40^{b}	66.88±3.57^{b}	63.63±5.41^{ab}	60.03±2.01^{a}*	64.16±4.84^{ab}
CA (min)	35.0±7.3^{ab}	35.0±7.38^{ab}	42.7±8.3^{b}*	33.5±6.6^{a}	35.0±7.5^{ab}	32.0±7.7^{a}
NO (μM)	0.252±0.085	0.293±0.100	0.253±0.079	0.250±0.081	0.214±0.065	0.234±0.067
TI (%)	95.05±6.34^{ab}	96.98±2.52^{a}	98.16±1.98^{ab}	97.69±2.47^{ab}	98.86±0.94^{b}*	96.96±5.64^{ab}
CP (U/ml)	9.61±8.28	8.03±6.25	5.07±4.49	9.22±7.31	8.71±4.92	9.48±6.08
ALP (U/ml)	7.13±1.24	7.02±1.14	7.71±1.48	7.10±1.54	7.53±1.58	8.18±2.02

Note. Values represent means ± standard deviation. Values within each bearing a different superscript letter are significantly different (ANOVA, $P < 0.05$). FI, feed intake; FW, final weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion rate; PER, protein efficiency ratio; GI, growth inhibition; CA, Complement assembly; NO, nitric oxide concentration; TI, Trypsin inhibition; CP, Ceruloplasmin activity; ALP, Alkaline phosphatase.

Acknowledgements

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POPULATION STRUCTURE ANALYSIS OF BIVALVE SPECIES *Arca noae* FROM THE CENTRAL ADRIATIC SEA

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Introduction

Along with the species *Pecten jacobaeus*, *Venus verrucosa*, *Mytilus galloprovincialis* and *Ostrea edulis*, the species *Arca noae* is one of the most important commercial bivalves overfished in the Adriatic Sea. Although much is known about the biology of the species, there is currently insufficient data on the intensity of fishing and the status of exploited populations on the east coast of the Adriatic. The minimum length of individuals (LML) allowed for fishing is 50 mm (Officiale Gazzete 63/2010), and the species reaches the indicated length at 3 to 7 years of age (Peharda et al., 2012). Although *A. noae* inhabits much of the eastern coast, recent studies indicate overfishing in certain areas. In the southern Adriatic basin (Maloston Bay), individuals below the legal minimum length accounted for 61% of commercial catches, and in the central Adriatic basin (Pašman Channel), the proportion exceeded 74% (Peharda et al. 2009). Therefore, the aim of this work was to determine the extent of fishing pressure on the population in Kaštela Bay in the central Adriatic Sea in order to gather information to assess the status of the population in this area.

Material and methods

The length composition of 50 individuals in the commercial catches was determined by the proportion of individuals shorter than the minimum length allowed for fishing (LML). In addition to describing the composition of individuals in terms of gender and size, the reproductive potential of the species and current selection pressure on individuals of a particular gender and length categories were analysed. Morphometric parameters were determined for all individuals and the gender of each individual was determined by histological methods.

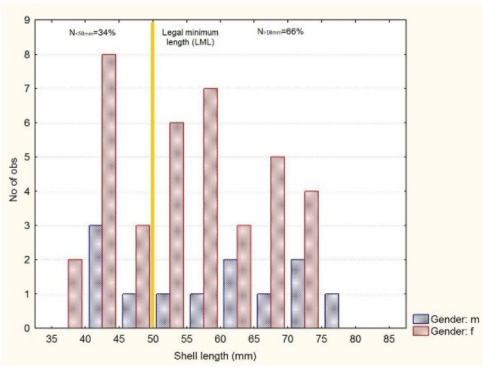

Fig. 1. Length frequency histogram of *Arca noae* individuals from the commercial catch in relation to the legal minimum length (LML)

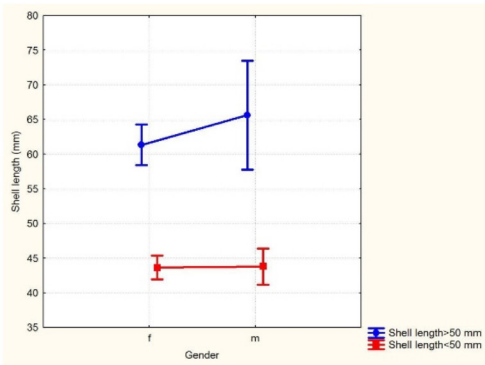

Fig. 2. Differences in mean length values of younger and older males and females from Kaštela Bay

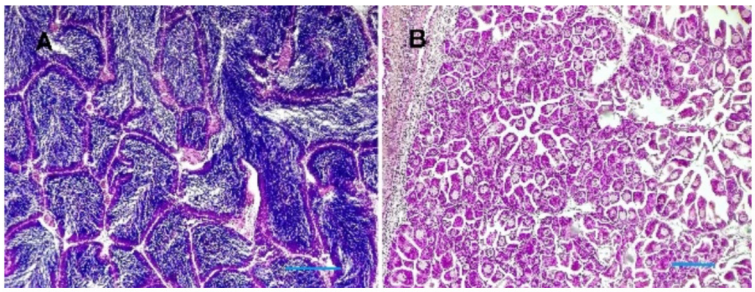

Fig. 3. Partially spawned male (A) and female (B) (scale bar: 300 µm)

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Results

Of the 50 individuals from the commercial catch, 12 (24%) were male, while 38 (76%) were female. 34% of all specimens were shorter than the legal minimum length (< 50 mm), of which 76.5% were female (Fig.1). Selection pressure was also observed on females with shell length between 50 and 60 mm. Analysis of the average length of captured males and females in both categories showed that captured males in the category above the legal minimum length were longer than females, while the lengths of males and females in the category below the legal minimum length were almost equal (Fig. 2). The reproductive potential of all individuals from both categories was 100% (Fig. 3).

Conclusion

Selection pressure was determined by gender analysis. The population is dominated by younger females, suggesting overexploitation of older females in the past and the current dominance of older males. The results also warn of the possibility of future overexploitation of individuals shorter than the legal minimum length and emphasize the importance of their role in maintaining population stability, as these individuals have the potential to participate in spawning, according to the reproductive potential analysis. The fact that *Arca noae* is not suitable for line-fish farming due to its low survival rate and slow growth (Župan et al., 2014) also contributes to concerns about overexploitation of this species.

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INFORMING AQUACULTURE MANAGEMENT USING *Vibrio* spp. ABUNDANCE AS AN ADDITIONAL INDICATOR OF WATER QUALITY

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Introduction

Bacterial presence in the water column can adversely impact fish health and, therefore, aquaculture production. For example, *Vibrio* spp. are ubiquitous heterotrophic bacteria found in aquatic and marine environments that can cause vibriosis, a potentially fatal disease in humans and aquatic animals (Baker-Austin et al, 2018). Pathogenic *Vibrio* spp. have already been causing mass fish die-offs, thus adversely affecting both farming and the environment; their impact is projected to increase due to climate change and other anthropogenic factors (Sampaio et al, 2022).

Water quality has generally been monitored using bacterial indicators such as heterotrophic plate counts, total coliforms, and fecal coliforms (Some et al, 2021). However, bacterial indicators such as *Vibrio* spp. have been ignored despite their potential importance in monitoring water quality in coastal areas. Although *Vibrio* spp. are regulated for food products (ISO 21872-1:2017), uniform guidelines and official regulations for monitoring their environmental presence in management strategies are still lacking.

Materials and methods

In this study, we examine the applicability of *Vibrio* spp. abundance as an additional indicator of water quality for informing science-based coastal management. To achieve this, we analyzed a three-year open dataset from Mali Ston Bay that included standard environmental and bacterial indicators, as well as *Vibrio* spp. abundance (Jug-Dujaković et al, 2022). To determine whether *Vibrio* spp. abundance provides additional information on water quality, we assessed the seasonal, spatial, and vertical variations of environmental and bacterial indicators such as HPC (heterotrophic plate counts), TC (total coliforms), *E. coli*, enterococci, and *Vibrio* spp. abundance, and their interrelationships. Specifically, we examined: (i) the extent of emissions from the fish farm, (ii) the environmental factors that impact *Vibrio* spp. abundance, and (iii) the variability of *Vibrio* spp. abundance in relation to other potential pathogens, including *E. coli*, enterococci, and heterotrophic bacteria overall. Differences between seasons (warm and cold), sites (fish farm and control) and depths (surface, 5m, 10m and bottom) for both environmental and bacterial indicators were investigated by permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). Relationships between environmental and bacterial indicators were assessed with Pearson's correlation analysis and RDA (Legendre and Legendre, 2012). Finally, the distributions of pathogenic bacteria (*E. coli* and enterococci) in the water column was compared to that of *Vibrio* spp. by using water quality abundance categories (as defined in the Official Gazette of the Republic of Croatia 73/08). Official standards for *Vibrio* spp. for assessing water quality were not yet available, so we estimated one using a threshold of 100 CFU/ml reported to result in bacterial transmission into organisms after prolonged exposure to *Vibrio* spp. (Kim and Lee, 2017). We assumed the 100 CFU/ml critical value represents a threshold defining 'sufficient' water quality, and extrapolated the other thresholds using classification for enterococci, who share the same critical value for 'sufficient' water quality.

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Results

Our results show no differences between the fish farm and control site in terms of environmental conditions, organic enrichment, and bacterial abundance. However, the abundance of heterotrophic bacteria, enterococci, and *Vibrio* spp. was unexpectedly higher during the cold season. *E. coli* and total coliforms, in line with traditional patterns, were more abundant during the warm season. Temperature was found to be the best predictor of *Vibrio* spp. abundance when examining the correlation among explanatory variables in RDA, followed by total phosphorus. This finding reinforces the significance of temperature and the often restrictive nutrients in driving the dynamics of bacterial abundance. According to the thresholds from the Official Gazette 73/08, water quality was generally excellent. The best score was achieved by *E. coli*, with over 95% of samples collected at each site meeting the criteria for excellent quality. Enterococci showed excellent quality in more than 91% of the samples, while less than 64% of samples tested for *Vibrio* spp. fell into the excellent category. The notably lower result for *Vibrio* spp. suggests that either (i) there is a genuine concern regarding the prevalence of potentially pathogenic *Vibrio* spp., or (ii) the thresholds adopted from the enterococci regulations may not accurately reflect the risk of disease.

Acknowledgements

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FEEDING RAINBOW TROUT (*Oncorhynchus mykiss*) WITH GUAR MEAL PROTEIN CONCENTRATE: WHICH IS THE BEST PERCENTAGE OF CONVENTIONAL PROTEIN SOURCE REPLACEMENT?

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Introduction

Since rainbow trout (*Oncorhynchus mykiss*) is one of the most significant freshwater fish species reared, efforts to improve this industry through genetic selection and sustainable diets are being made (Garcia-Ballesteros et al., 2021; D'Agaro et al., 2021).

Soy, rapeseed, wheat gluten and corn gluten are commonly used as protein concentrates in aquafeeds, due to their high digestibility (Parisi et al., 2020). Guar meal, deriving from the endosperm of an Indian cluster bean, the galactomannan polysaccharide Guar gum (*Cyamopsis tetragonalobus*), has already been used in aquafeed, increasing faecal stability (Janphirom et al., 2010). In the last years, it has been purified to reduce saponin, tannin, phytates and protease inhibitor concentration, that are considered to negatively affect growth of salmonids (Pach F. and Nagel F., 2017).

In this trial proprietary guar meal protein concentrate (MYCOPRIME®, Panghea SPA, Milan, Italy) replaced different percentages of conventional proteins in feed for rainbow trout, to investigate its effects on zootechnical performances during the fattening phase of rainbow trout.

Materials and Methods

A total of 2700 rainbow trout (mean body weight 50±1.4g) were reared at the initial stocking density of 15 kg/m^3 in 12 concrete tanks (6x1x0.5m). The principal water physical-chemical parameters (temperature, dissolved oxygen and pH) were recorded daily. Total Ammonia Nitrogen (TAN), nitrites (NO_2-N) and nitrates (NO_3-N) were determined weekly using a spectrophotometer (Hach mod-2005, Hach Company, Loveland, USA) following the American Water Works Association and Water Pollution Control Federation of American Public Health Association (APHA) standard methods (1995).

Trout were fed twice a day *ad libitum*; each diet was administered to fish of three tanks. The Control diet (CD) was a growing feed available for trout with 43% of proteins and 25.3% of lipids (% as it is). The two experimental feeds (D5 and D15) were formulated isoproteic and isolipidic; a partial replacement with guar meal concentrate (5% in the D5 and 15% in the D15) of fish meal, chicken meal and soybean meal was applied.

The trial was performed during a standard zootechnical cycle and lasted 90 days. Final mean weight (g) and Final mean length (cm) were recorded and Palatability was calculated. Water physical-chemical parameters, fish biometric parameters and final productive traits were submitted to one-way analysis of variance (ANOVA) with $p<0.05$.

Results and discussion

The water physical-chemical parameters showed a very similar trend in terms of temperature, dissolved oxygen and pH during the whole trial. The nitrogen compounds demonstrated significant differences in TAN, having the highest ammonia concentration in D15 tank (0.44 ± 0.01 mg/L) compared to CD (0.24 ± 0.09 mg/L) and D5 (0.22 ± 0.06 mg/L). Nitrites ranged between 0.01 ± 0.001 mg/L (D15) and 0.02 ± 0.002 mg/L (CD). Nitrates varied between 0.9 ± 0.1 mg/L (D15) and 1.1 ± 0.4 mg/L (CD) without significant statistical differences. Brinker et al. observed negative effects on fine solid particles using diets with guar meal replacement, that clogged biofilters, in a recirculating water system (Brinker A. and Fredrich C., 2012); this condition occurred also in tank D15.

Concerning the biometric parameters, the final mean weight of trout receiving D5 (201.00 ± 3.7 g) and CD (198.8 ± 3.8 g) showed similar results, significantly major in comparison with D15 (171.2 ± 5.1 g). Final mean length didn't show significant differences. Feed palatability resulted higher in CD and D5 diets in comparison to D15; in fact, presumably, trout fed with D15 reached the satiation before and this condition affected also the final zootechnical performances as shown by the lowest final mean body weight. This negative output could have been caused also by the reduction of the apparent digestibility of dry matter, crude protein, and crude lipid due to the highest inclusion of guar gum (Liu et al., 2022).

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Conclusions

Considering the zootechnical performances, the 5% inclusion of guar meal protein concentrate in fish feed resulted to be positive. Further analysis concerning fish welfare status and feed efficiency are going to be performed to confirm the satisfactory results.

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HOW MUCH DO OYSTERS FILTER MICROALGAE INSIDE AN IMTA POND? - FIELD TRIALS FOR MODEL CONSTRUCTION

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Introduction

Integrated Multi Trophic Aquaculture requires an equilibrium between farming different organisms in the same system. This requires an understanding of the biology and interactions between species. In earth ponds one of the main drivers of the dynamic of the system are microalgae and how the farmed species, either freshwater or seawater fish, bivalves and seaweeds, influence the population of microalgae. In Portugal since 2010 the EPPO has been studying the integrated culture of oysters in fishponds. From then EPPO has being building a larger database of technical, biological and water quality information to help create a model of IMTA for earth ponds. One key information for this database is the clearance rate of microalgae by oysters inside fishponds across a temperature gradient (reflecting the seasons). This poster will show the methodology to achieve this information in a reliable and affordable way.

Materials & Methods

All trials were performed in the earth ponds of the Aquaculture Research Station in Olhão, South of Portugal. The testing procedure involved using three acrylic cylinders: one served as a control, and the other two were replicas (Figure 1a). These cylinders were placed in the outlet of an earth pond fish culture, maintaining the typical environmental conditions of IMTA cultivation at EPPO (Cunha et al, 2019). The ponds used in all seasons comprised a total density of gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) of 0,8 Kg/m^3. To assess the filtration rates of oysters within the IMTA production system, specifically *Crassostrea gigas*, six individuals were selected for the assay. These individuals had dry weights ranging from 7.23 to 5.13 g.

Each of the three cylinders had a volume of 49.59 L, a water height of 1.75m, and a diameter of 0.19m. The cylinders were filled with water from the pond used and were continuously aerated to ensure constant water circulation and gas exchange with the atmosphere. Within each replica (C2 and C3 in Figure 1c), three oysters were placed in baskets suspended by ropes, approximately at the centre of the cylinders. The entire test spanned a duration of 3 hours, from 15:15 h to 18:15 h, during which environmental parameters within each cylinder were measured at 15-minute intervals.

Measurements included chlorophyll-a concentration (µg/L), pH, temperature (°C), and salinity, which were determined using a multiparameter system (model EXO 2, YSI/Xylem, USA). Additionally, the dissolved oxygen concentration (mg/L) was measured using a portable probe (model H9829, Hanna Instruments, USA) (refer to Figure 4, b). The dry weight and mineral content of the oysters, encompassing both meat and shells, were assessed by subjecting them to specific conditions in an oven (60°C for 24 hours) and muffle furnace (core: 525°C until white/grayish color, shell: 600°C for 2 hours), respectively.

Results & Discussion

Three different temperatures were test for oyster filtration rate: 16ºC, 20ºC and 26ºC. At the start of each test, chlorophyll a mean concentration for the three cylinders used was, respectively, 4,5 ug/L, 49,5 ug/L and 9,6 ug/L.

At 16ºC Chla concentration were not significantly different between control cylinder (without oysters) and both cylinders with oysters across the 3-hour period. At 20ºC oysters manage to decrease the microalgae growth inside the test cylinders by up to 34% compared with the control. Final Chla concentration after 3 hours at this temperature were 94 ug/L for Control and only 71,7 ug/L in test cylinders. At 26ºC, Chla concentrations in Control increase from 10,6 to 17,76 ug/L (an increase of 67%) and in test cylinders there was a decrease from 9,1 to 1,2 ug/L. This decrease shows that the oyster filtration rate was much higher than the microalgae growth, leading to a depletion of food for the oysters if the test continued a couple more hours.

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Although the initial microalgae concentrations for the three temperatures tested where significantly different as mentioned above, we could see that the filtration rate is higher at 20ºC compared with 16 and 26ºC ranges and similar to previous clearance rate tests of *Crassostrea gigas* (Bougrier *et al* 1995; Haurea *et al* 2003; Dupuy *et al* 2000; Nascimento *et al* 2022).

With this information on filtration rates for *Crassostrea gigas* we can see that temperature and microalgae concentration are key variables to estimate the oysters integration with fish and this varies substantially across seasons. Oyster density along the production cycle should be managed to avoid microalgae depletion at all seasons and especially when high oyster density and lower microalgae growth rates (usually below 18ºC) occur at the same time. Managing microalgae population across all seasons in an IMTA pond with fish and oysters is the main key to allow for better oxygen levels, proper performance of oysters and fish and avoidance of stressful conditions for all farmed species. Knowing how much oyster filtration rates affect these dynamics is vital to application of IMTA principles to pond aquaculture of fish and bivalves.

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ESTABLISHMENT OF ADEQUATE TAURINE LEVELS FOR THE WEANING OF THE GREATER AMBERJACK (*Seriola dumerili*)

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Introduction

The greater amberjack (*Seriola dumerili*) is a marine fish with great consumer acceptance than can reach up to 2 kg in 18 months. However, seriola's culture still face a series of bottlenecks that difficult a further expansion of this species aquaculture. Among them, the larval rearing phase faces great difficulties due to the limited bibliography of culture conditions and nutritional requirements, which produces a low quality of the juveniles, and therefore a limited market availability.

Taurine is an essential nutrient for larval development and growth, mainly in carnivorous marine fish such as the greater amberjack (Djellata et al., 2022). However, there are no available data on dietary taurine supplementation during the weaning period of this species.

Materials and methods

Four granulated microdiets containing 0.24 to 4.24 % of taurine levels were evaluated in 30 days post-hatching (dph) greater amberjack larvae until 44 dph. For that, 2700 larvae of 30 dph were handly distributed in twelve experimental tanks of 200 L volume in an open flow sea water system. During the first days the larvae were co-fed with enriched *Artemia* sp. metanaupli to be gradually weaned, and from day 39 until the end of the trial the larvae received only the microdiets. Each diet was offered in triplicate tanks from 8:00 am to 19:00 pm, and at 44 dph, survival, growth, the histology of liver and gut, the expression of growth and stress-related genes, and the incidence of skeletal anomalies were evaluated.

Results

There were no significant differences in survival because the administration of the different diets, however, regarding growth performance, the larvae fed 1.24% of taurine presented higher final weight and length, as well as higher total and daily weight gain.

Regarding the histological results, the level of vacuolization in liver was directly proportional to the level of taurine supplementation, with the highest levels reported for the larvae fed taurine at 4.24%. There were observed some intestinal lesions along the intestine without differences between treatments, however with the highest percentage of incidence (around 13%) in the larvae fed taurine at 4.24%.

Additionally, the larvae fed with 1.24 % taurine level had a higher expression of growth and stress-related genes (gh, igf-ii, crh and trh) at 44 dph.

The evaluation of skeletal anomalies revealed that treatment of taurine at 4.24 % also produced a higher incidence of total skeletal anomalies, followed by the treatment with the lowest taurine supplementation. The main anomalies founded were alterations in the vertebra of the pre-hemal and hemal regions, as compression or fusions of the vertebral bodies or kyphosis.

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Discussion and conclusions

The present results suggest the importance of adequate taurine supplementation in the weaning of the greater amberjack, as it impacts the growth, liver vacuolization, gene expression, and the incidence of skeletal anomalies in the fish. Despite including marine protein sources in the microdiets, a moderated level of taurine supplementation (1.24 g/100 g diet dry mass) is recommended for greater amberjack larvae. Additionally, excessive levels of dietary taurine (4.24 g/100 g diet dry mass) caused impairment in larval growth and quality.

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MONITORING *Neobenedenia girellae* INCIDENCE IN THE GREATER AMBERJACK (*Seriola dumerili*) IN CANARY ISLANDS

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Introduction

Parasitic infestations are a significant bottleneck for the further expansion of the culture of different aquaculture species as the greater amberjack (*Seriola dumerili*), producing mass mortalities and instability of the offer and production costs. *Neobenedenia girellae* is one of the main parasites which cause skin infection in carangids, feeding on their mucus and epithelial cells and causing haemorrhage, hyperproduction of mucus, inflammation, and epidermis thickening (Hirazawa *et al*., 2013; Fernández-Montero *et al*., 2019). Thus, an efficient approach for controlling these parasitic infestations is of great importance to developing the culture of *Seriola* sp., among them freshwater or formaldehyde baths and oral treatments with antiparasitic drugs (Hirazawa *et al*., 2004; Hirazawa *et al*., 2013). However, an integrated approach considering the local specificities is necessary to maximize the protocols' effectiveness. For those reasons, the present study aimed to assess the local specificities of *Neobenedenia girellae* life cycle in the context of the last challenges for climate change resilience in the Canary Islands, Spain.

Materials and methods

From April to July 2023, juveniles of greater amberjack placed in open seawater system were monitored every two weeks to determine the presence of *Neobenedenia girellae*.

For that the animals were anesthesized with clove oil:ethanol 1:1, and subjected to a freshwater bath for 4 minutes (Hirazawa et al., 2013). After the bath, the water was filtered thought a 125 μm net, and the net was examined under a stereoscope to determine the presence of adults or eggs of *N. girellae*.

In table I are resumed the conditions during this period.

Once the presence of parasites was determined, alive specimens were carefully collected from the skin surface with a scalpel and placed in plastic dishes (10 cm diameter) filled with natural seawater. Additionally, white segments of polyester threads (around 50 cm length), were placed in the tanks and monitored every 24 h to check the presence of *N. girellae* eggs. Polyester threads when then placed in 300 ml buckets filled with natural seawater. Water from both plates and buckets was removed and replaced daily with clean seawater and temperature was monitored. The time to hatching was monitored though daily visualization of the eggs under a stereoscope. Additionally, measurements of adults and eggs (n=30) were made under the stereoscope with the program Leica Application Suite (Leica Microsystems Ltd., Heerbrugg, Switzerland).

Table I. Experimental conditions.

	April-May	June-July
Juveniles mean weight (g)	173.29±23.81	249.59±49.71
Juveniles mean lenght (g)	22.41±1.03	26.01±1.97
Tanks temperature (°C)	20.5-21.5	21.5-23.8
Tanks volume (L)	300	1000

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Results

The first detection of parasitation of *N.girellae* on the juveniles was determined in early July, with water temperatures rising from 23°C, and severe parasitation levels occurred from that moment in only two weeks between treatments with freshwater baths. Collected *N.girellae* adults placed in plates spawned in 24 h (25-27 °C). The mean fecundity was of 58.82±33.59 eggs per day and adult. Adults survived for a maximum of 72 h without a host. Egg hatching occurred from 4 to 8 days at mean temperatures of 26.5±2.38 °C and 24.8±2.86 °C, respectively. The oncomiracidia died in a maximum of 48 h without a host.

The mean area and perimeter of the eggs were 0.01±0.00 mm^2 and 0.44±0.04 mm, respectively, being the mean total length of adults of 3.96±0.68 mm.

Discussion and conclusions

In the context of climate change and ocean warming, adapting to new conditions and challenges will determine the success of sustainable aquaculture (Glencross *et al*., 2023). Moreover, as most aquatic species' life cycles are greatly affected by environmental conditions, microbial and parasitic infestations are expected to increase, so resilient and adapted protocols must be developed.

Although data obtained for *N.girellae* fecundity and adult size is lower than those reported by other authors (Hirazawa *et al*., 2013; Fernández-Montero *et al*., 2019), the record temperatures achieved in the North Atlantic in 2023, with 2 degrees higher than expected (Climate Change Institute, 2023), has aggravated the population densities of *N.girellae,* producing severe infestations in only two weeks between control treatments. Therefore, the periodicity of preventive procedures must be adapted, being recommended to be made weekly under the conditions described. In this context, establishing adequate oral treatments is a necessary tool to control the increasing prevalence of parasitic infestations.

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CHARACTERISATION OF THE OLFACTORY ORGAN OF SENEGALESE SOLE (*Solea senegalensis*) USING SINGLE-NUCLEI RNA-SEQ

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Introduction

Senegalese sole *(Solea senegalensis*) is an emerging aquaculture species in Europe. However, despite large investments, bottlenecks in reproduction still curtail the expansion of this industry. This flatfish can reproduce in captivity when captured from the wild, but males reared in captivity (F1) fail to reproduce despite they produce viable sperm for fecundation. So reproduction is the main constraint in Senegalese sole aquaculture and consequently, the pursuit for potential solutions has been approached through different fields. It has been hypothesized that chemical communication might underlie sole courtship failure and that environmental factors associated with different life stories of F1 *vs* wild males may underlie the low performance of F1 males.

The fish olfactory organ, an essential player for chemical communication, is a multilamellar and rosette-shaped structure, composed of an epithelium embedded with olfactory sensory neurons expressing individual olfactory receptors. Despite its critical role in social relationships and individuals' interaction with their environment, the olfactory system remains understudied. In particular, while its role in mammalian reproduction is well-established, its involvement in fish reproduction is poorly characterized. The aim of this work is to improve our knowledge of the fish olfactory system, and in particular, in the context of reproduction. To do so, we conducted a single-nuclei RNAseq (snRNAseq) analysis of the olfactory organ, enabling the characterization of transcriptomic profiles at the individual cell level featuring the different cell populations and their heterogeneity.

Material and Methods

The olfactory organ was collected from 3 different Senegalese sole fish (1 wild male, 1 wild female & 1 F1 male - born in captivity). A total of 6 samples, two samples per individual considering dorsal and ventral olfactory rosettes, were dissected and flash frozen separately. Nuclei were extracted using a modified protocol (REF) and processed using the Single-cell/nuclei RNA-seq Chromium 10X pipeline. Single-nuclei RNA-seq libraries were sequenced in a Novaseq 6000 as 150PE reads. Raw sequencing was aligned against the most recent Senegalese sole genome (GCA_919967415.2, de la Herrán et al. 2022) using STARsolo (Kaminow et al. 2021) and analysed using the R package Seurat (Stuart et al. 2019).

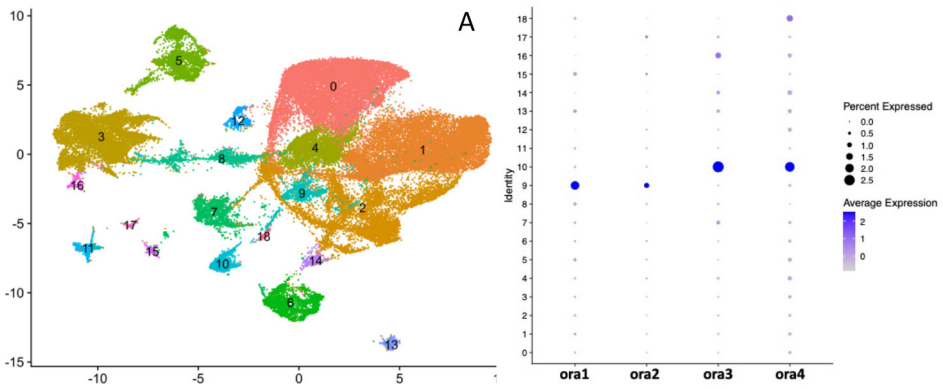

Fig. 1: (A) UMAP of the clustering. (B) Dotplot of ORAs expression in each cluster.

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Results

The six Senegalese sole single-nuclei libraries (wild female, wild male and F1 male x dorsal and ventral olfactory rosettes) were successfully sequenced, generating a total of 59,616 high-quality cells after filtering and quality controls. Cells were clustered according to their transcriptomic profile, resulting in the identification of 19 different cell populations present in the olfactory organ of Senegalese sole. A first inspection revealed no differences between the dorsal and ventral olfactory rosettes. The characterization of the different cell clusters showed a diverse set of cell types, including several groups of neuronal cells, immune cells, blood cells, secretory cells, ciliated cells, and fibroblasts, among others. This cell diversity along with the complex organization of the organ suggests that multiple biological processes are supported by the olfactory organ. In particular, we identified four ORA genes (ora1, ora2, ora3, ora4), which belong to an olfactory receptor (OR) family directly associated to chemical communication and pheromone perception. These genes exhibited low expression levels, which directly correlate to the rule 'one receptor-one neuron' applied to all ORs, and were concentrated in two clusters, cluster 9 (ora1, ora2), and cluster 10 (ora3, ora4). We characterized these clusters as neural cell types since ORs are always expressed in neurons (Fig. 1). Further analyses are in progress to functionally characterize the different cell populations, especially those neural subtypes potentially involved in reproduction.

Conclusions

The olfactory rosette of Senegalese sole is a complex and heterogeneous organ consisting of 19 cellular clusters with a diverse range of functions. The presence of different types of olfactory sensory neurons points to the importance of olfaction in fish, potentially underlying social behaviors such as reproduction.

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FISHPOND SEDIMENT COMPOSITION AND ITS POTENTIAL USE IN AGRICULTURE IN THE CZECH REPUBLIC

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Introduction

At present, there are approximately 24 000 fishponds in the Czech Republic. Decades of inappropriate management of the water catchment area, excessive erosion of arable land and an increase in fish production have led to an accumulation of sediment in ponds. Consequences of this can be a reduction in depth, depletion of dissolved oxygen and accumulation of nutrients, thereby limiting biological and ecological functions and fish production. The removal of sediment from ponds is necessary for fishpond maintenance. Mechanical removal is one of the options. Sediments are rich in nutrients and contain high levels of organic matter, carbonates and calcium. At the beginning of the 20th century, sediments were considered to be quality soil, but today, due to the possible presence of pollutants, they are classified as waste by legislation. This limits the potential application of sediments on arable land. The aim of our study was to evaluate the sediment composition and to compare it with the content of agricultural soils in the Czech Republic and assess the possibility of improving the soil quality.

Material and methods

Sediments were analyzed in 34 fishponds in the Czech Republic. All monitored fishponds are characterized as typical shallow ponds with mainly muddy sediments. The trophic state of the ponds ranged from mesotrophic to hypertrophic. The surface layer (0–15 cm) of the sediment was collected using an Ekman-Birge grab when fishponds were full and with a spoon, during the harvesting/emptying of fishponds. Dry mass of the sediment samples was determined and aqueous leachates from the dry mass were prepared in accordance with the ČSN EN 12457-4 standard. From the dried samples of the sediment, soil extracts were prepared according to the Mehlich III procedure (Zbíral, 2016). Part of the sample was burnt (550 °C, at least 6 hours) for determination of the organic share, and the burnt sample was used for the preparation of a soil extract with aqua regia in accordance with the process set out by Zbíral (2011). Phosphorus (P) and calcium (Ca) were determined from aqueous leachates; available P, Ca, magnesium (Mg) and potassium (K) were determined from Mehlich III; and total P and Ca content from aqua regia. The results are expressed in weight units of dry mass of the sediments used.

Results and discussion

The results obtained show a significant difference between fishponds, but also between samples from the same pond. Dry mass varied between 25 and 45%. Phosphorus (P) values also varied widely. In aqueous leachates, the average value was 5.36 mg.kg^{-1}. Available P in Mehlich III, which is commonly used to test soil composition, ranged from 2.2 to 104.3 mg.kg^{-1} with an average value of 25.1 mg.kg^{-1}. The average total P was 935.6 mg.kg^{-1}. The content of available P in sediment is lower than in arable soil, but the total P content in sediment is higher than in arable land. The average of available Ca was 21 g.kg^{-1} and a total Ca was 48 g.kg^{-1}. Large differences in Ca are affected not only by bedrock type but also by fishery management and lime application. Calcium content is much higher in sediments than in soil. The values of available and total Mg are higher in sediment than in arable soils, while the values of K in sediments are similar to those in arable land. One of the most important indicators of the quality of agricultural soils is organic matter content. The share of organic matter in soils is most often expressed as a percentage of organic carbon. Long-term monitoring shows a wide range of organic carbon content of agricultural soils in the Czech Republic, from 0.6 to 3.2%. In monitored fishponds, the average content of organic matter was 6.12%. Despite the significant differences between the individual fishponds, a high proportion of organic matter is evident when compared to agricultural soils, where the organic matter content is continuously decreasing.

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MODELING TROPHIC RELATIONSHIPS IN POLYCULTURE SYSTEMS FOR FRESHWATER FINFISH FARMING

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Introduction

The increasing demand for aquaculture products requires increasing both the productivity and sustainability of fish farming systems (Thomas et al., 2021). Polyculture has shown potential to do so because it can (i) use food waste better and (ii) increase nutrient recycling in the system, which can (iii) optimize resource-use efficiency and (iv) reduce environmental impacts (Aubin et al., 2021). However, nearly all polyculture systems are built empirically through trial-and-error, which is time consuming and does not allow a range of solutions to be explored. In contrast, studies that attempt to design optimized polyculture systems are rare, and even fewer aim to improve the system's productivity and sustainability simultaneously. Thus, the objective of this study was to develop a method to design more efficient and sustainable polyculture systems. To this end, we developed and modeled scenarios of species combinations using a food-web modeling tool and then selected the best scenarios as a function of performance indicators.

Materials and methods

The complementarity and compatibility of the species combined in this study had previously been assessed in experimental ponds in Le Rheu, France (SEPURE project). The ponds were 0.1 ha in area and 1 m deep. The species differed in feeding strategy: common carp *Cyprinus carpio* (omnivore), pike-perch *Sander lucioperca* (carnivore), roach *Rutilus rutilus* (zoo/phyto/planktivore and detritivore) and tench *Tinca tinca* (benthivore). We developed 10 scenarios of polyculture (each with 10 trophic groups) with no external food supply that differed in each species' percentage of total fish biomass at stocking. To assess the performance of these scenarios, we used Ecopath, a software package for modeling aquatic food webs (Christensen & Pauly, 2004). Biological parameters of these fish (e.g. specific growth rate, mortality rate, reproduction rate) and the productivity of macroinvertebrate, zooplankton, phytoplankton and detritus were extracted from the study of Aubin et al. (2021), which was conducted at the same study site. The individual weights of common carp, pike-perch, roach and tench used in the scenario were 10 g, 70 g, 150 g and 450 g respectively. The total stocking biomass was set to 87.5 kg/ha. Depending on the scenario, the percentage of fish species in the total biomass varied from 10-50% for carp, 5-10% for pike-perch, 22.5-48% for roach and 15-43% for tench. The duration of the rearing cycle was set to 270 days, which is sufficient for monitoring a temperate polyculture system. Each modeled scenario was assessed using indicators of agro-ecological performance that represented productivity, total system throughput (i.e. the amount of biomass flowing through the system over the rearing period), fish diversity, efficiency and recycling (Finn's cycling index).

Results

Over the rearing cycle, the scenarios had a total system throughput of ca. 701±6 g/m^2. The ecotrophic efficiency of the zooplankton, phytoplankton and detritus, which supported food resources in these unfed systems, depended on the fish species composition, varying from 0.52-0.61, 0.23-0.24 and 0.43-0.48, respectively. The species composition also influenced the percentage of biomass fluxes recycled in the system. The percentage of common carp in the scenarios was strongly and negatively correlated with Finn's cycling index ($r = -0.99$), unlike the percentage of other species such as roach ($r = 0.78$) and tench ($r = 0.87$), for which the correlation was positive. As the density of common carp decreased from 50% to 10%, the percentage of biomass fluxes recycled in the system increased from 17.8% to 18.1%.

Discussion

Ecopath food-web modeling seemed sufficient to assess scenarios of species composition. However, it has some limitations due to its use of linear equations (Christensen & Pauly, 2004), which may underestimate or overestimate the correlation between variables. In addition, the integration of environmental variables in the model is limited (Plagányi & Butterworth, 2004), which may limit its ability to explain certain parameters. Variations among performance indicators of the scenarios, especially ecotrophic efficiency and recycling, indicated relations among the trophic and functional roles of species in the system. When species have different feeding strategies, they do not compete with each other for food and share effects of their functional roles in the system (Wang & Lu, 2016). Differences in overall productivity among scenarios were small since the growth performance of fish and productivity of zooplankton, phytoplankton and detritus were extracted from another study (Aubin et al., 2021). However, the ecotrophic efficiency differed among scenarios, which allowed us

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to identify the most efficient scenario. The scenario with the best performance indicators had the lowest percentage of common carp (i.e. 10%), which was much lower than that in most polyculture experiments (i.e. usually more than 50%; Lin et al., 2022). Since these results come from a modeling exercise, experiments are needed to validate the productivity and sustainability of the recommended scenario.

Conclusions

Ecopath's trophic network modeling allowed us to analyze multiple scenarios of species composition in a polyculture system. The scenario with the lowest percentage of carp (10%) appeared to be the most sustainable, based on ecotrophic efficiency and Finn's cycling index.

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PHOTOPERIOD MANIPULATION ON A YEAR-ROUND BASIS AND DIET SUPPLEMENTATION WITH AN ALGAE BLEND AFFECTS DIFFERENTLY TURBOT *Scophthalmus maximus* OVULATION AND EGG ANTIOXIDANT STATUS

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Introduction

Turbot, *Scophthalmus maximus*, is an economically important flatfish species, produced in China, Iceland, Norway, and Southern European countries including Spain, France, and Portugal. This species still presents reproductive dysfunctions when held in captivity, namely the absence of spontaneous spawning. Breeders need to be hand-stripped when mature and eggs artificially fertilized. In routine procedures, photoperiod is manipulated to obtain eggs and sperm in a year-round basis. However, egg quality might differ across seasons, as well as the number of ovulating females. In this sense, diet may be an effective approach for improving and standardizing egg quality and ovulation success.

The incorporation of micro- and macroalgae in aquaculture feeds has been linked to immune system strengthening, antiviral and antibacterial action, improved gut function, and stress resistance, in addition to providing protein, amino acids, fatty acids, vitamins and minerals (Chiu et al., 2001). Thus, the objectives of this study were to evaluate both the effect of photoperiod manipulation and diet supplementation with an Algae Blend (Spirulina and Iodine-rich Macroalgae), in egg quality and ovulation success in turbot.

Materials and Methods

Captive-reared broodstocks (mean weight ~ 6 kg) from the company Flatlantic® were used, reared in 15 m^3 tanks at a constant water temperature all year round (14.0 ± 0.5 ºC) and under simulated natural photoperiod. Exp. 1: Each photoperiod simulated the natural seasonal oscillations, displaced to overlap the spawning period (15h of light) to the four seasons of the year: spring, summer, autumn and winter. In 2022, females from broodstocks corresponding to each season were checked for ovulation at the corresponding month of 15h light. These females were fed *ad libitum* on a commercial feed (Sparos Lda.). Exp. 2: In 2023, females from four tanks corresponding to the displaced photoperiod of spring season were checked at 15h of light -spawning season- and 15:30h of light -end of spawning season-. Each two tanks were fed a different diet: (1) on the same commercial feed (Control) and (2) on a diet supplemented with 5% Spirulina and 1% Iodine-rich Macroalgae, fortified with nutrients such as Astaxanthin, Vitamin C, and Vitamin E (Sparos Lda.) (Algae).

In ovulated females, eggs were hand-stripped and egg diameter, ovarian fluid pH and osmolarity were evaluated. The total antioxidant status and presence of enzymes with antioxidant activity in eggs, particularly of superoxide dismutase (SOD) and glutathione peroxidase (GPX), were measured using kits purchased from Randox Laboratories Ltd. (UK). Blood samples were also obtained, and plasma levels of 17β-estradiol (E_2) were analysed using commercially available ELISA kits (Cayman Chemical Company, USA).

Statistical analyses were carried out using SigmaPlot v14 (Systat Software Inc., Richmond, CA, USA). Data for all the broodstocks were compared using One-way ANOVA followed by Holm Sidak's test or an Anova on Ranks followed by Dunn's test (no normal distribution). Chi-square test was applied to compare ovulation rates. Data was expressed as means ± SD and significance was set at $P < 0.05$.

Results

The presence of ovulated eggs differed among broodstocks reared under different photoperiod displacements and feedings ($\chi2 = 27,523$ with 7 degrees of freedom; $P = <0,001$). In exp. 1, the highest number of ovulated females was observed in the 15h light of the spring broodstock (100 %), followed by the summer (62.5 %) and winter (57.1 %) broodstocks, while no ovulated female was obtained in autumn. In exp. 2, the broodstock fed with the Algae diet had the same percentage of ovulated females as the summer broodstock, when sampled at 15h light, while control females did not ovulate. At 15:30h light, ovulation rate in Algae females decreased (25 %) while in control group there was an increase (12.5 %). E_2 levels

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were significantly higher ($P < 0.05$) in the control group (2.8 ± 1.1 ng mL^{-1}), when compared with Algae females (1.0 ± 1.2 ng mL^{-1}) at 15:30h.

No significant differences were observed in egg diameter, ovarian fluid pH and osmolarity, either in exp 1 or 2. The activity of SOD in eggs was lower ($P < 0.01$) in Algae females (1.9 ± 0.7 units mL^{-1}) than in those from 2022 fed on the commercial diet (3.2 ± 1.1 units mL^{-1}). The activity of GPX was unaffected within groups. The total antioxidant status was higher ($P < 0.05$) in Algae females eggs (1.2 ± 0.24 mmol L^{-1}).

Discussion and Conclusion

The present study showed a significant variation in female ovulation rates among broodstocks, which might compromise the success of larvae production throughout the year, even though egg quality appeared to be consistent. On the other hand, providing an Algae Blend supplemented diet clearly enhanced ovulation rates in comparison to the commercial diet, since control group appeared to have a delay in ovulation, only starting at end of the spawning season (15:30h light). Higher E_2 levels in this group also suggested a delay in concluding gonadal growth, considering E_2 is present in high levels during vitellogenesis and diminishes at final oocyte maturation and ovulation.

Antioxidant enzymes provide a first line of defence against the effects of reactive oxygen species (ROS) that may damage cell membranes, and alter RNA and DNA (Félix *et al.,* 2020). Fish eggs are rich in lipids, with relatively high levels of polyunsaturated fatty acids, which makes cellular membranes vulnerable to ROS. Therefore, adequate antioxidants, such as those found in micro and macroalgae, are expected to help protect cellular components. SOD was expected to exhibit increased activity in eggs from females fed the Algae diet, indicating greater protection against superoxide anion peroxidative action; nonetheless, the opposite was observed. On the other hand, total antioxidant status was significantly higher, which is more informative than individual enzymes, since a decrease in one may be compensated by an increase in another. In any case, although the Algae Blend used possesses antioxidant properties, its beneficial effects may not be restricted to these qualities.

As main conclusions we may say that (i) photoperiod manipulation affects ovulation differently when applied in different seasons, and (ii) the Algae diet guaranteed greater ovulation rates, coinciding with an accelerated ovulation, and improved the total antioxidant content of eggs. This suggests that diets supplementation can be a powerful tool to standardize egg production in turbot, counteracting its reproductive dysfunctions.

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THE POTENTIAL OF *Palmaria palmata* INCLUSION IN DIETS FOR EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES

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Introduction

The increasing global population and growing preference for healthy and natural dietary choices have increased the demand for fish for food, boosted the aquaculture production industry, and led to finding solutions to the limited availability of fishery-based products [1]. Modern aquafeeds have shifted from relying on fish meal as a primary ingredient to agricultural protein proteins such as soy, rapeseed, and corn gluten. However, these alternative proteins are often associated with increased land and water usage and a high carbon footprint in aquaculture. Thus, it is important to find new ingredients that ensure more environmental and economic sustainability of aquaculture.

Macroalgae have captured the attention of producers and fish nutritionists because production does not rely on land or freshwater and can be sustainably harvested from coastal regions with minimal environmental impact. Furthermore, macroalgae contain moderate protein levels and a high abundance of beneficial bioactive compounds, such as antioxidants, carotenoids, vitamins, and minerals [3]. These compounds could potentially benefit fish welfare and improve the nutritional value of fish fillets for human consumption. However, a high percentage of fiber hampers the digestibility of these ingredients in fish. Breakdown of the macroalgal cell wall could enhance nutrient bioavailability and access to digestive enzymes, leading to better nutrient absorption and fish growth [4,5].

The red macroalga *Palmaria palmata* is the most abundant red algae species in Northern Europe [6] and has been successfully cultivated as an extractive species in integrated multi-trophic aquaculture systems (IMTA). *P. palmata* has a relatively high protein content, around 20%; however, its potential as a feed ingredient for aquafeed has been little studied. The present study aimed to assess the effect of untreated and/or alkaline hydrothermally pretreated *P. palmata* on the growth performance and feed utilization of European seabass.

Material and Methods

Treatment of macroalgae

Samples of *P. palmata* provided by AlgaPlus (Aveiro, Portugal) were to alkaline hydrothermal pre-treatment (1N NaOH, solid-liquid ratio of 4:3, autoclavation at 121ºC for 30 minutes).

Growth trial

A plant-based practical diet (corn gluten, wheat gluten, soybean meal, pea protein concentrate, and wheat meal) was formulated to contain 48 % crude protein and 18 % crude lipids and was used as the control. Four other experimental diets were formulated similarly to the control but included 7.5 or 15 % of untreated or treated *P. palmata* at the expense of the plant feedstuffs mixture.

The growth trial was conducted in a RAS system with 15 tanks of 500 L water capacity, thermoregulated to 22 ºC. Groups of 16 European seabass juveniles (initial body weight of 38 g) were randomly distributed in each tank and triplicate groups of fish were fed each experimental diet twice a day until satiation, six days a week, for 11 weeks. Utmost care was taken to avoid feed waste and ensure all feed supplied was consumed. At the end of the growth trial, fish from each tank were weighed after a light anesthetized (2-phenoxyethanol; 0.3 ml l^{-1}) following 24h of feed deprivation to assess growth performance.

Statistical analysis

All data were checked for normality and homogeneity of variances and normalized when required. Specific non-orthogonal contrasts analyses were performed to compare the control diet vs. the *Palmaria palmata* treatment and inclusion level. All statistical analyzes were performed using the IBM SPSS Statistics software version 26 (IBM, NY, USA).

Results

At the end of the feeding trial, compared to the control diet, the dietary inclusion of 7.5% of untreated and 7.5 or 15% of treated *P. palmata* did not affect the growth performance, feed intake, and feed utilization efficiency of European seabass juveniles. However, including 15% of untreated *P. palmata* increased weight gain and daily growth index.

In conclusion, the alkaline hydrothermal treatment of *P. palmata* did not contribute to improve growth performance and feed utilization. The dietary inclusion of 15% untreated *P. palmata* replacing traditional agriculture feedstuffs improved the growth performance of European seabass. This preliminary study highlights the potential of *P. palmata* as an aquafeed ingredient contributing to the sustainable development of the aquaculture sector.

Acknowledgment

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ALKALINE HYDROTHERMAL TREATMENT OF *Codium Tomentosum* ENHANCED THE OXIDATIVE STATUS OF EUROPEAN SEABASS *Dicentrarchus labrax* JUVENILES

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Introduction

Aquaculture is responsible for approximately half of the world's supply of fish for human consumption. Therefore, it is important to guarantee the nutritional quality of the fish through aquafeeds capable of meeting the nutritional requirements of fish and ensuring the sustainable development of aquaculture. Thus, finding new suitable green alternatives to replace the traditional protein sources in aquatic feeds is of utmost importance. Plant feedstuffs are the most used alternative ingredients in aquafeeds. However, these ingredients increase the pressure on land-based feed production systems and are often associated with poor growth performance, feed utilization, and antioxidant status of fish.

Macroalgae emerge as an alternative to plant feedstuffs and are produced without needing arable land, freshwater, and the expensive fertilizers associated with terrestrial crop production [1,2]. Green seaweeds, the most abundant macroalgae, are an important marine biological resource. Their bioactive components may modulate several biological processes, such as antioxidant activity, immunoregulation, and anti-inflammatory response [3].

Fish have evolved a strong defense system against oxidative damage. This defense system includes non-enzymatic components, such as glutathione (GSH), and enzymatic mechanisms, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase (GR). Their primary function is to counteract the harmful effects of reactive oxygen species (ROS) and ensure overall oxi-redox cellular equilibrium and thus contributing to fish health and well-being [4, 5, 6]. In this study, we evaluated the impact of the dietary inclusion of 7.5% untreated or alkaline hydrothermal pre-treated *Codium tomentosum* on oxidative defense mechanisms in the liver and intestine of European seabass (*Dicentrarchus labrax*) juveniles.

Material and Methods

Alkaline hydrothermal pre-treatment of *Codium tomentosum* was performed using NaOH (solid-liquid ratio of 4:3), autoclaved (121ºC) for 30 and 60 min. Four isoproteic (48% dry matter basis) and isolipidic (18% dry matter basis) diets were formulated: a control diet with no inclusion of macroalgae, and three other diets with the inclusion of 7.5% of *Codium tomentosum* untreated or treated for 30 or 60 min, as aforementioned.

The growth trial was run in a RAS system with 12 tanks. Triplicate homogenous groups of 16 European seabass (initial body weight of 38g) were fed the experimental diets by hand, twice daily, 6 days a week, until apparent visual satiation, for 11 weeks. At the end of the growth trial, the liver and intestine from 3 fish per tank were collected and stored at -80ºC until enzymatic assay quantification. Oxidative stress biomarkers were determined in the liver and intestine after the homogenization in phosphate buffer (0.1 M pH 7.4). The activity of glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), glutathione peroxidase (GPX; EC 1.11.1.9), and glutathione reductase (GR; EC 1.6.4.2) was measured. Lipid peroxidation (LPO) was determined in the liver and intestine as malondialdehyde concentration.

Results

In the liver, independently of the treatment, the dietary inclusion of *C. tomentosum* decreased CAT activity, compared to the control.

Intestinal SOD activity was lower in fish fed the pretreated *C. tomentosum* than in fish fed the control diet. Dietary inclusion of *C. tomentosum* pretreated for 60 minutes also decreased LPO levels compared to the control.

Overall, the current results indicate that dietary inclusion of 7.5% pre-treated *C. tomentosum* with 1N NaOH for 60 minutes may contribute to enhance the antioxidant responses of European seabass.

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EXPLOITING A MACRO- AND MICROALGAE BLEND AS DIETARY SUPPLEMENTATION TO ENHANCE IMMUNE STATUS IN TURBOT (*Scophthalmus maximus*) BREEDERS

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Introduction

Europe's bioeconomy focuses on harnessing renewable resources from both terrestrial and marine environments to meet the increasing demands for food, animal feed, and bio-based products. The aquaculture sector, particularly marine aquaculture, has emerged as the fastest-growing food production sector globally, striving to ensure sustainable food security in response to the rising protein needs driven by a growing global population. However, despite its rapid growth, European aquaculture, including both marine and inland operations, faces challenges in keeping pace with global production growth, requiring the exploration of innovative solutions.

Among the flatfish species, turbot (*Scophthalmus maximus*) is highly valued for human consumption and produced mainly in Southern European countries like Spain, France, and Portugal, as well as in Iceland and Norway. Yet, turbot farming encounters obstacles, particularly in achieving high and consistent larval survival rates. This limitation is partly attributed to the quality of breeders, which can be improved through the supplementation of feeds during reproductive events using antioxidant and immune-stimulating raw ingredients. In line with the bioeconomy concept, this study focuses on developing new feeds for turbot breeders, incorporating sustainable marine compounds like microalgae and macroalgae.

Materials and methods

This study was conducted at the commercial aquaculture facility – Flatlantic, SA, where turbot breeders were fed two experimental diets for 4 months. The male breeders had an average weight of 5.24 ± 0.8 Kg, while the female breeders had an average weight of 7.49 ± 1.35 Kg. Briefly, the experiment involved four different broodstocks, where two broodstocks served as the control group, receiving the breeders' baseline diet without any supplementation (Control). The other two broodstocks were fed an ALGAE diet, a specially formulated diet enriched with a blend of Macro- and Microalgae. The ALGAE diet included 5% Spirulina and 1% Iodine-rich Macroalgae (*Laminaria digitata*), fortified with additional nutrients such as Astaxanthin, Vitamin C, and Vitamin E. This dietary supplementation aimed to enhance the immune status of the turbot breeders and study the potential modulation of different diets during the breeding season.

Skin mucus and blood samples were collected from the breeders in two different dates at the end of the feeding period, coinciding with the breeding season. These samples were used to assess the immune status of the breeders and analyse the effects of the ALGAE diet on their immune status.

An overall multivariate analysis combining raw data from humoral parameters analysed in turbot skin mucus and blood plasma (using PCA-DA) was performed to discriminate the physiological effects caused by the experimental diets excluding the sampling times. Sampling time was excluded from this multivariate analysis for noise reduction.

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Results

The first two discriminant functions accounted for 98.84% of dataset variability. Group discrimination was significant (Wilk's lambda = 0.0299, $p < 0.0001$) highlighting the differences between experimental diets and gender. This discrimination was loaded by the expression of IgM in skin mucus, and peroxidase, bactericidal, and lysozyme activity measured in blood plasma.

Regarding plasma humoral parameters, no clear differences were observed. However, it was observed a tendency for higher lysozyme activity in fish fed the ALGAE diet, particularly in female breeders. And a tendency for a higher peroxidase activity in fish fed the CTRL diet. Overall, it was also observed a higher bactericidal activity in male breeders particularly those fed the CTRL diet.

Interestingly, IgM values in skin mucus increased in fish fed the ALGAE diet compared to those fed the CTRL diet, particularly in female breeders.

Discussion and conclusion

The present study adds valuable insights into the effects of incorporating a blend of micro- and macroalgae in the diets of turbot breeders. The findings from this trial indicate that the inclusion of the ALGAE blend does not have any adverse effects on the performance and survival of turbot broodstock. Moreover, it demonstrates a modulatory effect on the immune status of the breeders. 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 to say that IgM plays a relevant role in maintaining microbiota homeostasis at these sites. The observed modulation of immune parameters, especially IgM, in turbot breeders fed with the ALGAE blend seems to indicate a potential enhancement in their immune response. This finding suggests that dietary supplementation with micro- and macroalgae could contribute to improving the immune status of turbot breeders, which is crucial for their overall health and resilience against pathogens. Further research is planned using skin mucus proteomics to gain a comprehensive understanding of the immunomodulatory effects of the ALGAE blend on turbot breeders in commercial aquaculture settings. Skin mucus is a critical component of the fish's immune system, serving as the first line of defence against pathogens. Analysing the skin mucus proteome can provide valuable insights into the specific proteins and peptides involved in the immune response and their interactions with the ALGAE blend.

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EXPLORING THE IMMUNE MODULATION POTENTIAL OF DIETARY TRYPTOPHAN SUPPLEMENTATION IN GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Amino acids, the foundational elements of proteins, transcend their conventional role in protein synthesis, acting as precursors to vital metabolites that shape various physiological processes. Tryptophan (Trp), an amino acid of notable significance, extends its impact beyond protein building, serving as a precursor for pivotal compounds such as serotonin and melatonin in fish. Serotonin, stemming from tryptophan metabolism, influences behavior, mood, and immune responses, introducing a novel dimension of amino acid functionality. Additionally, the conversion of tryptophan to melatonin holds implications for stress resilience and circadian rhythm regulation. This study explores the immunomodulatory potential of dietary tryptophan supplementation in gilthead seabream (*Sparus aurata*), aiming to unveil its multifaceted influence on immune response dynamics and broader physiological processes in fish.

Materials and methods

Triplicate groups of fish (6.28 ± 0.28 g) were either fed a control diet (CTRL) with a balanced AA profile, or the CTRL diet supplemented with graded levels of Trp (i.e. 0.5% and 1% of feed, TRP1 and TRP2, respectively) for a 4-week feeding period. After 2 and 4 weeks, fish were euthanized and blood was collected for blood smears, plasma for humoral immune parameters, whole gut for oxidative stress biomarkers, anterior gut and hypophysis for the measurement of transcripts. After the 4-week feeding period, fish were intraperitoneal injected with inactivated bacteria and the inflammatory insult was monitored with samplings at 4-, 24- and 48-hours post-injection.

Results

The study's outcomes revealed that dietary treatments had no significant impact on growth performance, with no distinctions observed in final body weight, daily growth index, specific growth rate, voluntary feed intake, feed efficiency, or feed conversion ratio. Blood immune parameters during the feeding trial demonstrated a decrease in white blood cell count from 2 to 4 weeks, alongside increased hemoglobin at 2 weeks and reduced peripheral blood leucocytes at 4 weeks.

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In response to inflammation, blood cell dynamics and hemoglobin showed an increase. Plasma cortisol and humoral immune parameters exhibited variations, with no differences between dietary treatments. Gut oxidative stress markers remained steady during the feeding trial, while SOD increased and catalase and tGSH decreased during the inflammatory response. Gene expression analyses highlighted the modulation of *csf1r* in the gut by dietary treatments. In the brain, no significant differences were observed between dietary treatments, although variations were noted over sampling times. In the hypophysis, *pomca1* was downregulated in fish fed TRP2 diet during the inflammatory response, while *pomca2* was upregulated in fish fed tryptophan-supplemented diets, regardless of inclusion level.

Discussion and conclusion

Tryptophan, aside from its protein building role, holds significance as a precursor for metabolites influencing fish immune systems, including serotonin and those generated through the IDO pathway. While previous research has explored tryptophan's potential as a growth modulator or nutraceutical ingredient, its functional role in challenging contexts remains less understood. In this context, the study aimed to elucidate its effects. Results showed that the inclusion of tryptophan did not significantly alter the hematological profile, general health biomarkers, or oxidative stress markers in the anterior gut.

Similarly, no significant effects were observed in cortisol levels, a well-established stress marker, both during the feeding trial and post-injection immune stimulation. However, when immune mechanisms were activated by bacterial injection, an immune response was evident regardless of dietary treatments. An increase in lymphocytes and neutrophils highlighted the immune system's response to acute stress and infection. Interestingly, an upregulation of the colony stimulating factor 1 receptor (*csf1r*), associated with macrophage differentiation, was noted in fish fed diets supplemented with tryptophan. Moreover, *pomca1* expression significantly decreased after 48h post-insult in fish fed TRP2, suggesting mediation and regulation of the stress response.

While the results suggest a mild effect of the tested tryptophan inclusion levels without a stimulus, the study highlights the potential for tryptophan's immunotolerance role, particularly under immune stimulation, as evidenced by its modulation of oxidative stress and health biomarkers in the anterior gut of gilthead seabream. Overall, the study sheds light on the intricate interplay between dietary tryptophan supplementation, immune response, and physiological processes in fish.

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PREDICTING FISH BODY COMPOSITION: CAN WATER AND ASH INPUTS IMPROVE ESTIMATIONS?

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Introduction

In aquaculture, the body composition of fish is an important aspect to be measured or estimated, as it can affect the fish health, flesh quality and fish market value. Usually, fish body composition is measured through chemical analysis following standardized methods (e.g., AOAC 2005). However, these methods can be both time-consuming and expensive. In turn, mathematical models can be an alternative and complementary tool to estimate body composition since they are less time-consuming and less costly than chemical analysis.

SPAROS developed a free tool – ficoEst (https://webtools.sparos.pt/ficoest/) - to estimate the body composition of farmed fish, in terms of crude protein, crude lipids, water, phosphorus and energy content. This tool comprehends different types of models - BC1, relies on body weight as an input; BC2, uses body weight and water percentage as inputs; BC3, uses body weight, water and ash percentage as inputs - as a cost-effective alternative to chemical analysis.

In this study, we evaluated the performance of the model from Raposo et al. (2023) when calibrated for other species besides Nile tilapia (i.e., Atlantic salmon, gilthead seabream, European seabass and rainbow trout) against the estimates from ficoEst, to assess if including information on water and ash content improves the models' accuracy in estimating other body composition components.

Materials and methods

The models from ficoEst tool (BC1 – only body weigh as input, BC2 – body weight and water as inputs and BC3 – body weight, water and ash as inputs) and Raposo et al. (2023) model (BCRA) were calibrated with data collected from literature and from trials developed in the framework of projects coordinated by SPAROS and its R&D partners. Regression analysis was performed for each body component and the models were evaluated both qualitatively, by visually observing model behaviour, and quantitatively, by calculating the mean absolute percentage error (MAPE) between observed and predicted values for each body composition component. Additionally, a method of cross-validation with 5-folds and 10 repetitions was used to assess model performance. Models were further validated using independent datasets (i.e., not used during the calibration process). Furthermore, to assess the uncertainty of the observed values, the mean percentage error (PE) was calculated based on the mean±sd values of the pairs that were used to calibrate and validate the models.

Results

Overall, the model that relies on body weight, water and ash as inputs (BC3) displayed lower validation errors when compared with other tested models. Particularly for Nile tilapia, BC3 model outperforms all others for all body composition components. However, regardless of the type of model, the validation errors were generally higher for Nile tilapia, specifically when predicting crude protein (MAPE ≈ 8%), crude lipids (MAPE ≈ 44%) and energy (MAPE ≈ 19%).

Discussion and Conclusion

The higher MAPEs observed in estimating the body composition of Nile tilapia may be related to the greater variability of strains used worldwide and due to different inclusion of males/females in the trials. Additionally, for the Nile tilapia published datasets the analysis of the sum of components shows greater deviations, and the correlation coefficients between crude protein and body weight and crude lipids and water are lower, when compared to other species. Furthermore, the ash estimates demonstrated higher MAPEs, potentially because of laboratory errors and renormalized data reported in

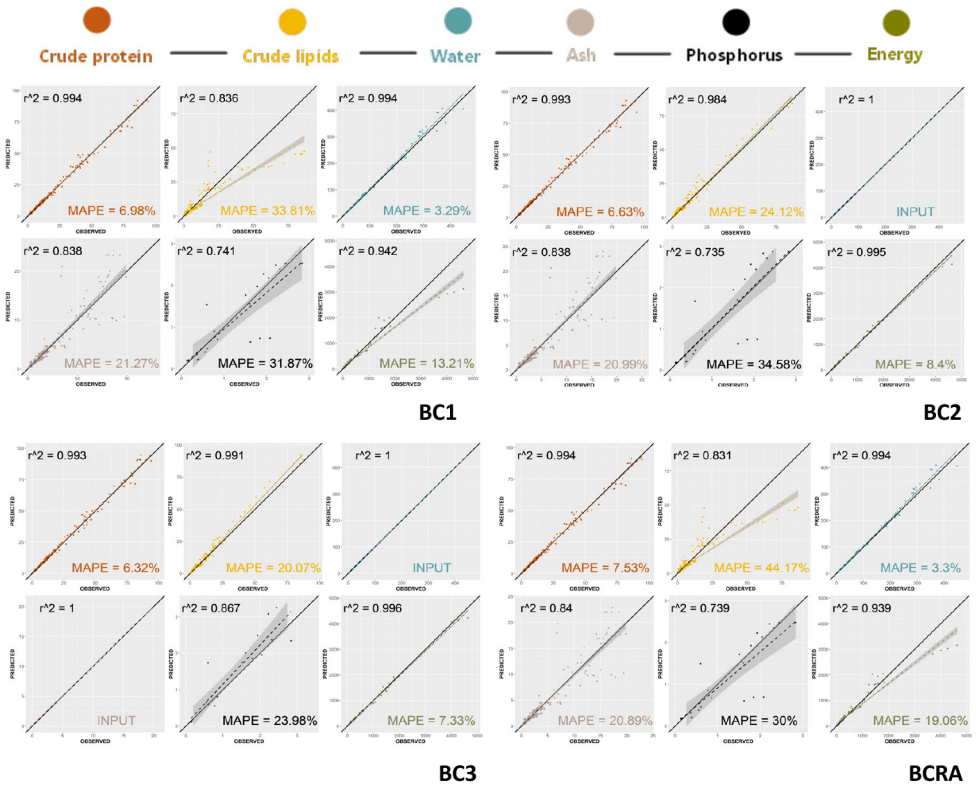

Figure 1 – Scatterplots displaying the prediction errors (MAPE) after model validation.

literature. The fact that the BC3 model was more accurate in predicting fish body composition, indicates that accounting for the abundance of water and ash can improve model performance.

Overall, the ficoEst tool is a valuable resource for estimating fish body composition, particularly when direct measurement is not feasible due to economical or logistical constrains.

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MODELLING GROWTH AND BODY COMPOSITION: A COMPARATIVE ANALYSIS OF COMMERCIAL FISH SPECIES

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Introduction

A wide range of species are produced in aquaculture, which implies a great variability among aquatic species in terms of nutritional requirements compared to what is seen in livestock production. This poses a great challenge for fish nutritionists and aquafeed producers. Objective criteria, based on the use of body composition and nutrient/energy budget modelling, can be used to explore similarity relationships between species and thus contribute to mitigate this challenge.

To predict the body composition in fish, usually isometric and/or allometric models are used. The isometric model assumes fish composition is proportional to their weight, while the allometric model accounts for a non-proportional relationship between the two variables. In nutrition studies, assumptions of isometric behaviour for protein and/or ash in fish may not hold for dynamic body composition evaluations.

Nutrient-based models, consider energy and protein intake to predict growth and body composition. These models assume that maintenance metabolic expenditure can be defined by exponents, which determine the change in metabolic rate as a function of body weight. However, there is a disagreement among authors regarding the definition of these exponents for the metabolic weight of fish, which makes it unclear whether the same exponents should be applied to different species.

In this work, commercial fish species such as, Atlantic salmon (*Salmo salar*), gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Oncorhynchus mykiss*), turbot (*Scophthalmus maximus*) and Senegalese sole (*Solea senegalensis*) were compared based on their body composition and growth analysis. The goal was to assess certain assumptions and hypotheses present in the literature, such as the use of isometric or allometric models to describe body composition, the applicatiom of "universal" or species-specific metabolic body weight exponents, the use of constant or non-constant protein/energy efficiency ratios, and the assumption of similarity between species with similar morphological and physiological traits (e.g., flatfish species).

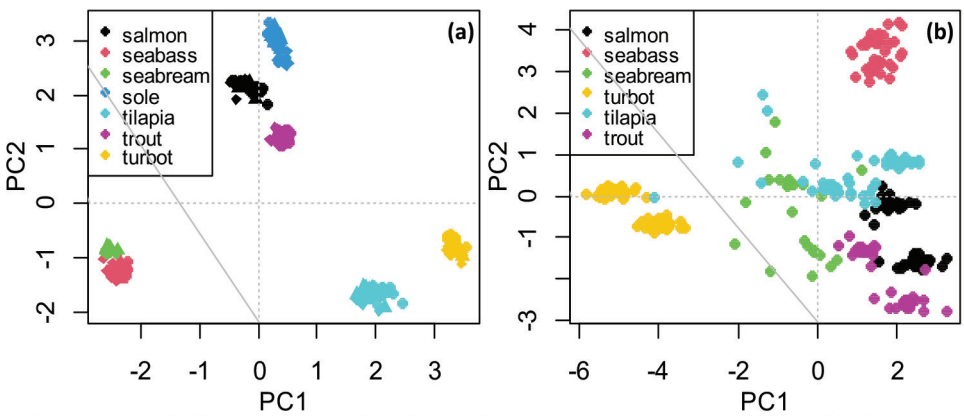

Figure 1 - Principal component analysis (PCA) of leave-one-out estimates of general body composition (a) and growth (b) parameters for each species.

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Materials and methods

Data was collected from literature on the body composition and growth for the aforementioned species. For analysis of growth parameters, datasets on *in vivo* growth trials were used to estimate fasting maintenance and retention efficiency parameters, through linear regression techniques, for all species, except for sole due to limited data.

The study evaluated the similarity relationships between species in terms of body composition and protein/energy budgets using principal components analysis (PCA). The respective parameter estimates of the species were plotted along the first two principal components and scatter plots of the individual parameters were generated. Additionally, the predictions of the calibrated models (species-specific) were compared between different species.

Results

PCA analysis shows that seabream and seabass share similarities in their body composition parameters, as do salmon and trout. However, the flatfish species, turbot and sole, seem to have different body composition parameters. In turn, sole appears to be more similar to salmonids, whereas turbot displays certain similarities with tilapia.

In terms of general growth parameters, it seems that tilapia, seabream, salmon, and rainbow trout have some similarities, while seabass and turbot seem more different. Salmon and rainbow trout are closer to each other, while tilapia is closer to seabream. Turbot has the most distant estimations for fasting maintenance costs. Salmonids have higher retention efficiency for protein and energy, while Mediterranean species have lower retention efficiency for protein. Seabream has higher energy retention efficiency than seabass, but both have low protein retention efficiency.

Discussion and Conclusion

Results suggest that the similarity within salmonids and Mediterranean species are strong and consistent across parameters and thus clearer in the PCA projections. Despite having some differences in growth, Mediterranean species show similarities, especially in early life. However, flat species do not group as clearly. The variability observed between species in terms of model parameters and predictions may be related to taxonomy, physiological stages, ecological features, fish activity, body mass, as stated in previous studies. Results also suggest the metabolic body weight exponents for energy and protein are likely to be species-specific and differ between species, ranging from 0.6 to 0.9, which agrees with previous studies that challenge the theory of universal metabolic allometry.

This study enables the evaluation of certain assumptions in literature. Moreover, it shows that species belonging to the same family, geographical area, or with similar diets or body shapes may or may not exhibit similar body composition and growth patterns. Therefore, conducting the analysis of body composition and growth parameters between species is useful, as they complement each other, providing a full view of their developmental patterns, and allows to identify both similarities and differences between the species.

Acknowledgements

A. Raposo acknowledges financial support by Grant PD/BDE/150525/2019 (SANFEED Doctoral program, with support by FCT and SPAROS Lda, Portugal). This work was also funded by project 47175_FICA, supported by Portugal and the European Union through FEDER/ERDF, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020.

MULTIOMICS ANALYSIS OF GILTHEAD SEABREAM (*Sparus aurata*) LIVER MOLECULAR STRESS RESPONSE UNDER DIFFERENT AQUACULTURE CHALLENGES

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Introduction

The ability of fish to respond to environmental challenges is critical for their survival and the maintenance of healthy populations. Therefore, to ensure a sustainable aquaculture while meeting the increasing demand for fish, it is essential to prioritize the welfare of farmed fish by minimizing the stress levels associated with aquaculture practices. The study of the molecular mechanisms of fish stress appraisal is paramount to achieving this goal and avoid negative impacts on fish health and ultimately on the aquaculture productivity. In this study, we performed, an integrated multiomics characterization of the fish liver, a central organ during stress adaptation, to provide a holistic assessment of the molecular stress response at different organizational levels. Widen our understanding into the physiological changes occurring in the fish organism during stress adaptation can leverage the industry with valuable scientific knowledge for developing forthcoming species-specific welfare assessment protocols.

Methods

Sparus aurata adults were exposed to different suboptimal rearing conditions in three separate trials: overcrowding (45 kg/m^3), repetitive net handling (4 times/week), and hypoxia (15% DO). A control group was also included, consisting of fish reared under optimal conditions for the species. By the end of the trials, fish were sampled (n = 6) and liver extracts were prepared for further transcriptomics (RNA-seq), proteomics (label-free shotgun) and metabolomics (untargeted LC-MS) analyses. In transcriptomics, after reference-guided transcriptome assembly, differential expression analysis was performed with DESeq2 (adjusted p-value < 0.01, log2|fold-change| >1). In proteomics and metabolomics, pairwise comparisons between identified proteins (protein FDR < 1%; peptide FDR < 0.1%) and metabolites, were achieved by a student's t-test, $p < 0.05$; FDR controlled at 0.05. Gene set enrichment analysis (GSEA) and overrepresentation (ORA) analyses based on GO, KEGG and REACTOME databases were performed.

Results and conclusions

A total of 946 genes, 397 proteins and 121 metabolites were differentially regulated between challenged and control fish across the three trials. Integration and functional analysis of these selected features suggested a scenario of challenge-specific responses at the transcriptome, proteome, and metabolome levels. Net handling and hypoxia triggered a more impactful effect on hepatocytes than overcrowding, inducing an overall metabolism reprogramming to shift energy towards stress response processes and cell cycle arrest, mainly driven by ribosomal assembly stress, DNA replication stress, endoplasmic reticulum stress, and downregulation of insulin growth factor signalling. These results support the high phenotypic plasticity of this species and its differential responses to distinct challenging environments at different molecular levels. Additionally, the study provides valuable resources for characterizing and identifying potentially novel features essential for gilthead seabream resilience and aquaculture production efficiency concerning fish welfare.

BIOMASS PRODUCTION AND REPRODUCTION OF *Hediste diversicolor* FED WITH BREWER´S SPENT GRAIN.

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Introduction

The "ragworm" *Hediste diversicolor* is a suitable species for industrial aquaculture because of its adaptability to wide environmental conditions, feeding flexibility and high growth rates. It is usually used as fishing bait or as feed supplements for farmed fish and crustaceans. The farming of this species is relatively simple and high yields can be obtained when using fishmeal and fish oil based feeds. However, this type of feed is expensive and unsustainable. Different low-cost feeds have been tested such as clam faeces, sludge from fish farming recirculation systems or biogas side stream. We propose a low cost product such brewer´s spent grain (BSG), the main by-product of the brewing industry. BSG is mainly composed of fibres and protein (Mussatto, 2014). Therefore, the aim of this work was to gain information on the growth and reproduction of *H. diversicolor* fed with BSG.

Material and methods

Growth**:** Experimental units (EU) consist on three polycarbonate trays (width: 0.35 m; height: 0.30 m; length: 0.54 m) provided with aeration, top water inlet (2.5 l/min), bottom drainage anda 12 cm high quarry sand substrate (0.25 - 1.0 mm grain size). Ragworms (N = 720, 4000 individuals / m^2) of wet body weight (BW) 46.7 ± 5.5 mg, (mean ± SD), obtained from a captive population stock, were placed in the experimental units (biomass per tray = 33.7 g). Ragworms were fed with freshly produced BSG in an artisanal brewery, which was frozen until use (humidity 75%). Triturated BSG were distributed six days a week ad libitum. Bi-weekly, wet BW was estimated and the amount of food adjusted. At the end of the trial (culture day 79), all ragworms from each EU were harvested and the following indices were calculated as growth performance variables: Mean wet BW, percentage of ragworms larger than 500 mg, survival (%) and specific growth rate (SGR= $(\ln BW_f - \ln BW_i)*100/ T_f-T_i$). Dissolved oxygen (DO) were always > 90 %, and temperature, salinity and photoperiod were natural (13.8±1.0 ºC, salinity 36 ppm, 9 to 10.5 hours of light).

Reproduction: 400 ragworms larger than500 mg from the previous trial were selected as broodstock and placed in two stocking tanks (height 0.5 m; area 1m^2, 20 cm high sand substrate) (Rasines et al. 2023). One tank was fed, *ad libitum*, with dried BSG (105ºC, 5 hours) and the other with fish feed. Samples were taken every two weeks from the top two cm of the substrate to detect and quantify the number of juveniles. Temperature, salinity and photoperiod were natural.

Results and Discussion

Ragworm body weight increased over time (Figure 1) but slowed between days 46 and 57 due to inadequate feeding management; BSG debris accumulated on the surface of the sand making it difficult for polychaetes to access fresh food. From that time onwards, BSG debriswas removed periodically.

Although the SGR and production data were lower than those obtained with fish feed (Rasines et al. 2023, Nesto et al. 2012), they were quite promising because a significant biomass production per m^2 can be obtained with a low-cost feed (Table I).

There were reproduction processes with both types of feeding, so it is not necessary a change of feeding for the broodstock. The first juveniles were detected between 2 and 2.5 months. Both the time and the number of juveniles were in the range of those obtained when fish feed is used throughout the production process (Rasines et al. 2022) (Table II).

In conclusion, BSG is a suitable food for the growth and reproduction of *Hediste diversicolor*. Further studies are needed to characterise its nutritional profile and to advance in the optimisation of cultivation techniques.

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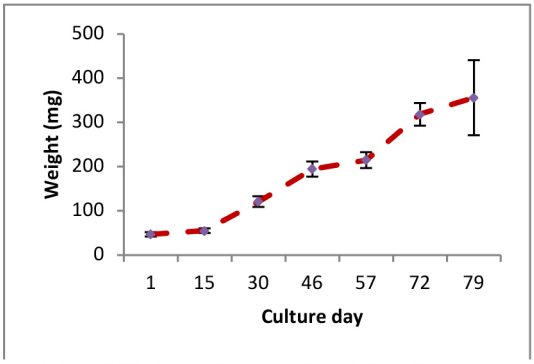

Fig. 1: Growth in weight of *Hediste diversicolor* fed on brewer´s spent grain (BSG)

Table I:Medium body weight, percentage of individuals larger than 500 mg, survival percentage, SGR and production determined when all individuals were harvested.

BW final (mg)	>500 mg (%)	Survival (%)	SGR (1-79)	Production g/m²
360±85	26±16	60±11	2,6±0,3	806±120

Table II. Reproduction of *Hediste diversicolor*. Time to detect the first juveniles and number of juveniles produced from a broodstock (individuals larger than 500 mg) obtained by grow-out with BSG and then fed on BSG or fish feed.

Food	BW (mg)	N	Detection of first juvenile	Nº juveniles
BSG	618±113	200	2 months	7667
Fish Feed	619±129	200	2,5 months	7456

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Acknowledgments

This study is part of the ThinkInAzul programme and has been funded by the Spanish Ministry of Science and Innovation with European Union NextGenerationEU funds (PRTR-C17.I1) and by the Government of Cantabria (Spain).The authors wish to thank the craft brewery Rocker Beer®

EXPLORING THE CHEMICAL NATURE OF THE SPAWNING-INDUCING PHEROMONE IN PACIFIC OYSTER, *Magallana gigas:* 'OLFACTORY' *VS* BIOLOGICAL ACTIVITY

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Introduction

The synchronization of bivalve spawning involves chemical communication. Sperm carries a chemical signal that induces spawning in females: a spawn-inducing pheromone (SIP) (Galtsoff, 1938; Taylor et al., 2018). In the oyster *Pinctada maxima*, SIP has been characterized as proteinaceous and multicomponent, associated with sperm membrane (Taylor et al., 2018). Following this, the current study investigated the 'olfactory' and biological activity of male SIP in the Pacific oyster, *Magallana gigas*, through an electrophysiological technique and a bioassay, respectively. In addition, we also investigated the possible proteinaceous nature of SIP.

Material and methods

To isolate and chemically characterize oyster pheromones, sperm milt obtained by stripping the gonads was centrifuged to obtain different fractions. After centrifugation, the supernatant (supernatant 1) was carefully transferred to another vial and the pellet was resuspended in seawater: part was stored and used as a fraction - resuspended pellet- and the remaining part was homogenized with a glass Teflon homogenizer and centrifuged to obtain the supernatant of homogenized pellet (supernatant 2). Each fraction was assessed for olfactory activity using the electro-osphradiogram (EOsG) (Rato et al., 2023), to identify which fraction(s) contain the pheromone. The voltage signal of the EOsG was amplified (x5000) with the low-pass filter set at 30 Hz and then, the signal was digitised and recorded on a PC running Axoscope TM software. L-cysteine (10^{-3} M) was used as a positive control (or standard) whereas charcoal-filtered seawater was the blank or negative control. The EOsG peak amplitude was measured in millivolts. All recorded responses were blank-subtracted and normalized to the standard (10^{-3} M L-cysteine).

That a given compound can evoke an olfactory response does not necessarily mean that it has full biological activity, i.e., can induce spawning in conspecifics. To address this, a bioassay was performed in which oysters were exposed to the different sperm milt fractions, both singly and in combination, with exception of the resuspended pellet. Since chemical stimuli alone were not sufficient to induce spawning, oysters were induced to spawn by thermal shock (15°C - 30°C, at intervals of 1h) in combination with chemical stimulation, in which each of the fractions, plus fresh and frozen stripped sperm were used as chemical stimulus.

To investigate the possible proteinaceous nature of SIP, the protein profile of each sperm fraction and of an unfractionated sample (total) was analysed by SDS-PAGE electrophoresis (12% polyacrylamide), quantified by Bradford Protein Assay and the results correlated with EOsG and the bioassay.

Results

The olfactory responses did not reflect the biological activity. Although supernatant 1 evoked strong olfactory response, it failed to induce spawning on its own. Supernatant 2 alone, and in combination with supernatant 1, did induce spawning but not to the same extent as untreated milt. Frozen milt had similar biological activity to freshly stripped milt, inducing spawning in 87% of the oysters.

The SDS-PAGE revealed different protein profiles in each of the fractions, with supernatant 1 revealing a distinct band of ~35 kDa, whereas pellet and supernatant 2 fractions showed clear bands with a lower molecular weight of about 15 kDa. Each of these bands could also be seen in the unfractionated samples (total).

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Protein quantification revealed maximums of 0.42 $mg.ml^{-1}$ and 0.34 $mg.ml^{-1}$ in the supernatant of the frozen milt and in its unfractionated sample, respectively. The minimum protein concentration was seen in the supernatants 2 and 1 of fresh milt, varying between 0.08 $mg.ml^{-1}$ and 0.09 $mg.ml^{-1}$.

Conclusions

The chemical signal responsible for inducing spawning in conspecifics likely involves a low molecular weight protein (15 kDa) but is probably multi-component. It possibly includes peptides or proteins (by extrapolation from other molluscs).

The fact that a given fraction evoked a strong olfactory response is not indicative of its biological activity; two or more components are necessary to induce spawning.

That frozen sperm milt had a similar biological activity to fresh sperm milt may be useful and profitable for bivalve hatcheries as they can store it for use in a later spawning season.

The low protein concentrations may indicate that oysters are extremely sensitive to conspecific signals and, since oysters inhabit dense beds close to conspecifics, the concentration of such signals in the natural habitat may be relatively high.
Future work will focus on the chemical isolation and identification of the male pheromone components and, if proteinaceous, on isolation and sequencing.

Acknowledgements

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YEAST CELL WALL DERIVED FROM *Saccharomyces cerevisiae* DIFFERENTIALLY SHAPE INTESITNAL INNATE IMMUNITY AND MUCOSAL TISSUE RESPONES OF ZEBRAFISH (*Danio rerio*)

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Introduction

YCWs are proven immune response modifiers holding an important position among multifarious paraprobiotics and, in fish, have been implicated in the maintenance of (intestinal) mucosal health and integrity, disease resilience and animal performance. The major structural components of YCWs are *β-glucans and* mannanoligosaccharides (MOS) which act as microbe-associated molecular patterns (MAMPs) that can be recognized by the pattern recognition receptors of the host immune system. YCW can vary in their biochemical composition and molecular structure due to the yeast species, strain, fermentation, and down-processing conditions. Such diversity of MAMPs structure and profile could elicit distinct host immune responses and to date has been under evaluated. The current investigation aimed to characterise the biochemical and molecular properties of four proprietary *S. cerevisiae* YCW fractions of discrete origin. Our hypothesis was that the biophysical characteristics of given *S. cerevisiae* will elicit distinct immune responses, which has not been previously addressed using a purposely designed comparative study.

Materials and Methods

A 5-week trial (Plymouth, UK) tested 5 dietary groups in triplicate using wild-type adult zebrafish ($BW_i = 0.80 \pm 0.02$ g; 25 fish/tank) and a basal diet formulated to NRC requirements for cyprinids (36% protein, 8% lipid). Treatments consisted of 1] non-supplemented basal diet (Control), and basal diet supplemented with 2] YCW1, 3] YCW2, 4] YCW3, 5] YCW4. All YCW fractions were supplemented at 2.0 kg/T of feed and consisted of contrasted proprietary YCW strains provided by Lallemand SAS (France). YCW fractions were assessed by sulfuric acid method. Diets were fed at fixed rate of 4% biomass daily over 3 daily rations. At the end of the trial, intestinal and skin histomorphometry (n = 9 fish/ treatment) as well as the intestinal gene expression analysis (n = 6 fish/ treatment) was assessed. For gene expression analysis differences between control and experimental groups were assessed by non-parametric permutation test with significance accepted at $p < 0.05$. All other data was analysed by One-way ANOVA and significance accepted at $p < 0.05$.

Results

The biochemical analysis revealed significant differences in percentage composition of β-glucan and alpha - mannan polysaccharides in each YCW fraction (Fig 1; $p < 0.05$). Histological appraisal revealed significantly elevated GCD, IELs and acidomucins in YCW1, 2 and 3 groups containing higher levels of mannan content compared to YCW4 (Fig 2; $p < 0.05$). Gene expression analysis revealed differential modulation in markers for innate immune pattern recognition receptors (PRRs) and signal transduction factors, transcription and innate cytokines that suggest polarisation of responses to Th1, Th17, Tr1 and $Foxp3^+$-Tregs according to each YCW group (Fig 3).

Conclusion

This study identifies marked intra-species variability in the molecular properties of *S. cerevisiae* yeast cell wall fraction. The α-mannan content was associated with GCD and IEL hyperplasia suggestive of fortifying intestinal barrier integrity and immune competence. The gene expression analysis revealed marked modification in the expression pattern of PRRs when fed contrasted YCW fractions. Furthermore, the gene expression patterns for transcription factors and cytokine responses suggest the preferential mobilisation of distinct T-helper cell subsets for Th1, Th17, TR1 and $Foxp3^+$ -Tregs, indicating a particular potential for each YCW fraction to confer protection against infectious agents and, or non-infectious pathologies. Accordingly, this study highlights the potential of considering the biophysical characteristics of the YCW to apprehend their specific immune properties and elicit targeted immune functionalities towards precision functional nutrition in Aquaculture.

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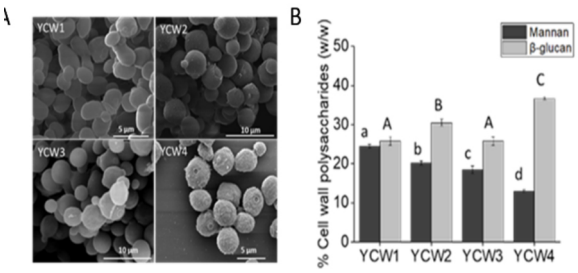

Fig 1. YCW fractions characterised by differences in cell surface structure and composition: A) Scanning electron microscopy images of each YCW fraction; B) YCW biochemical composition of bioactive compounds, as total percentage of α-mannans and total β-glucans for β-1,3 and β-1,6-glucans present in each YCW fraction. Different letters indicate significant differences between treatments $p < 0.05$).

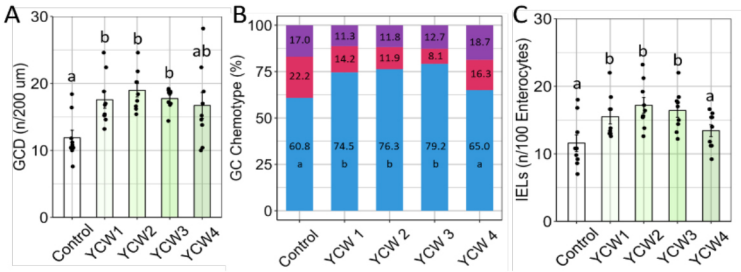

Fig 2. Fortification of the intestinal barrier in YCW1, 2 and 3 groups by elevations in goblet cell density (GCD), acidomucins secreting cells and intraepithelial leukocytes (IELs) compared to the control and YCW group 4. Different letters indicate significant differences between treatments ($p < 0.05$).

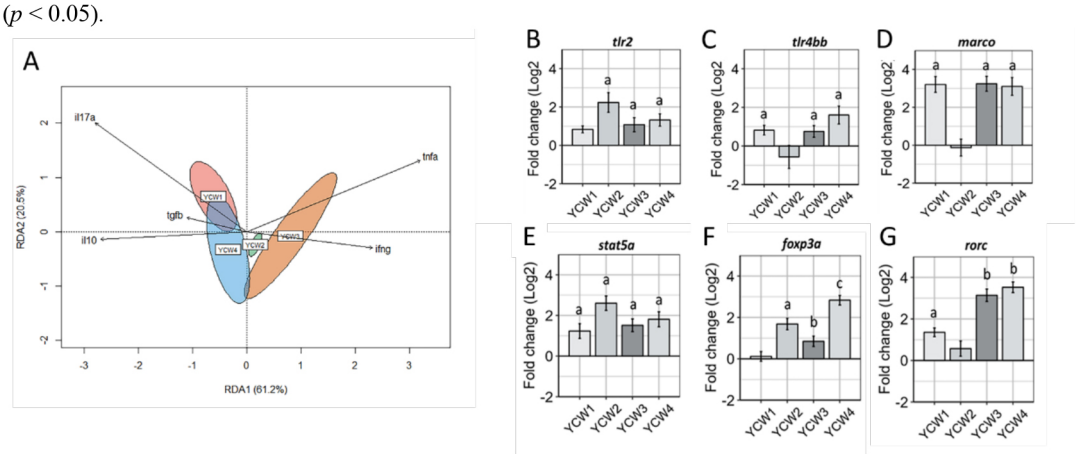

Fig 3. Gene expression analysis reveals differential expression patterns for innate cytokines as shown by the redundancy analysis plot (A). Figures B-D show differential gene expression data for PRRs. Figures E-G show differential gene expression data for transcription factors. Different letters indicate significant differences between treatments ($p < 0.05$).

THE EFFECTS OF PARTIALLY DEFATTED BLACK SOLDIER FLY *(Hermetia illucens)* MEAL ON ENVIRONMENTAL SUSTAINABILITY OF JUVENILE SIBERIAN STURGEON (*Acipenser baerii*) REARING

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Introduction

The rapid development of insect-derived feed materials production scale and quality increases the possibility and justification of their use in commercial diets for fish each year. For a number of species initial studies which assess the potential of fish meal replacement was performed. Fish tolerance for even high doses of insect meals was assessed with positive results for this novel group of feed ingredients. However, in most of the studies, there is a lack of calculations analyzing the sustainability of fish rearing based on combined feed composition and fish growth performance results.

Materials and method

900 juvenile Siberian sturgeons (5 g each) were used in the 60-day-long feeding trial. They were divided into 9 treatments using 5 tanks per group and 20 fish per tank. The control diet (CON) contained 300 g/kg of fish meal. In 8 following experimental treatments 75, 150, 225 and 300 g of *Hermetia illucens* partially defatted meal (BSFM) was introduced. In four diets (H75, H150, H225, H300) the level of crude protein in BSFM was based on nitrogen analysis with the assumption that crude protein = 6.25 x N. In another four diets (HK75, HK150, HK225, HK300) the BSFM protein content was calculated on the basis of amino acid levels using a modified conversion factor (Kp). After 60 days of feeding sustainability indexes including feed conversion efficiency and fish in – fish out ratio were calculated, and all the data were analysed using analysis of variance and post hoc Duncan's test.

Results

The highest feed conversion efficiency (body weight gain/feed intake) was reached in HK75, HK150 and HK300 treatments in which efficiency was higher up to 300 g/kg of used feed than in CON treatment. The FIFO ratio was the lowest in diets containing 300 g/kg of BSFM with no difference among protein calculation methods.

Conclusions

Partially defatted Black Soldier Fly (*Hermetia illucens*) larvae meal is a feed resource which may not only meet the nutritional requirements of fish but also improve the sustainability of Siberian sturgeon production.

This study was carried out as part of the project entitled: Innovative application of Hermetia illucens protein and fat in Acipenseridae fish aquaculture (LIDER/2/0018/L-12/20/NCBR/2021) financed by The National Centre for Research and Development

SHORT TERM EFFECTS OF ELEVATED NITRITE AND NITRATE ON PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) SURVIVAL

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Animal density in indoor shrimp production is continuing to increase as producers seek to maximize production from their aquaculture systems. Increasing animal density and therefore feeding rates, results in faster accumulation of nitrogenous waste in systems with limited water exchange. Pacific white shrimp (*Litopenaeus vannamei*) are considered to be resilient to most forms of nitrogenous waste and have higher tolerances than many other aquatic species. Although shrimp may be tolerant to elevated levels of ammonia, nitrite, or nitrate individually, there is little information on the combined effects of these nitrogenous wastes on shrimp, particularly nitrite and nitrate. This study was designed to evaluate shrimp survival when subjected to elevated levels of both nitrite and nitrate.

In this experiment 36 64-L tanks were stocked with 10 shrimp each at a salinity of 15 ppt. Three different concentrations of nitrite-N (0, 10, 20 ppm) and four different concentrations of nitrate-N (0, 200, 400, 600 ppm) were used, resulting in a total of 12 treatments with three replicates each. The treatments were labeled based on the targeted amount of nitrite and nitrate in mg/L: 0/0, 0/200, 0/400, 0/600, 10/0, 10/200, 10/400, 10/600, 20/0, 20/200, 20/400. 20/600. The experiment was conducted for 11 days, at which point the total number of surviving shrimp were counted.

When examined individually, nitrite had a significant impact on survival at both the 10 and 20 ppm levels. By itself, nitrate levels of 600 ppm significantly decreased shrimp survival. Furthermore, the interaction between the two compounds was found to have a significant negative impact on shrimp survival. Combined levels of nitrite over 15 mg/L and nitrate over 400 mg/L showed mortality rates approaching 50%. This has important implications for shrimp producers, as many producers maintain high levels of nitrate and frequently experience fluctuations in nitrite levels. It seems that the two compounds should not only be considered as individually toxic, but in combination as well.

CAN BIOACTIVE PEPTIDES PRESENT IN PROTEIN HYDROLYSATES IMPROVE STRESS RESPONSE IN MEAGRE (*Argyrosomus regius)* JUVENILES SUBMITTED TO ACUTE STRESS?

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Introduction

Functional feeds are currently being proposed as a disease management tool under intensive farming conditions. These feeds, are designed not only to fulfil nutrient requirements for growth, but also strengthen immunity and stress resistance. The dietary inclusion of protein hydrolysates (PH) has been shown to improve fish growth, antioxidant activity and immunity. Protein hydrolysis can give rise to bioactive peptides with immune-modulatory effects. Low molecular weight peptides (< 3kDa) are described as having antimicrobial, immune-stimulating, anti-inflammatory and antioxidant properties. The present work aims to assess the effects of different PH on stress resilience, immune response and oxidative stress of meagre (*Argyrosomus regius*) juveniles prior and after acute stress.

Material & Methods

A practical commercial-like diet was used as control (CTR), whereas 3 other diets were formulated based on CTR to contain a 3% inclusion level of a short chain purified bioactive peptide in diet BPP, 3% shrimp PH in diet SPH and 3% feather meal PH in diet FMH. Diets were randomly assigned to triplicate groups of 90 fish (IBW approx. 9.5 g) that were hand fed to satiation 4 times a day for 14 days. After 2 weeks of feeding, fish were subjected to an acute stressful event (i.e., 5 min chasing with a net). Intestine samples were taken for gene expression, immune parameters and oxidative stress analysis, immediately prior to stress and one day following acute stress. Plasma samples were also taken for cortisol and glucose levels measurement, prior and one hour after stress.

Results & conclusion

Results demonstrate a stress induced response in the experimental animals. Thus, increased plasma glucose levels 1h after stress and increased gut lipid peroxidation 24h after stress were measured irrespective of the dietary treatment. Antioxidant functions in the gut were also affected by stress with a dietary modulation of the response. Both catalase (CAT) and superoxide dismutase (SOD), show a decrease in activity 24h post-stress, with SPH fed fish showing higher SOD and CAT activities than FMH group. In the case of SOD, enzyme activity is also higher than CTR and activity values are equivalent to those measured prior to the stimulus. This, might indicate a positive effect exerted by SPH diet on meagre stress response. Further analysis on gut and plasma samples, namely immune response and gene transcription, are currently underway to obtain an overall picture of fish stress response and health status.

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DIETARY INCLUSION OF SARDINE COOKING WATERS: IMPACT ON APPETITE REGULATION, GROWTH AND SENSORY PROPERTIES OF EUROPEAN SEABASS

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Introduction

Plant-based ingredients have been considered suitable options for replacing marine-based ones in aquafeeds (Naylor et al., 2021), but this may compromise diet palatability (Geurden et al., 2013), potentially affecting fish growth and flesh quality. To overcome this, feed attractants have been suggested. These compounds, like alcohols, aldehydes, and organic acids, were reported to stimulate intake in mammals (Chen et al., 2017; Takács et al., 2018). They can be obtained from agri-food by-products, within a circular economy framework. One example is the 9 m^3 liquid waste generated by the canning industry per tonne of canned fish (Ferraro et al., 2013). The objective of this work was to extract aromas from the effluent waters of a canning industry and incorporate them in plant-based diets for European seabass. The aim was to evaluate their potential to modulate intake, growth and fillet organoleptic characteristics.

Materials and methods

Sardine cooking wastewaters were collected and either used directly (CW-A) or after processing by vacuum distillation (VD-A) or liquid/liquid extraction with soybean oil (LLE-A). Although the resulting extracts had different chemical profiles, the most abundant compound in all fractions was the 1-penten-3-ol. This was hence selected as marker and extracts containing 2 µg/g of this compound were incorporated in isolipidic and isoproteic diets (CW, VD and LLE) for European seabass. A non-supplemented diet was used as control (CTRL). Each diet was assigned to triplicate fish groups (initial weight 95.7 ± 13.5 g), hand-fed twice daily until apparent satiation in a recirculating saltwater system at 21 °C. After 18 weeks, growth performance and nutrient utilisation were evaluated. Flesh colour and texture were assessed instrumentally and by sensory analysis using a consumer panel. Moreover, the expression of neuropeptides involved in feed intake regulation (neuropeptide y – *npy*, agouti-related peptide – *agrp2*, cocaine and amphetamine-related transcript – *carpt2* and pro-opiomelanocortin – *pomca*) in the brain was analysed. Metabolites in plasma and liver of fish were also quantified.

Results and Conclusions

Fish fed LLE displayed a significantly higher voluntary feed intake than those fed the CW diet, although neither differed from CTRL. LLE also resulted in increased feed conversion ratio. Final weight, whole-body composition, and nutrient gain were similar among diets. The expression of intake-regulating neuropeptides was not significantly affected by diets, but a slight upregulation of the orexigenic *npy* was observed for the LLE diet. In addition, fish fed this diet displayed the lowest plasmatic glucose and highest hepatic glucose and triglycerides values, although the remaining metabolites (lactate, non-esterified fatty acids, cholesterol and glycogen) were unaffected. Thus, the changes in feed intake are probably due to a combined effect of the homeostatic (nutrient-driven) and hedonic (pleasure-driven) regulation. No differences were found in skin or muscle colour among treatments. Despite a lower hardness in fillets of fish fed LLE when compared to those fed CTRL, no significant differences were perceived by the sensory panel; global liking of samples was similar among treatments, being all generally well accepted. Additionally, the samples' taste and odour were characterized as "characteristic fish" and "soft" for all treatments. Overall, results suggest that aromas from sardine cooking wastewaters can modulate feed intake, but further optimization of either the processing and/or incorporation levels seems required to maximize their effectiveness as feed intake stimulants for application in aquafeeds.

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EXPLORING THE INFLUENCE OF SARDINE COOKING WATER EXTRACTS ON THE SHORT-TERM REGULATION OF FEED INTAKE IN EUROPEAN SEABASS

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Introduction

In fish, feed intake regulation is mediated by an interaction between homeostatic and hedonic signals (Soengas et al., 2018). The homeostatic control relates to biological needs regarding nutrients and energy (Soengas, 2021). On the other hand, the hedonic control is associated with reward-based routes, which are triggered by highly palatable food (Díaz-Rúa et al., 2022). Hedonic control may override homeostatic and maintain a drive to eat, due to the sensory pleasure felt by eating. Increasing the palatability of diets, such as those with high levels of plant-based ingredients, is therefore an important matter in aquaculture, as to achieve the best growth performance of fish. In addition, to evaluate the potential of a diet to affect the hedonic regulation of feed intake, long-term growth trials may be inadequate, since the need to maintain nutrient and energy homeostasis becomes more relevant over time. In parallel, the habituation effect to the diets occurs and masks the impact of diet palatability. Thus, studying feed intake stimulants, preferably obtained from by-products (considering a circular economy approach), on short-term trials may shed light on their impact on diet palatability and hedonic regulation of feed intake. In this work, we evaluated aromatic extracts from sardine cooking wastewaters as feed intake stimulants in plant-based diets for European seabass, focusing on the first feeding response towards the experimental diets.

Materials and methods

Sardine cooking wastewaters were collected and either tested directly (CW-A) or after vacuum distillation (VD-A) or after liquid/liquid extraction with soybean oil (LLE-A), resulting in distinct chemical profiles. The most abundant compound in the three extracts was 1-penten-3-ol, being thus chosen as marker and included at 2 µg/g in isolipidic and isoproteic diets (CW, VD, LLE). A practical plant-based diet (12.5% fishmeal and 4% fish oil) without any supplementation was used as control. Each diet was assigned to six homogeneous groups of European seabass juveniles (96 g). Fish were sampled 2 and 6 hours after a single first meal distributed to apparent satiation. Metabolites including glucose, lactate, cholesterol and non-esterified fatty acids were quantified in the plasma. Additionally, the mRNA abundance of neuropeptides involved in the regulation of feed intake, namely neuropeptide Y (*npy*), agouti-related peptide (*agrp2*), cocaine and amphetamine-related transcript (*carpt2*) and pro-opiomelanocortin (*pomca*) was quantified in the hypothalamus and telencephalon.

Results and Conclusions

Feed intake after the first meal was significantly higher for the control diet than for the supplemented diets. No significant differences on plasma metabolites were observed. Although the expression of neuropeptides involved in feed intake regulation was statistically similar among diets and sampling points, LLE and VD diets displayed the lowest expression of the *agrp2* in the telencephalon, whilst LLE diet led to the highest expression of *pomca*, 2 hours after feeding.

The similar profile of plasma metabolites among diets suggests that the regulation of feed intake in the tested fish was driven more by hedonic factors, such as reward or pleasure-driven, rather than homeostatic factors, such as nutrient-driven. The slightly lower expression of orexigenic (inducing feed intake) neuropeptide *agrp2* in fish and higher expression of anorexigenic (supressing feed intake) neuropeptide *pomca* may be regulating factors associated with decreased feed intake observed in fish fed LLE and CW diets, but other factors might have also contributed to such results. Overall, the sardine cooking wastewaters extracts, in the tested concentration, had no positive effect on the short-term feed intake response of European seabass, which suggests that feed palatability was not enhanced. Further optimization of the extracts' production process, and/or inclusion levels in diets should be considered in both short and long-term trials to improve effectiveness of the extracts as feed attractants.

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THE COMPARISON OF THE EPITHELIAL SKIN TRANSCRIPTOME RESPONSE IN ATLANTIC SALMON (*S. salar*) INFESTED WITH SEA LICE (*C. rogercresseyi*) IN SEAWATER CAGES REVEALS DIFFERENTIAL BIOLOGICAL FUNCTIONS RELATED TO SEASONALITY

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Introduction

The Atlantic salmon (*S. salar*) industry covers approximately 90% of the total salmonid culture, with an annual worldwide production of ~1,000,000 tonnes. Chile is the second largest Atlantic salmon worldwide producer, representing its second most important economic activity. Among their challenges, the industry has to deal with sanitary issues, such as sea lice (*C. rogercresseyi*) infestations causing significant economic losses and social consequences for the aquaculture industry worldwide. Sea lice is an ectoparasite that infests the skin mucosa of Atlantic salmon in Chile. Despite its relevance, few studies have focused on evaluating the health status of the skin mucosa of Atlantic salmon reared in sea cage farms. In addition, environmental fluctuations such as temperature, a parameter of particular relevance in fish due to their poikilothermic characteristics, have not been considered either. For this reason, in this study, we evaluated the epithelial skin mucosa transcriptome in summer (at the maximum seawater surface temperature) and autumn (at the descending ramp temperature between the highest temperature and the lowest temperature) of Atlantic salmon infested with *C. rogercresseyi*.

Materials and methods

We performed a transcriptomic profile of the epithelial skin mucosa in Atlantic salmon infested and non-infested with sea lice sampled from the same seawater cage of a farm located in the fjords of the Aysén Region (Chile). We conducted the sampling in two different seasons: summer (during the peak of maximum water temperature; 10 weeks after seawater transfer) and autumn (at the half-descending ramp temperature;23 weeks after seawater transfer). For transcriptomic analysis (RNA-Seq), total RNA was obtained from the epithelial skin tissue. We used a pooling strategy for the non-infested and infested fish group (n= 3 pools per condition; n= 5 fish per pool).

We verified the quality of each sequencing library with FastqQC (Andrews, 2010), a software package that estimates the number of uncallable and low-quality bases. We mapped the skin mucosa transcriptome to the *Salmo salar* reference genome (Ssal_v3.1) using STAR (Dobin, 2013), a high-performance community standard aligner. We used transcripts per million (TPM) values as gene expression levels for all the analyses. The differential gene expression analysis was based on the negative binomial distribution using the DESeq2 package (Love, 2014). Pathways enrichment analysis was performed using the Gene Ontology Consortium database (Gene Ontology Consortium, 2019) and STRING database (Szklarczyk, 2019).

Results

We mapped our data to a total of 14,831 genes. We observed a different expression profile between phenotypes (infested; non-infested) and seasons (summer; autumn). We identified only 19 differential expressed genes (DEGs) in the summer. By contrast, in autumn, the DEGs augmented to 102 DEGs. The representation of common DEGs between summer and autumn was minimal, with only 3 DEGs. For the exclusive DEGs, the summer showed 11 upregulated and 6 downregulated DEGs. On the other hand, the autumn showed 66 upregulated and 33 downregulated DEGs.

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We compared the functionality of the upregulated and downregulated DEGs between the infested and non-infested Atlantic salmon. The enrichment analysis showed the preference of the extracellular matrix process for the upregulated DEGs during summer. Conversely, the downregulated DEGs during summer showed no enrichment. In autumn, we observed upregulated DEGs associated with cellular response to corticotropin-releasing hormone stimulus, metabolic processes, cell proliferation, and nitric oxide biosynthetic process regulation, among others. On the other hand, the downregulated DEGs were associated with processes like riboflavin transport and mitochondrion organization.

Conclusions

Our analysis revealed that sea lice infestation does not appear to dominate the differential expression profile in summer, suggesting that both infested and non-infested samples are more concerned with seawater environmental adaptation. On the contrary, during autumn, the infested and non-infested Atlantic salmon show a differential expression profile and biological processes in response to sea lice. In this way, it stands out the physiological response to stress orchestrated by the cellular response to glucocorticoid stimulus.

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EFFECTS OF ENVIRONMENTAL CONDITIONS ON MYSID AQUACULTURE – A MULTIGENERATIONAL STUDY

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Mysids play a vital role as a food source in aquaria and fisheries, and they are considered a valuable experimental model for studying crustacean biology due to their shrimp-like characteristics and favorable traits. However, our understanding of their physiology and biochemical responses in controlled culture systems is still limited. In this study, we focused on the marine mysid *Neomysis awatschensis* and reared them in a controlled laboratory system to investigate various parameters. We examined the growth, survival, molting, levels of 20-hydroxyecdysone (20E), and biochemical responses, such as antioxidant defense and cholinergic system, in response to consistent fluctuations in environmental factors across five generations. Our findings revealed that temperature fluctuations had a significant impact on growth parameters, including total length, antennal scale length, expod length, endopod length, and telson length. Specifically, the levels of 20E were significantly elevated during the postnauplioid stages when exposed to temperature changes. The number of newly hatched juveniles produced by the mysids was greatly influenced by temperature changes across generations. Furthermore, we observed that lower salinities below 15 practical salinity units (psu) negatively affected both growth and survival, while no significant modulation was observed at salinities above 25 psu. The combined effects of low temperature and salinity were identified as the most significant environmental factors impacting multigenerational mysid culture. It is worth noting that although the survival rate was slightly reduced at a pH of 7.0, it did not significantly affect the overall culture performance. The information obtained from this study is valuable for optimizing mass mysid culture in controlled systems. Understanding the specific environmental factors that influence mysid growth, survival, and reproduction can aid in the development of effective strategies for mysid aquaculture and enhance our knowledge of their physiology and responses to environmental conditions.

ARE CONSUMERS READY TO ACCEPT AQUACULTURE PRODUCTS?

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Introduction

Aquaculture is a growing sector that plays a fundamental role as a healthy source of food and nutrients for the world's population. Fish and aquatic foods are associated with numerous health benefits, not only as a source of protein, but also as an exceptional and diverse supplier of bioavailable fatty acids and micronutrients. Moreover, is the main, and almost unique source of long-chain omega-3s, and its consumption is recommended by the World Health Organization[1].

Consumers' perception of food is evolving towards a greater awareness of the relationship between nutrition and health[2]. This is both a challenge and an opportunity for the agri-fod industry, which must develop innovative products that meet consumers' expectations, such as functional foods. Farmed fish can be considered an excellent candidate for a functional food since it combines the innovation of the sector with the possibility to tailor muscle composition with health beneficial compounds. It is, however, important that consumers understand and trust the benefits of consuming functional foods and know that the health claims are supported by scientific evidence[3].

In order to assess consumer acceptance of differentiated aquaculture products a comprehensive survey was conducted internationally, as part of the OmegaPeixe Project.

Objectives

To carry out a comprehensive consumer survey, both nationally and in relevant European countries, a questionnaire was developed with the main purposes of:

1. Assess the level of consumer confidence in aquaculture products.
2. Identify trends in fish consumption.

Material and methods

An online questionnaire was designed, according to Hill[4], to allow for the collection of information on the target audience - socio-economic characterization data, consumption data of fish and aquaculture products, level of trust and preference for aquaculture products. The questionnaire was disseminated by the project promoters, through their commercial and institutional contact networks, as well as through social networks. Additionally, relevant entities from the aquaculture sector and NGOs were invited to participate in the dissemination of the questionnaire to make it as comprehensive as possible. Responses were collected over a 5-month period from 16th September 2021 to 16th February 2022. The information collected was statistically treated in IBM SPSS Statistics 28.0 program. For comparison between groups, Pearson's chi-square test ($\chi 2$) was performed to evaluate the association between nominal qualitative variables, with the following assumptions: sample number greater than 20; 80% of the categories with expected frequency greater than 5. To evaluate the strength of association of the chi-square test, Cramer's V test was used for nominal variables and Eta test for ordinal variables.

Results

In this study 1349 valid questionnaires were obtained, with respondents aged 18-82 years, 61.7% living in Portugal and 30.9% living in Spain, 4% in the other countries of the European Union, 2% in the other countries of Europe, and almost 2% of the respondents were in other Countries Outside Europe. More than 80% of respondents were active and the average monthly household income was over 3000 € for 21.1% of the respondents, and 33.4% spend more than 400 € monthly on food. Additionally, 88.3% have never worked in the aquaculture sector and 97.4% were fish consumers. Moreover, in total, 41.9% consumers preferred wild fish, 41.3% showed no preference, while 7.2% preferred aquaculture fish, and 9.5% fish from organic aquaculture. Consumers in the age groups of 18-27 and 28-37 were less likely to choose wild fish, and more choose "no preference" answers as to how it was produced, than in the other age groups. On the other hand, the age group 58-67 years showed more preference for wild fish. Respondents with High School diploma consumed less aquaculture fish, while College degree respondents chose more "no preference" answers. Furthermore, results showed that working or having worked in the aquaculture sector significantly favoured fish from aquaculture production over wild fish.

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Conclusions

A low number of consumers showed preference for fish from aquaculture, indicating that consumers may not be ready to accept aquaculture products yet. Nonetheless, younger consumers, with higher levels of education showed higher level of acceptance towards eating fish from aquaculture. Moreover, consumers that work or have worked with the sector showed higher levels of consumption of aquaculture fish, suggesting that an increase in the dissemination and awareness of this industry could lead to an increase in acceptance and consumption of farmed fish by the general population.

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MICROBIAL BASED EARLY-WARNING TOOL FOR H_2S OCCURRENCE IN RAS- A PRINCIPLE DEMONSTRATION

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Introduction

Fish in RAS is threatened by severe risks associated with the water quality, even though it is designed for optimizing growth conditions. Sudden mass mortality of fish is one of the major risks in RAS. In the past years, an increasing number of such incidents has been reported and most cases have been associated with H_2S[1]. To-date monitoring of H_2S is not commonly implemented in RAS due to limitations in analytical methods to detect low concentrations relevant for fish health. This entails development of more precise analytical chemistry-based methods, or sophisticated methods for indirect detection of the hazardous gas. It has been shown across several fields that microbial communities and their composition can be an excellent proxy for predicting the state of a given environment[2]. Data obtained by next generation sequencing (NGS) from environmental samples typically contains information on thousands of microbial taxa, which allows for the employment of supervised machine learning (SML) techniques. In the recently finished "MonMic" project, we have found that microbial communities are an excellent indicator for perturbations in RAS. Building upon that knowledge, we are exploring the potential of microbial communities as indicators for H_2S in RAS.

Material and methods

Atlantic salmon (*Salmo salar*) post-smolt were randomized and stocked into control and exposure groups, and acclimated for two weeks in RAS prior to the H_2S introduction. The control treatment comprised of one tank and one biofilter, while the exposure treatment included two tanks connected to one biofilter. The tanks and biofilters had a capacity of 0.8 m^3 and 0.350 m^3, respectively. The exposure group was stocked with two different densities of fish biomass, 10 and 30 kg/m^3, and the control group was stocked with 30 kg/m^3. After the acclimation period, both exposure tanks were exposed to artificially added H_2S for ten consecutive days. Sodium sulfide (Na_2S) was used to artificially introduce H_2S. The starting concentration of H_2S was 1,25 µg/L. Doubling concentrations of H_2S from previous exposure were used for subsequent exposures until a stress response in the fish was noticed. The final exposure of 160 µg/L was performed after nine days of the challenge. Different exposures were conducted after 24 hours, enabling fish recovery from the previous exposure. H_2S concentrations were monitored in real-time employing the SeaRAS AquaSense System (AQS).

Results

There was a difference in microbial communities at the start of the experiment between the two parallels (prior to H_2S exposure) (figure 1A). Communities of both treatments were also dynamic over time. Partial least square discriminant analysis (PLS-DA) and Random Forest (RF) SML have been used to attempt to classify H_2S exposed and non- exposed samples. Both approaches yielded excellent predictions, with PLS-DA classifying at 99%, while RF at 99.5% accuracy. Most prominent features contributing to the classification strength from both approaches were filtered out and classified (figure 1B).

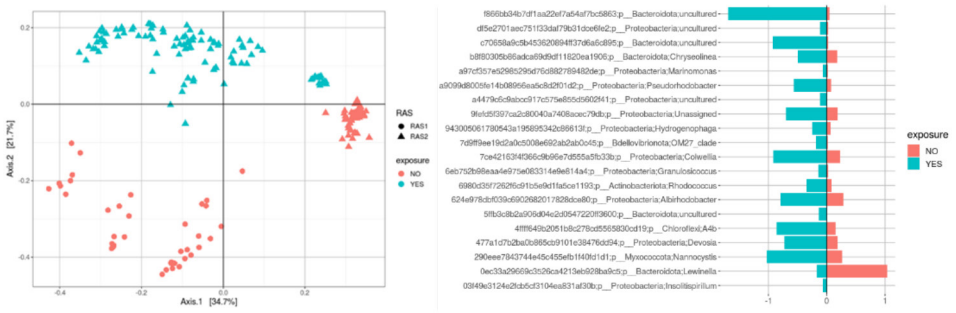

Figure 1. Beta diversity analysis of all samples annotated on basis of different RAS and exposures (A). Most prominent features with mean relative abundances across two treatments contributing to classification of H2S exposed samples for RF (B) approach.

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Discussion

Although the control and exposure RAS were identical from design, and the same water was used to fill the systems, communities between them were different (figure 1A). In fact, this is not uncomon for identical RAS, as very subtle differences in operations, water chemistry, etc. can lead to such contrasts. There was also a time component influencing community dynamics in both treatments which seemingly hinders clear conclusions to what extent H_2S has influenced community alterations in exposed RAS. Nevertheless, a larger time-wise spread of community dynamics in the exposure group compared to the control is an indication that these larger alterations of microbial community may be indeed influenced by the introduction of H_2S, rather than the standard community development. Application of supervised machine learning indicates that there is a pattern in microbial communities refelcting the presence of H_2S. Indeed, many of the prominant features are belonging to the taxa associated with sulfur oxidation, e.g., *Marinicella*, *Pseudomonas*, *Hydrogenophaga, Ahrensia, Hoeflea, Phycisphaeraceae*, or are associated with the metabolism byproducts of sulfur oxidation (e.g., *Colwellia, Marinomonas*). It has to be mentioned, however, that the experimental desing was setup to simulate acute H_2S conditions, with short-lasting peakseasing off quickly. We may speculate that with a setup simulating chronic exposure, community shifts and signatures would be even more pronounced.

Conclusion

1) Two identical RAS show spatial and temporal differences in microbial communities. 2) Microbial community based SML model exhibits a potential for H_2S prediction in experimental RAS. 3) Features contributing to SML model distinction of H_2S exposed and non-exposed samples can be associated with sulfur metabolism.

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THE COMBINATION OF METAGENOMICS AND METABARCODING DATA TO EXPLORE THE SPATIOTEMPORAL DYNAMICS OF MICROBIAL COMMUNITIES IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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Introduction

In recirculating aquaculture systems (RAS), an enclosed aquaculture system for cultivating animals in a controlled manner, microbial communities are critical for maintaining water quality and animal health (Rieder *et al.*, 2023). Despite their systemic importance, the composition of the microbial communities within most RAS remains largely unknown. Establishing accurate, standardized, and user-friendly methods to study microbial communities in RAS may lead to better-designed RAS, improved management practices, and natural ways to prevent disease outbreaks, thereby securing sustainable food production (Moschos, Kormas and Karayanni, 2022).

Methods

This study investigated the effects of sampling, data generation, and analysis strategies on the inference of microbial community composition in RAS. To understand the key aspects of microbial community structure and dynamics, we collected water and biofilm samples (Fig 1A) from tanks (Farm A & B) and the biofilter compartment (Farm B) within two perch freshwater RAS (Fig 1B). Furthermore, to understand the advantages and disadvantages of different sequencing resolutions, we compared two amplicon sequencing approaches, short-read with Illumina Miseq and long-read with PacBio, and amplicon-free shotgun metagenomics with Illumina NovaSeq 6000 (Fig. 1D-F).

Results and Discussion

In the **short-read dataset**, the community composition varied between farms, the tank and biofilter compartment within farm A, the different sample types (biofilm vs. water), and between biofilms of different ages (> 1 week vs. < 1 week) (Fig 2). In the **long-read dataset**, in a semi-quantitative manner, we could identify the most abundant species in each farm and sample type and found complementary results to the Illumina amplicon sequencing concerning spatial patterns. For example, tank biofilm and tank water samples had the most considerable overlap of species, while between the biofilm sample groups, environmental farm conditions had a more substantial impact on shaping the communities than sample type (Fig. 3). The **shotgun metagenomics** sequencing yielded impressively comparable results to Illumina amplicon sequencing. Among the top 0.5% phyla, similar bacterial phyla appear. In addition, we can detect taxonomic groups that the short amplicon approach is blind to, such as Ascomycota (sac fungi) and Streptophyta (green algae) (Fig 4). The fungal signal will be interesting to study further and understand interactions between bacteria and fungi because the dynamics between these two groups play a role in animal health.

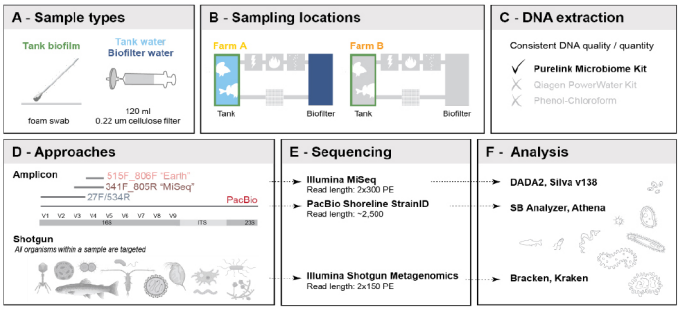

Figure 1: Project design including sampling locations, DNA extraction method, and sequencing and analysis methods.

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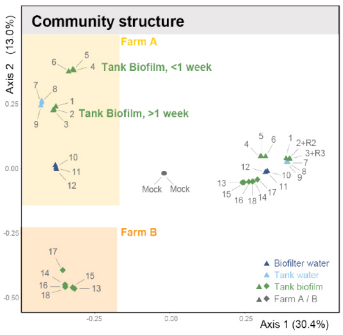

Figure 3: Multi-dimensional scaling analysis on the **short-read dataset**, indicating the dissimilarities of the microbial communities at the phylum level between farms, compartments, sample types, and differently aged biofilms.

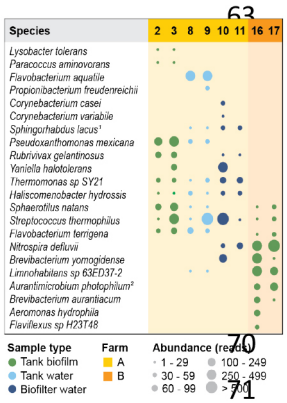

Figure 2: The top 10 species abundance and distribution according to the **long-read dataset.** Similar spatial pattern detected in the short-read

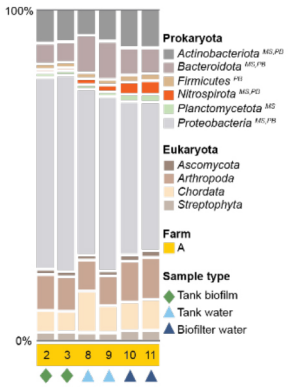

Figure 4: Relative abundance of the top phylum for the **metagenomic dataset**. Similar top phylum detection with amplicon sequecing

Conclusion

The results show that **microbial communities in RAS are highly dynamic** and that management routines create a state of continuous succession and recolonization that can be detected with various sequencing methods. Also, **different compartments feature unique microbial communities**, despite the permanent water circulation, demonstrating the immense impact of environmental parameters on the community composition.

The results presented here contribute to the overall understanding of the microbial community and dynamic and complex interactions in RAS. They also indicate that further research of microbial communities in aquaculture will be helpful to farm management (e.g., biofilter start-up or disease prevention), to understand basic biological principles (e.g., the link between environmental stressors and microbiome dysbiosis), and to clarify medical relevant interactions (e.g., between host-microbiome-environment interaction and disease development).

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GENETICALLY SUPERIOR SEA BASS (*Dicentrarchus labrax*) AND NUTRITIONAL INNOVATIONS: EFFECTS ON GUT MICROBIOTA

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Introduction

The sustainability of the aquafeed sector depends on an adequate and efficient response to the increasing demand for traditional fish-derived raw materials as aquaculture production increases. During the last decades, novel raw materials, including meals from terrestrial animal by-products, or oils from microalgae, have been proposed as alternatives to fishmeal (FM) and fish oil (FO). In parallel, great efforts have been spent for selective breeding to improve the productivity and sustainability of cultured fish species. Accordingly, the present study, in the frame of AquaIMPACT project (Horizon 2020), aimed to investigate in European sea bass (*Dicentrarchus labrax*), the genetic- and nutritionally-mediated effects upon gut mucosal health and microbiota. Different genetic stocks of fish fed with novel feed formulations completely devoid of FO and with low inclusion levels of FM, containing different additives, were tested.

Materials and methods

The feeding trials were carried out at the Parque Científíco-Tecnológico Marino of the University of Las Palmas de Gran Canaria (Telde, Canary Island, Spain). Two batches of European sea bass populations: selected for high growth (HG), and wildtype (WT), produced at MARBEC-IFREMER, were used. In the first trial, as described in details by Montero et al. (2023), 294 dph sea bass larvae (t=0) were randomly distributed in cylinder-conical tanks (10 tanks for each genotype and dietary group, 500 L or 1000 L) at an initial density of 50 fish per tank. Fish were fed to apparent satiation with either a control or a "future" diet (Table 1), in which FO was totally replaced by a combination of poultry and DHA oil, and 50% of FM was replaced by poultry meal inclusion (Skretting ARC, Norway). Fish were sampled at t=0, and at the end of the feeding trial (609 dph).

In the second trial, HG and WT sea bass were fed the "future" diet until achieving the initial experimental size of 16 g. Then, fish were randomly distributed in 24 tanks of 500 L (34 fish/tank; 12 tanks for genotype) and fed diets supplemented with 3 different functional additives (INVE, Belgium) as follows: 2 weeks at high dose followed by 10 weeks at low dose. The functional additives used were a probiotic mixture (PROB), organic acids mixture (ORG), and a phytogenic (PHYTO). In this case, fish were sampled for the scheduled analyses only at the end of trial (after 12 weeks of feeding).

In both experimental trials, from each sampling point, 0.5 cm samples of proximal and distal intestine regions (n= 6 fish genotype/diet) were collected for GALT gene expression analysis by qPCR, whereas the mucosa associated microbiota was obtained by scraping the entire intestinal mucosa (n= 6 fish/ genotype/diet) with a sterile cotton swab. The high-throughput sequencing of 16S rRNA gene on MiSeq platform (Illumina) was performed to characterize the gut microbial communities as described in Rimoldi et al. (2019). The raw data obtained following sequencing were analyzed using the QIIME software. A Two-way ANOVA was applied to test for differences in gene expression and bacterial taxa relative abundances. Significance was set at $p < 0.05$.

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Table 1. Ingredients of the reference "Future" diet (CTRL).

Ingredients (%)	
Corn gluten	5.0
Hi Pro Soybean meal [1]	6.0
Wheat gluten	10.2
Faba bean dehulled	8.0
Wheat	19.95
Soy protein concentrate[3]	15.0
Fish oil	0.0
Fish meal [5]	10.0
Rapeseed oil	8.98
Phosphate	0.35
Vitamin & mineral mix	0.3
Poultry meal	10.0
Poultry oil	1.37
DHA oil	2.75
Lecithin	2.0

Results and discussion

Results from the first feeding trial showed differences in the expression of GALT-related genes in distal intestine. In particular, the expression of cytokines *il-1β*, *tnf-α*, and *il-10* was different, showing an interaction effect diet x genotype. With regard to gut microbiota, diet had a lower influence than genotype. Indeed, regardless of the diet, WT fish showed higher species richness than HG genotype. Furthermore, the gut microbiota of HG fish shared a reduced individual variability, indicating an enhanced capacity to cope with changes in diet composition. A significant genotype effect was found for specific bacterial taxa, such as *Paracoccus* genus and other genera belonging to Moraxellaceae family, which were more abundant in WT fish, regardless of the diet. Among them, the relative abundance of *Paracoccus* genus was positively correlated with the higher proinflammatory cytokine *il-1β* expression found in distal intestine of WT sea bass.

In terms of GALT-related gene expression, the second trial revealed a genotype x diet effect only for *il-1***β** in distal intestine. In terms of gut microbiota, discriminant analysis did not show a clear separation between fish fed the future diet and fish fed additive supplemented diets. Despite that, relative abundances of specific taxa varied among experimental groups. Specifically, fish fed ORG diet presented higher relative abundance of *Streptococcus* in both genotypes, whereas fish fed PHYTO had higher abundance of Lactobacillales. Interestingly, *Bacillus*-based PROB had bactericidal activity on *Pseudomonas* and *Acinetobacter* genera. This result confirmed, our previous evidence, that the *Bacillus*-based probiotics have the capacity to modulate gut microbiota in European sea bass despite of a lack of colonization of the host's intestinal mucosa (Moroni et al., 2021).

Acknowledgements

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EFFECT OF STRESS ON DIFFERENT AGE GROUPS OF CULTURED *Labeo victorianus* CAUSED BY PHYSICO-CHEMICAL WATER QUALITY PARAMETERS

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Given the growth of the aquaculture industry and concerns over negative effects of stress on fish, it is important to research how cultured fish respond to stress. The duration and intensity of stress can lead to fish mortality, disease outbreaks, poor growth performance, and reproductive failure.

This study aims to investigate the impact of physico-chemical water quality parameters on the stress response of different age groups of Labeo victorianus, a cultured fish species. Four treatments will be conducted, each consisting of 100 fish of different age groups (5g, 20g, 50g, and 100g) housed in four tanks and replicated three times. The fish will be fed high-quality feeds with a crude protein content of 30% throughout the experimental period.

Blood samples will be collected from each age group every two days, and the cortisol, glucose, sodium, and chloride ion concentrations will be analyzed. This process will continue for 10 days, with subsequent blood samples taken every two days to monitor the effects of stress. Physico-chemical parameters of each experimental set-up will also be measured by taking three water samples from each setup before extracting blood samples from the experimental fish.

The analyzed blood and water samples from each treatment will be compared to evaluate the response of different age groups of Labeo victorianus to different water quality parameters. This study will contribute to a better understanding of how fish respond to stress and may inform strategies to mitigate negative impacts on cultured fish.

GENOMIC ANALYSIS OF AMOEBIC GILL DISEASE CAUSING *Neoparamoeba perurans* IN ATLANTIC SALMON AQUACULTURE

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Introduction

Amoebic Gill Disease (AGD) is a severe infection of farmed Atlantic salmon (Salmo *salar L.*) with the first reported case in Tasmania-farmed salmonids in 1986. The causative agent was confirmed via *in situ* hybridization to be *Neoparamoeba perurans*. Since its emergence, AGD has continued to spread across the globe at an alarming rate with initially reported cases in Ireland in 1995, Norway and Scotland in 2006 and Chile in 2007. The spread is concerning, both in its speed and in the outsized impact it can have on the salmon farming industry due to the high financial costs associated with both treatment and losses. Over 40 years since its discovery, we still know little about how *N. perurans* spreads between farms, production cycles, regions and countries. An understanding of parasite transmission dynamics could inform fallowing and control in salmonid aquaculture. Any genomic analyses required to achieve *N. perurans* molecular epidemiology, however, are frustrated by characteristically high levels of intracellular bacterial contamination.

Currently, a handful of *N. perurans* genes have been successfully sequenced (18S rRNA, cytochrome oxidase I, ITS, etc) but with insufficient genetic resolution to be of use the farmer or regulator. A A draft genome of *N. perurans* has been generated at the Llewellyn lab, University of Glasgow, and has enabled hundreds of new sequencing markers to be developed. These markers can be sequenced directly from gill swabs at low cost to provide epidemiologically relevant information., A highly multiplexed testing strategy is under develpment with the intent to sequence a series of AGD samples from Ireland and Scotland 2019-2023 for molecular epidemiological analysis.

Method and Materials

Gill swabs were analysed from 2019 and 2022 from Atlantic salmon affected by AGD for qPCR barcoding that were provided from aquaculture industry partners in Scotland. Further non-invasive gill swabs from infected individuals were obtained from aquaculture industry partners in 2023 for additional qPCR barcoding. DNA extractions were conducted on all obtained gill swab samples and amplified through qPCR to determine the presence of the 18S rRNA gene of *N. perurans*.

A multiplex PCR was developed using up to >300 primer pairs per reaction to amplify genes across the nuclear, mitochondrial, and kinetoplast genome on samples that displayed a positive presence of *N. perurans* from qPCR. PCR products were barcoded and cleaned. Library preparation was conducted as per Nanopore specifications. PCR products were then sequenced using the Nanopore MinION system. Basecalling was completed per MinKNOW 23.04.3 protocol. Sequences were then aligned with phylogeny estimated and analysed in RStudio, MEGA, and Figtree.

Results

Extracted DNA of 149 gill swabs from Atlantic salmon in Scotland and Ireland revealed 108 (72.5%) to be positive for *N. perurans* presence at or before 30 amplification cycles of the 18S rRNA gene and quantified (ng/µl) as seen in Figure 1A & B. From this data, best candidates for DNA sequencing based on DNA quantity, and epidemiological considerations (location & time) were chosen to both maximise the success of genome sequencing and allow for phylogenetic investigation inclusive of spatiotemporal changes.

Sequence data is still being sequenced through Nanopore and awaiting results back to begin alignment and phylogeny estimates. Samples for sequencing based on qPCR results with a cut-off limit of 30cq are to be brought forward for sequencing to enhance the viability of genomic sequencing results.

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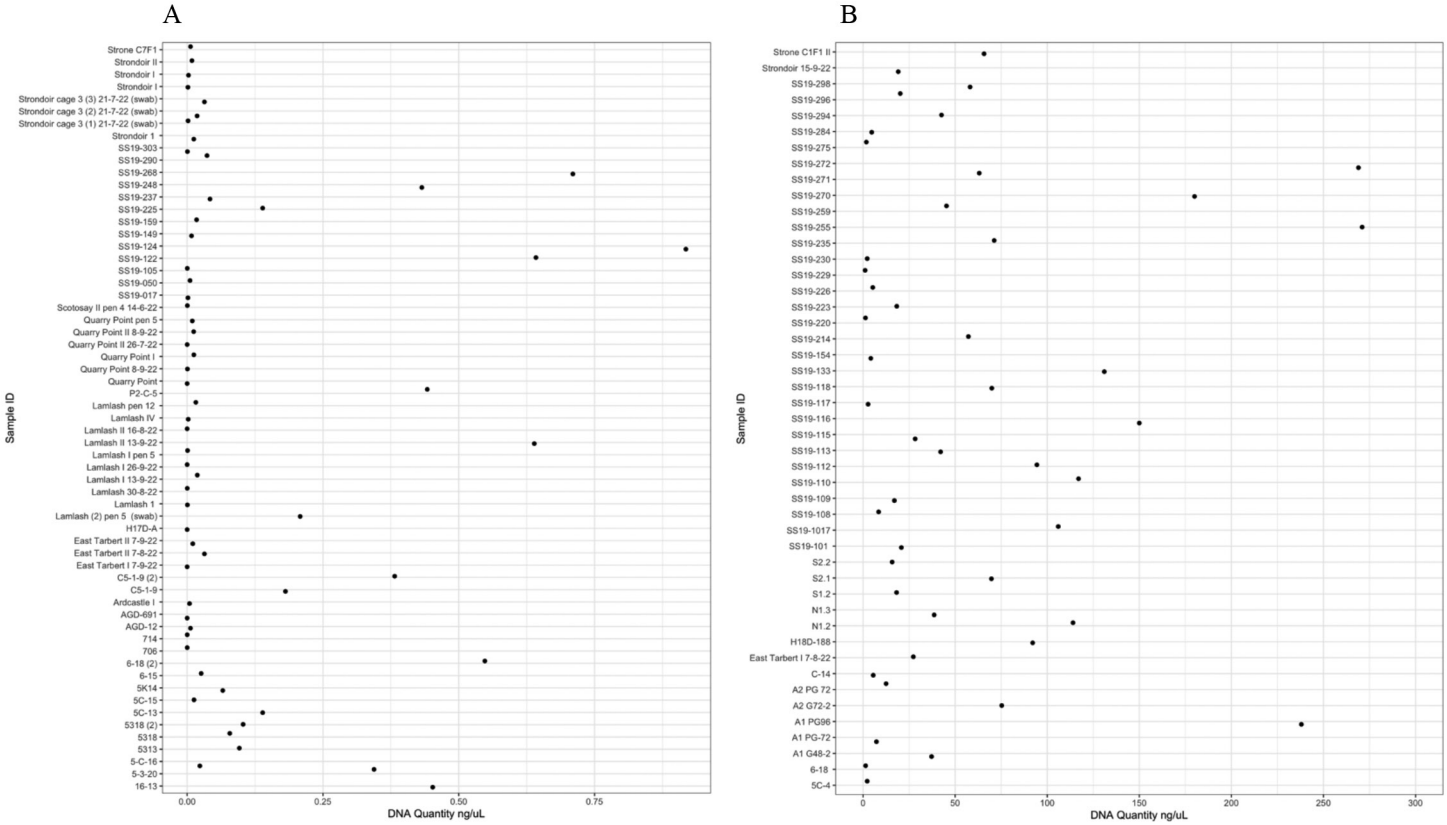

Figure 1: (A) Plot of gill swab samples with DNA quantities between 0 -1 ng/µl per RT-PCR output. (B) Plot of gill swab samples with DNA quantities greater than 1ng/µl per RT-PCR output.

Discussion

To date, there is no effective means of attributing AGD infections to source. Although currently incomplete, this current work has taken important steps towards achieving this by demonstrating that high-resolution genetic information may be readily derived directly from gill swab samples to inform AGD treatment and control.

HEALTH PROMOTING PHYTOADDITIVE FOR GUT HEALTH IMPROVEMENT ACROSS FISH SPECIES: GILTHEAD SEA BREAM *Sparus aurata* AND TILPIA *Oreochromis sp.*

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Introduction

After the latest discoveries on how the gut health influences the general wellbeing in humans, the fish intestine is getting more and more attention. The gut acts as a selective barrier allowing nutrients in and blocking the entry to pathogens.

The inclusion of new ingredients in fish feed formulation represents a challenge for the correct functionality of the gut. Not only the formula has to be nutritionally balanced but it should also promote the health of the intestine.

Feed additives are designed to provide a benefit for the fish, helping in the transport, absorption and/or incorporation of nutrients. In order to evaluate such a benefit, the Ussing chamber has been used as diagnostic tool to assess the tissue electrical properties of the gut (electrophysiology). This technique allows the analysis of the intestine *ex-vivo* if the tissue is kept alive, and reflects its *in vivo* functionality.

In this work, electrophysiology and histology have been used to evaluate the impact of a feed additive on the gut health of a marine fish, gilthead seabream (*S. aurata*) and a freshwater fish, tilapia (*Oreochromis sp.*).

Material and methods

Gilthead seabream: fish of 98,07±1,02 g average individual body weight were distributed in 6 tanks of a marine (32 psu) recirculating aquaculture system (RAS) held at 23ºC. A group of 25 animals were stocked per tank. Fish were fed either a control diet (diet A) or the same diet to which a gut health promoting additive (Sanacore GM®, Adisseo) was added (diet B). Feeds contained 10% FM and the additive was included at 0,5%. Feeds were isoproteic and isolipidic (CP/CF = 45/18). Fish were fed manually twice per day 6 days per week. Trial lasted 8 weeks.

Tilapia: fingerlings of 9.95±1,02 g average individual body weight were stocked in 12 tanks of a freshwater RAS. Fish were fed a positive control diet (diet T+), a negative control diet (diet T-) and a diet S, containing the additive (Sanacore GM®, Adisseo). Diet T+ contains 10 % FM and diets T- and S had 0% FM inclusion. Diets were isoproteic and isolipidic (CP/CF = 33/5). Fish were fed automatically distributing the daily ration during 12 hours per day. Trial lasted 8 weeks.

Epithelial electrophysiology in Ussing chambers

The anterior intestine, was isolated and mounted as previously described (Gregorio et al., 2013) with apical (luminal) and basolateral (blood side) sides of the tissue identified on a tissue holder of 0.25-0.30 cm^2 and positioned between two half-chambers containing 2 ml of physiological saline. Bioelectrical parameters for each tissue were recorded continuously during the *in vitro* period onto Labscribe3 running in a MacIntosh computer using IWorx188 and Lab-Trax-4 data acquisition systems, from the time of mounting for 90 min. Epithelial resistance (Rt, $\Omega.cm^2$) was manually calculated (using Ohm's law) from the current deflections induced by a bilateral +2 mV pulse of 3 s every minute.

Results & conclusions

Gilthead seabream: diet A and B had a similar performance in terms of SGR and FCR as well as in somatic indexes. Significant difference was found in the transepithelial resistance of fish fed the two diets (Fig.1).Tissue resistance in the intestine of healthy seabream juveniles of this size is expected to be higher than 150 Ω cm^2. The time-response in vitro in the Ussing chamber experiment shows a typical progression to plateau values in groups A and B. Fish fed diet B showed a significantly higher average values of tissue electrical resistance than fish fed diet A.

Tilapia: there are very few reports on epithelial tissue resistance in the intestine of tilapia. Values obtained in previous experiments with tilapia species points to values between 45-75 Ω. cm2. Values barely changed during the time *in vitro*, and no significant differences between groups were observed after statistical analysis. However, fish fed with the diet S showed an increase of the epithelium resistance in tilapia although no significant difference was found between diet S and diet T+. Diet T- without additive inclusion showed the lowest Rt values (Fig.2).

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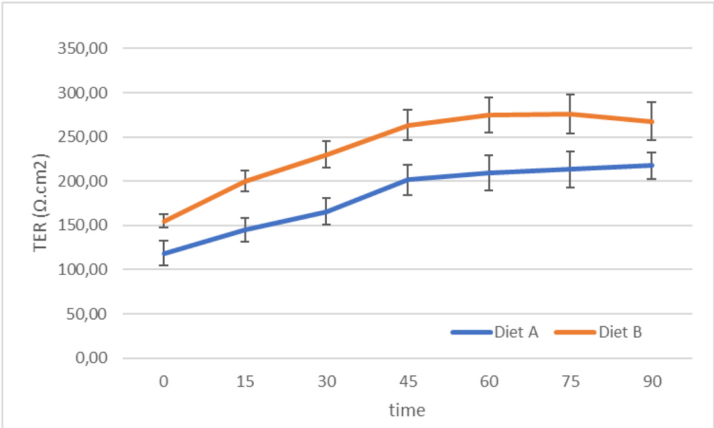

Figure 1. Tissue resistance (Rt, Ω cm2) in the anterior intestine of sea bream juveniles recorded in vitro during experimental testing of 90 min. Each line shows the average + SEM (n=12 fish per diet).

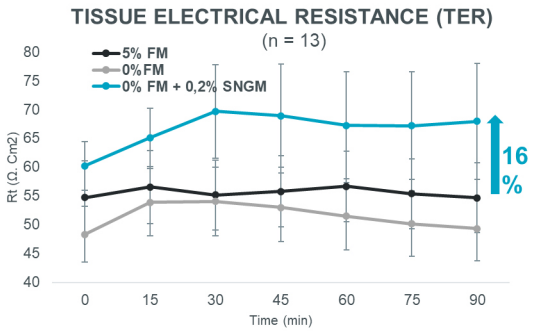

Figure 2. Tissue resistance (Rt, Ω cm2) in the anterior intestine of tilapia fingerlings *in vitro* during experimental testing of 90 min (5%FM is diet T+; 0% FM is diet T- and 0%FM+0.2% SNGM is diet S). Each line shows the average + SEM (n=13 fish per diet).

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FROM A CELL MODEL TO A FISH TRIAL: IMMUNOMODULATORY EFFECTS OF HEAT-KILLED *Lactiplantibacillus plantarum* AS FUNCTIONAL INGREDIENT IN AQUAFEEDS FOR SALMONIDS

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Introduction

During fish farming, fish are exposed to several stressor conditions that can lead to reduced growth, increased mortalities and large economical losses. These problems can be mitigated by the use of functional feeds, leading to a more sustainable aquaculture and improved animal welfare. The use of functional novel aquafeeds containing paraprobiotics (dead/inactivated probiotics) can be an important solutions to these problems. *Lactiplantibacillus plantarum* strain L-137 is a common bacterium found in fermented Southeast Asian dish made from fish and rice. Use of the heat-killed form of this bacterium (HK L-137) in aquaculture species is associated with improved growth performance and immunoregulation, representing a cost-effective way to prevent mortality. The effects of Feed LP20™ (20% HK L-137) were already proven in species such as Nile Tilapia, striped catfish and bighead catfish. Thus functional feeds containing HK L-137 also have potential to improve growth performance and health in Atlantic salmon.

Materials and methods

For the *in vitro* model, we used an intestinal epithelium cell line from rainbow trout (RTgutGC) stimulated with pre-digested HK L-137 to mimic *in vivo* gastro-intestinal digestion. We consider the distal intestine as a good model to study the effect of functional feeds, since this is where the active compound is in contact with the mucosa-associated lymphoid tissue (MALT) in this organ (GALT), which can coordinate both local and systemic immune responses.

For the *in vivo* dietary trial with Atlantic salmon, pre-smolts (average weight 27.3g) were fed *ad libitum* once a day (6 h) one of the five experimental diets (triplicate tanks, 55 fish per tank, 61 days): a commercial-like diet without any growth-promoting additives (control diet, CD); control diet supplemented with 20, 100 and 500 mg of Feed LP20™ kg^{-1} feed (LP20, LP100 and LP500, respectively); a positive control diet (MG) - control diet supplemented with 2 g of β-glucan kg^{-1} feed (Macrogard®).

Results and Discussion

In vitro results showed that the barrier function of the cell monolayer was strengthened, suggested by the reduced permeability of Lucifer yellow after 6 h exposure (**Figure 1A**). An improved barrier can improve digestive function and decrease the vulnerability to bacterial infection. At the protein production level (**Figure 1B**), there was an increased production of IL-1β both after 6 and 24 h after exposure and a decreased production of Anxa1 after 24 h exposure, indicating an activation of pro-inflammatory process. No difference in the expression of E-cadherin was observed.

At the *in vivo* dietary trial, HK L-137 did not compromise fish growth performance. Intestinal microbiota composition was slightly affected, but without relevant changes in alpha and beta-diversities. This was expected since the *L. plantarum* was inactivated and the inclusion levels were low, preventing colonization. Interestingly, indirect ELISA assay in distal intestine showed that fish fed higher inclusion level of HK L-137 also have a lower production of Anxa1 (**Figure 2A**), as seen in *in vitro* model, and no difference in production of IL-1β or E-cadherin. Fish fed LP500 also had a higher production of total IgM in plasma, which was not changed specifically by HK L-137 (**Figure 2A**). The increase of total IgM could be an increase in natural antibodies relevant for defense and homeostasis. Transcriptomic analysis in distal intestine tissue showed that increasing inclusion level of HK L-137 in diets induced an increase in differentially expressed genes. Lowest inclusion level (LP20) induced an up-regulation of 5 terms related with cell structure, adhesion, communication and proliferation and immune regulation (**Figure 2B**, terms in bold). The up-regulation of the same terms was conserved also in the higher inclusion levels, in addition to other terms related with barrier function, immune response and metabolism (**Figure 2B**). No differences were found between dietary groups regarding intestinal villi height.

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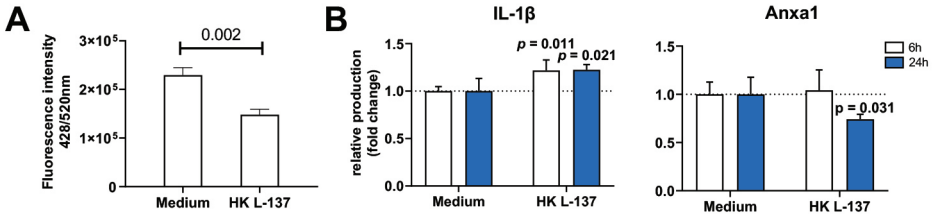

Figure 1. Results from RTgutGC cell line after HK L-137 exposure. (A) Lucifer yellow permeability in monolayer - 6 h; (B) indirect ELISA - 6 and 24 h.

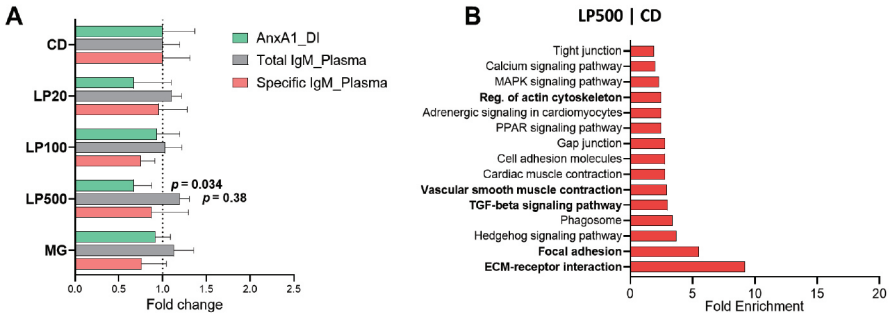

Figure 2. (A) Protein detection by indirect ELISA in distal intestine and plasma; (B) Up-regulated terms by ShinyGO, comparison between LP500 and Control diet.

Conclusion

In the current study, we investigated the potential of HK L-137 at both *in vitro* and *in vivo* levels. Our results suggest that HK L-137 has immunoproperties by modulating cytokines and effector molecules, suggesting its potential in functional feeds for salmonids. The similarity between the two models indicate that an *in vitro* model is indeed a useful tool to study the immunomodulatory properties of a novel fed component. This also is a faster, cheaper approach that also promote animal welfare. The current study was performed on healthy fish in fresh water deprived of major stressor factors. This creates a baseline for future work with HK L-137 in Atlantic salmon. A follow-up live pathogen challenge trial with salmon is on-going, where we are evaluating the ability of HK L-137 to reduce mortalities after seawater transfer.

STRAIN-SPECIFIC MATERNAL NON-GENETIC INHERITANCE AND OFFSPRING PERFORMANCE IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Rainbow trout (*Oncorhynchus mykiss*) is the most important branch of the freshwater aquaculture sector in Europe, responsible for over 75% of the production. Despite of that, the sector suffers from specific problems such as low disease resistance (Tobback et al. 2007) and variability of reproductive performance (Bonnet, Fostier, and Bobe 2007) that are contributing to the stagnation of the production in Europe (FEAP 2020). Lack of genetic information on the strains produced nowadays also put at risk the production and species management (FAO 2020). The sector thus calls for improvement of the productive chain in order to provide food security in a sustainable manner. The selective breeding performed nowadays can be highly enhanced by gathering objective data from various production phases where linkage between the reproductive performance and future performance of the offspring is largely ignored (Janssen et al. 2015). Non-genetic inheritance (NGI) mechanisms are responsible for all heritable features that breeders transmit to offspring that are not directly part of the DNA sequence but still interact or derive from it (Adrian-Kalchhauser et al. 2020). Fish eggs content, in this context, constitutes maternal NGI with major impact in early embryonic development and also later consequences. Investigating these mechanisms can help to shed light on maternal impact on offspring performance and they could also, in a long term run, be considered during selective breeding programs. This project aims to characterize three different molecules responsible for NGI in females and to which extent they are implicated in offspring performance by studying breeders, embryos and juveniles from three farmed strains of rainbow trout.

Materials and methods

Experiments were conducted to evaluate (1) egg quality and thus, maternal investment for reproduction, offspring performance regarding (2) growth and (3) disease resistance and (4) NGI potentially implicated in (1)-(3). More specifically, proteins, mRNA and miRNA profiles in eggs. Additionally, immune response related genes in liver, spleen and gills of fish from (3) will also be assessed.

To assess egg quality, eggs from six females from each strain were fertilized using a pool of sperm of males from the same strain. Fertilization, eyed stage, hatching and mortality rates were recorded for (1). Hatched embryos from all females from one strain were pooled and once larvae reached swimming-up stage, total biomass from each strain was calculated. Larvae were placed into glass tanks for (2) in triplicate. Weekly, larvae were weighted and measured (n=30) for food offer correction and growth evaluation. Mortality during this period was also recorded. When fish reached average 6g individual weight, 162 fish were sampled for (3). They were divided in control and infected group in triplicate (n=27). Both fish groups were subjected to a bath for 1h. Infected group bath contained *Yersinia ruckeri* at a concentration of 4.5×10^8 while control group contained only tank water. After, all fish were placed back into their original tanks. Fish tissues that will be used for molecular analysis were sampled and snap frozen before, at three, five and seven days post-infection in both control and infected group (n=6). Mortality and major infection signs were recorded up to 14 days post-infection.

For (4), a portion of unfertilized freshly stripped eggs from same females as the above mentioned experiments were snap frozen for molecular analysis. These samples will be used for RNA and protein extraction and subsequent mRNA, miRNA and proteins profiling that will be compared between and within strains.

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Results

High quality eggs were obtained from the two strains already evaluated. Fertilization (93.4±3.8 and 85.8±3.1%), eyed stage (84.7±5.2 and 90.5±3.6%), hatching (97.2±1.0 and 97.5±2.2%), egg mortality (2.8±1.0 and 2.6±2.2%) and larvae mortality rates (1.1±0.5 and 0%) did not present differences between strains A and B. In growth performance, however, difference was observed between both strains from the fourth week. No mortality difference during this period was observed between strains. Concerning bacterial challenge, mortality in strain B started earlier and is already double of the one observed in strain A. Trial is still in progress. Trials for third strain have started and molecular analysis of fish tissues will be performed in May/June. Thus, zootechnical and molecular data will be available for presentation at the conference.

Discussion

Commonly used in farmed mammals, progeny tests are largely applied to select the best breeders and thus improve overall stock quality (Rosa 2013). Selection based on offspring performance is a valid option also for fish selective programs once enough information on progeny is available. Our results will contribute in providing this knowledge in terms of early survival, growth, late mortality and disease resistance. NGI has the potential for changing offspring gene expression and, as a consequence, change offspring phenotype and their interaction with environmental challenges. From this perspective, it is of highest importance to identify mechanisms responsible for expression and/or heritability of particular traits in relation to the genetic background and experience of the population studied. Therefore, the knowledge on maternal NGI implicated in the production of high quality offspring in different fish populations may contribute to improve selective breeders programs and hence aquaculture sustainability.

Acknowledgments

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ARE THE INITIAL STAGES OF FISH INGESTING OR ABSORBING MICROPLASTICS IN THE WILD ENVIRONMENT?

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In recent years, several studies showed that the initial stages of fishes are able to ingest microplastics or absorb them through the gills. These plastic particles with less than 5 mm can cause gut blockage and limit food intake but also can have negative effects on growth, development, reproduction and even lead to death. Although an increasing number of studies focus on the ingestion and effects of microplastics, most studies are laboratory-based, and field studies are still very limited. Therefore, the present work aimed to evaluate if microplastics are being ingested or absorbed by wild larval stages of fishes, collected from the Douro Estuary (northern Portugal), an estuarine nursery ground for marine fish species. Monthly surveys were performed over 1 year (2021-2022) to collect fish larvae and microplastics from water samples. Samples were sorted, fish larvae identified and two fish species relevant for human consumption were chosen to evaluate their ingestion rate, namely the Senegalese sole, *Solea senegalensis* and the European sardine, *Sardina pilchardus*. A total of 30 fish larvae of *S. senegalensis* and 100 fish larvae of *S. pilchardus* were analyzed to extract microplastics, using a previously optimized protocol where the organic matter is degraded with H_2O_2 at 65°C. Microplastics present in the water were also quantified, and all microplastics retrieved from water and larval fish samples were characterized in terms of size, color, shape, and the polymer identified through FTIR. So far, results showed that all the microplastics present in fish larvae were fragments or fibers of 0.01 - 2.0 mm in size, and of 8 different colors. In contrast, a higher variety of shapes was observed in water samples, including fibers, fragments, films, foam, and paint, in more than 10 colors and ranging between 0.01 - 5 mm in size. These preliminary results indicate that microplastics present in fish larvae are similar to the ones from the estuarine water, although only the small size plastic fragments or fibers were ingested/absorbed by fish larvae. This study will allow to investigate microplastic occurrence in the initial stages of fishes from an estuarine nursery area, evaluating the incidence of microplastic present in fish larvae and comparing these results with the microplastics collected in the water.

Acknowledgments:
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EFFECT OF CAROTENOID DIETARY LEVELS IN THE IMMUNE AND OXIDATIVE RESPONSE OF *Paracentrotus lividus* (LAMARCK, 1816) SEA URCHINS

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Introduction

The European purple sea urchin *Paracentrotus lividus* is a keystone species of benthic coastal communities and an important marine resource in southern Europe (Bertocci *et al.,* 2018). The growing interest in increasing aquaculture production has been driving the research for diets able to supply species nutritional requirements and produce high quality roe. A well-balanced diet can also contribute to maintaining an efficient immune system. Like other invertebrates, the sea urchins cannot biosynthesize carotenoids *de novo* (Tan *et al.,* 2020), but these are directly involved in the immune and antioxidant defense system. The carotenoids are functional nutrients that stimulate the activity of antioxidant enzymes, increasing the efficiency of detoxification after immune activity (Babin *et al.,* 2015). Simultaneously, by taking over the antioxidant enzyme activity and eliminating free radicals, carotenoids reduce the need for these enzymes and overall burden (Tan *et al.,* 2020). A high dietary carotenoid content enhances immunity by supporting endogenous enzymes (catalase and superoxide dismutase) and detoxifying free radicals produced during immune activity. Despite the important physiologic role, In sea urchins, coelomocytes are the main cellular components responsible for the immune response (Banks, 2014) seconded by the proteases and lysozymes, representing a barrier against bacterial proliferation due to their peptidase activity (Fernández-Boo *et al.,* 2018). The main objective of the present study was to analyze the effect of high and low carotenoid levels on the immune and oxidative response of *P. lividus* sea urchins.

Material and Methods

Adult sea urchins (N = 153) with mean diameter of 38.57 ± 2.99 mm were collected on a rocky beach (Porto Batel, Peniche) and maintained in three recirculatory aquatic systems (RAS) equipped with three 40 L glass tanks each (N = 17 individuals/tank). For 8 weeks, the animals were fed three times a week with three jellified diets: a diet rich in carotenoids (HC); a diet low in carotenoid (LC); seaweed-based control diet (Control). The high content of carotenoids in the diets was accomplished by adding pumpkin and dried *Nannochlropsis* sp to a vegetable-based diet. At the end of the nutritional trial, the sea urchins were exposed to a time-course pathology challenge, where they were inoculated with *Vibrio parahemolyticus* to stimulate the individual's immune system. Sea urchin's weight, condition, immune and oxidative status were measured at the beginning and at the end of the nutritional trial. In detail, the sea urchins were measured and weighed, their gonads were removed and weighed to determine gonadosomatic index and to quantify the activity of oxidative stress related enzymes (Catalase – CAT; Superoxide dismutase - SOD; Lipid peroxidation - LPO). For the analysis of cellular and humoral immune parameters, coelomic fluid (CF) was collected individually to identify and count coelomocytes under 400x magnification microscope. For the analysis of humoral parameters were used the turbidimetric assay to quantify the lysozyme concentration, the azocasein hydrolysis assay for protease activity (Fernández-Boo *et al.,* 2018) and to quantify the nitric oxide content, the Griess reaction procedure (Tafalla *et al.,* 2003).

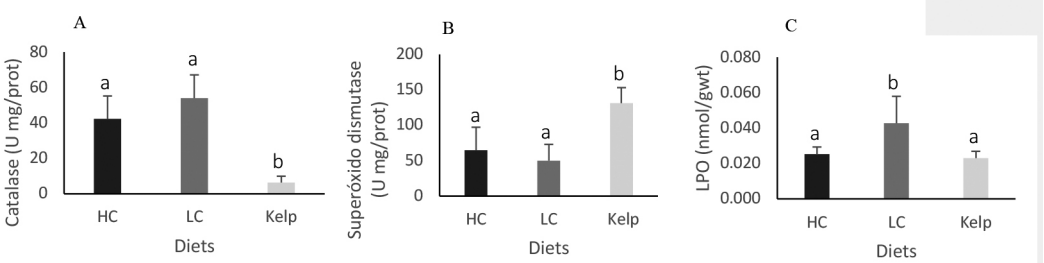

Figure 1- Values of oxidative stress parameters quantified in *Paracentrotus lividus* gonads at the end of the assay, A - Catalase; B - Superoxide dismutase; C- Lipid peroxidation. a and b indicate significant statistical differences ($p < 0.05$)

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Results

In the end of nutritional trial, there was an increase of coelomocytes in the sea urchins fed with HC and LC diets (respectively) when compared to the initial value (), while in the control diet there was a decrease in the total number of coelomocytes (). From these coelomocytes, and similarly in the three dietary groups, the most abundant were the phagocytes (HC: 58%; LC: 65%; Control: 69%), followed by the colorless granulocytes (HC: 18%; LC: 17%; Control: 16%). For the humoral parameters, the lysozyme (HC: 0.86 μg/mL; LC: 0.82 μg/mL; Control: 0.42 μg/mL) and protease (HC: 13%; LC: 14%; Control:13%) did not differ between the three diets studied. On the other hand, remarkable differences in the parameters of oxidative stress were observed between dietary treatments. The production of CAT was higher in individuals fed with HC diet (42 U mg/protein) and LC diet (54 U mg/protein) in relation to those fed with control diet. The opposite occurred with the production of SOD. Here, the animals fed with control diet produced a high amount of this enzyme (131 U/mg), compared with animals fed LC diet (50 U/mg). The occurrence of lipid peroxidation was more visible on sea urchins belonging to the treatment with the LC diet (0.043 nmol/g wt).

Discussion

The cellular and humoral immune parameters analyzed in the sea urchins CF were not affected by the dietary carotenoids' levels selected. Regarding the antioxidant defense system, there was an effect of the carotenoids included in the diets provided to the sea urchins. The high content of carotenoids in an organism, as part of the integrated antioxidant system, enhances immunity by assisting endogenous enzymes (for example, catalase and superoxide dismutase) (Tan *et al.,* 2020). Peroxidation is a common cellular damage caused by oxidative stress, because of the reaction of ROS with lipids. However, the effect of this cellular damage can be minimized by the activation of cytoprotective enzymes, such as superoxide dismutase (SOD) and catalase (CAT) (Amorim *et al.,* 2020). In the present study, the urchins fed with LC diet presented low SOD, higher CAT values and then higher lipid peroxidation values than the HC and Control diet. This could mean that the production of catalase wasn't enough to prevent the occurrence of lipid peroxidation in this treatment.

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OFF-FLAVOR REMOVAL THROUGH DEPURATION: A CASE STUDY ON NILE TILAPIA (*Oreochromis niloticus*)

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Introduction

Approximately 82.1 million tons of aquatic animals are produced per year (FAO, 2020). Sustainable aquaculture, such as Recirculated aquaculture system (RAS), is envisaged as one of the solutions to achieve a further increase in global seafood production. However, even though RAS farms can produce high-quality fish, "musty" and "earthy" off-flavours often taint fish, which is a well-known issue for fish farmers, inflicting significant financial losses as well as a negative perception of the RAS-farmed fish (Moretto et al., 2022). The main volatile compounds that contribute to those flavours in fish are geosmin and 2-methylisoborneol, but other compounds may additionally play a role (Jones et al., 2022). The traditional procedure to deal with off-flavour is the depuration of the fish in cleaner water (Podduturi et al., 2021). In this project sensory analytic instrumental procedures were applied for the characterization of aroma-active compounds in Nile Tilapia (*Oreochromis niloticus)* fillets to determine the aroma profile and composition of RAS fish and comprehend the impact of the depuration process.

Methods

Samples were collected in March 2022 from a recirculated aquaculture farm (Gårdsfisk, Sweden). Three fish replicates were caught randomly from the production and depuration tank. All samples were directly stunned, hand filleted, skinned and frozen at -20 °C until use for analysis. For efficient extraction of the volatile compounds, the procedure was adapted from Mahmoud 2017 (Mahmoud et al., 2017). Briefly, 50 g portions from the fish fillets were taken and minced into approximately 1 cm^3 cubes and extracted once with an aliquot of 100 mL of dichloromethane by stirring at room temperature for 30 min. Subsequently, the extract was collected separately and distilled using solvent-assisted flavour evaporation (SAFE). After SAFE, the sample was dried over anhydrous Na_2SO_4. Prior to further chromatographic analysis, the total volume of each sample was reduced to approx. 100 µL via Vigreux distillation and micro distillation at 50 °C. The aroma analysis was performed using gas chromatography-olfactometry/flame ionization detector (GC-O/FID) equipped with DB-FFAP and DB-5 columns. The comparative aroma extract dilution analysis (cAEDA) was performed using the DB-FFAP column. Moreover, gas chromatography-mass spectrometry (GC-MS) and two-dimensional gas chromatography with mass spectrometry and olfactometry (2D-GC-MS/O) analysis were carried out. The identification of the odour-active compounds was based on the comparison of odour quality, retention indices on two capillaries with different polarities (DB-FFAP and DB-5) and mass spectra with those of authentic reference compounds. Retention indices were calculated for each odour-active compound by means of a reference series of homologous alkanes (C6-C30). Furthermore, the aroma profile analysis was performed according to ISO 13299:2016–09 and complemented by the determination of the overall intensity of the sample's odours.

Results

The odour attributes fatty, green, mouldy, earthy, fishy and sea water-like/algae-like were selected by the panellists for the description of the fish aromas. Using combined sensory and instrumental techniques, 108 and 79 aroma-active compounds were olfactorily detected in Nile Tilapia from tank and depuration, respectively. Of these compounds, 77 were identified by comparison of the respective retention indices, mass spectra and odour qualities with their corresponding reference compounds, while 31 compounds could not be resolved due to their low concentration.

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Discussion

In this study, SAFE technique followed by sensory-instrumental analysis was successfully applied to identify odorant compounds in fillets from Nile Tilapia before and after depuration. A higher number of odour-active substances was detected in the fish sampled before depuration. For a detailed comparison of quantitative aspects, further studies involving quantification of the aroma substances would be required. This was, however, not the aim of the present study which focuses on a non-targeted approach for the characterization of odour-active substances that might be related to aquacultural off-odours. Recirculating water in RAS may favour bacterial growth and the accumulation of the substances they produce, often including off-flavours with earthy and musty notes. Little is known about the odorant characterization of fish and the potential impact of the individual odour-active compounds on the overall aroma in the fish matrix (Jones et al., 2022). Therefore, the identification of potent aroma compounds in Nile Tilapia is described to help mitigate the prejudice of the tainting of RAS fish.

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HOW DOES OCEAN ACIDIFICATION AFFECT THE GROWTH AND BONE MINERALIZATION IN GILTHEAD SEA BREAM (*Sparus aurata*)?

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Introduction

Climate change presents a risk to aquaculture productivity, particularly through rising temperatures and ocean acidification (OA). In particular, OA can have profound implications for the growth and development of many marine organisms. It has been demonstrated that low pH causes mortality or inhibits the growth, increases plasma cortisol and glucose levels, and decreases plasma chloride ions and osmolality in several species of fish (Reid et al., 2019). While OA has been demonstrated to impair normal calcification in marine invertebrates, its impact on vertebrates bone-cartilage calcification is largely unknown. In one species of elasmobranch an increase on skeletal density in response to acidification has been observed. However, the consequences of marine acidification on teleost fish have not been completely demonstrated (Di Santo, 2019). The aim of this study was to determine how CO_2 injection-induced acidification in the water modifies the insulin-like growth factors (IGFs) system members' gene expression in muscle, liver and bone, as well as the development and mineralization gene markers' expression in bone of gilthead sea bream (*Sparus aurata*).

Material and methods

Gilthead sea bream juveniles were obtained from the commercial hatchery Piscimar (Burriana, Spain) and were maintained in the animal facilities of the Faculty of Biology at the University of Barcelona (Barcelona, Spain). Fish were randomly distributed into 200 or 400 L tanks in a seawater recirculation system under a 12 h light/12 h dark photoperiod, 38‰ of salinity, and 22 ± 1 ºC. During the trial, one group was maintained at pH 7.9 (control) and the other at pH 7.3 via CO_2 injection. Each experimental group was represented by triplicate tanks (two 200 L and one 400L but with equivalent biodensity). After 68 days of exposure, 15 fish per condition were anesthetized and sampled. Body weight (BW) and body length (BL) were measured, and condition factor (CF) was calculated. Somatic indexes including hepatosomatic index (HSI), viscerosomatic index (VSI), and mesenteric fat index (MFI) were estimated, and the specific growth rate (SGR) was determined from a number of tagged fishes. Samples of liver, white muscle and bone were obtained and directly frozen in liquid nitrogen for gene expression analysis. The expression of components of the IGFs system were analyzed in all the tissues, and different bone-related genes were analyzed in bone by qPCR.

Results and discussion

After a 39 days culture period, the fish in the high pH condition exhibited significantly higher values of BW, BL and SGR compared to the fish in the low pH condition. By the end of the experiment, the fish subjected to low pH displayed lower values in BW, BL, SGR, and MFI, but no significant differences were observed in SGR. Regarding the gene expression, no statistically significant differences were observed in the liver for the genes analyzed, However, in both bone and muscle, there was a downregulated expression of the binding protein *igfbp4* in fish subjected to the lower pH condition. Furthermore, in white muscle, under low pH, there was an increase in *igfbp5* expression. Changes in the expression of these binding proteins might result from feedback regulatory processes. Thus, although under the low pH condition, a decrease in fish growth was observed in comparison to the control pH-reared fish during the first part of the trial, this condition did not disrupt the expression of key genes from the growth hormone / IGFs axis. The expression of the analyzed osteogenic genes remained unaltered, likely due to a blood buffering mechanism as a response to the low environmental pH. Overall, this study provides information about how OA could impact fish growth, potentially reducing their growth performance.

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THE IMPACT OF CO_2 ON THE GROWTH AND DEVELOPMENT OF GILTHEAD SEA BREAM (*Sparus aurata*) OSTEOBLASTS

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Introduction

Ocean acidification and warming have been shown to modify the calcification rates of marine invertebrates' shells. More recently, it has been described that increased CO_2 levels, which cause ocean acidification, can also affect skeletal mineralization of elasmobranch (Di Santo, 2019). In addition, as a physiological response to high CO_2 levels, the plasma pH decreases, leading to acidosis (Drábiková et al., 2023). Primary fish cell cultures derived from bone are a useful model to study the effects of CO_2/pH on skeletal mineralization. The aim of this study was to determine how different CO_2 concentrations modify proliferation and mineralization of gilthead sea bream (*Sparus aurata*) osteoblast cells in culture.

Material and methods

To this end, vertebra bone-derived cells from this species were incubated during 30 days in either growth medium or osteogenic medium at 23ºC and, three different CO_2 levels: 0%, 1.5% and 2.5% to generate distinct pH levels. Cell proliferation was assessed every 5 days using the MTT assay and mineralization by means of Alizarin Red staining.

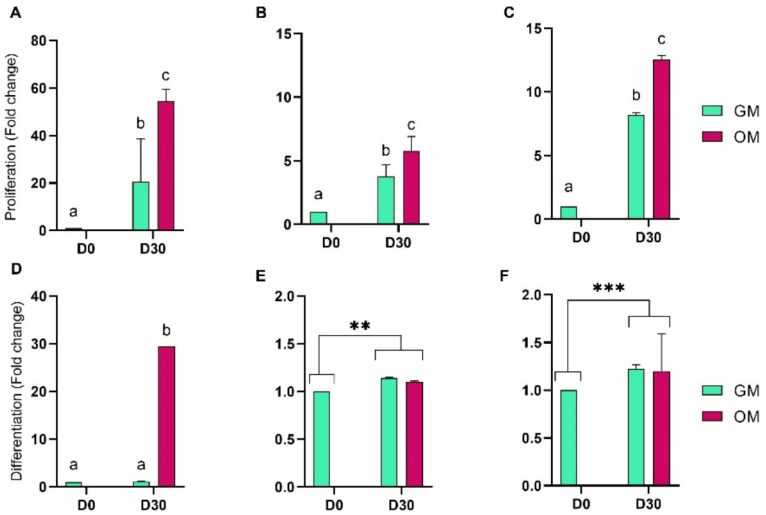

Figure 1. **(A-C)** Proliferation and **(D-F)** differentiation of bone-derived cells at days 0 (D0) and 30 (D30) of culture development with 0% (A and D), 1.5% (B and E) and 2.5% (C and F) of CO_2. Data are shown as means ± SEM respect to D0. Statistical differences were determined using a two-way ANOVA ($p<0.05$) and a Tukey's *post-hoc* test when the interaction between the two factors was significant and indicated by different letters ($p<0.05$). When only the day was significant the differences are indicated by asterisks ($p<0.05$).

(Continued on next page)

Results and discussion

Data showed that at a CO_2 concentration of 1.5% the pH remained close to the gilthead sea bream physiological level (7.54±0.07) and under the 2.5% and 0% CO_2 conditions, there was a slight acidification (7.33±0.03) and alkalinization (8.02±0.12) of the medium, respectively. Regarding proliferation and mineralization, significantly higher levels of both processes were detected in cells incubated with osteogenic medium at 0% CO_2 only at day 30 (Fig 1A, 1D), compared with the other culture conditions. However, at 2.5% and 1.5% of CO_2, significant increases in proliferation from day 5 and in mineralization from day 10 were found, respect to the corresponding day 0, regardless of the incubation media (growth medium or osteogenic medium), that were still evident at day 30 (Fig 1C, 1F for 2.5% and Fig 1B, 1E for 1.5% of CO_2, respectively).

Overall, this study provides information about how the CO_2/pH levels can affect osteoblasts growth and mineralization and help to elucidate if a reduced pH detrimental to the culture of fish bone cells. Moreover, these results suggest that ocean acidification caused by the increased anthropogenic CO_2 emissions could affect the growth and development of gilthead seabream skeleton *in vivo*.

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TRANSCRIPTIONAL RESPONSE OF GILTHEAD SEA BREAM (*Sparus aurata*) MYOBLASTS TREATED WITH AMINO ACIDS AND IGF-1: REGULATORY NETWORKS OF MRNASMIRNAS-LNCRNAS

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Introduction

The skeletal muscle of teleost fish is a very plastic tissue that integrates external and internal inputs to adapt to a changing environment. In vertebrates, muscle growth includes the activation of the satellite cells, their proliferation, fusion, differentiation, and maturation in a process known as myogenesis that is regulated mainly by the myogenic regulatory factors (Myf5, Myod1, Myf6 and Myog). Furthermore, muscle growth and development are conditioned by the balance between protein synthesis and degradation, which are controlled by the PI3K/AKT/mTOR pathway that can be activated in response amino acids (AA) or insulinlike growth factor 1 (Igf-1).

In the last decades, research mainly in mammalian models has demonstrated that the noncoding RNAs (ncRNAs) also play a key role in regulating the myogenesis but little is known about their role in fish muscle development. The ncRNAs are a group of RNAs that, generally, do not codify for proteins but perform various regulatory functions in cellular processes, and include the microRNAs (miRNAs) and the long non-coding RNAs (lncRNAs). MiRNAs regulate gene expression by recognition of the complementary sequence present in the target mRNA, either promoting its degeneration, deadenylation or preventing its translation into proteins. LncRNAs can increase or decrease the transcription and function of genes through different strategies, such as direct interaction with the DNA, RNA or even proteins.

To our knowledge, the integrated role of lncRNAs and miRNAs in regulating the response of fish skeletal muscle to pro-growth signals such as AA and Igf-1 has not yet been studied in fish. Hence, this work uses a RNAseq approach to address the present lack of knowledge by generating a transcriptome and microRNAome from gilthead sea bream myoblasts in response to AA or Igf-1, and study the interactions between miRNAs, mRNAs and lncRNAs to better understand the role of ncRNAs in the myoblast's transcriptional response to pro-growth signals.

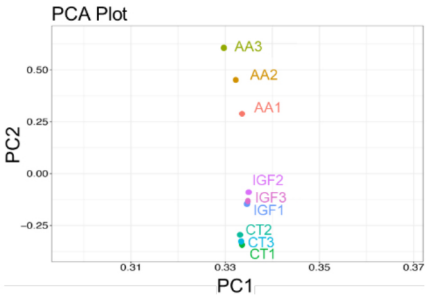

Figure 1. Principal Component Analysis (PCA) of transcriptome of untreated myoblasts (CT) or treated with AA or Igf-1. Principal components were obtained using the log2 normalized counts for the genes in each sample.

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Materials and methods

The myoblasts were isolated from gilthead sea bream fingerling (≈ 5g) and cultured in complete growth medium until day 8 when they were incubated for 12 h in a free AA and FBS medium to reduce gene expression to basal levels. Then, cells were incubated for additional 24 h in free AA medium (CTR group), medium with AA (AA group), or medium with recombinant Igf-1 (Igf-1 group) [free AA medium supplemented with Igf-1 from gilthead sea bream at 100 ng/mL. After the treatments, total RNA was extracted using Trizol, and transcriptome and microRNAome were obtained through the NovaSeq 6000 platform and the HiSeq 4000 platform, respectively. To determine the effect of the treatments, a principal components analysis (PCA) was performed using all genes identified in the transcriptome of the different conditions. Gene Ontology (GO) analysis was performed using the STRING online tool. Pearson correlations on transcription levels between differentially expressed genes for mRNAs-miRNAs-lncRNAs were determined, and sequences interactions were predicted using RNAhybrid v.2.2.1 and LncTar software. Differences in transcription levels between treatments were considered significant when log2-fold change was > 1.5 and corrected *p*-values (False Discovery Rate, FDR) was < 0.05. For Gene Ontology analysis, differences between categories were compared against the zebrafish database and considered significant when FDR < 0.05.

Results and discussion

The PCA analysis showed that the samples from each of the different conditions clustered together in three distinctive groups (Figure 1), indicating the effect of each treatment. Igf1 cluster was closer to the CT cluster than the AA, suggesting that the global transcriptomic profile of the myoblasts treated with Igf-1 was closer to the CT profile than to that of AA.

The number of mRNAs affected by the pro-growth treatments was significantly greater compared to the ncRNAs. The treatment with AA had a higher impact on transcription (1815 genes significantly changed), compared to the addition of Igf-1 (389 genes significantly changed).

Both treatments stimulated the transcription of genes related to muscle differentiation (GO:0042692) and sarcomere components (GO:0030017), but AA stimulated more the DNA replication and cell division (GO:0007049).

Over 402 different miRNAs were expressed in gilthead sea bream myoblasts but with three (*miR-21*, *miR-146* and *miR-22b*) clearly dominating the transcription landscape. In addition, over 100 miRNAs had their transcription significantly changed in response to the pro-growth treatments, including several muscle-specific miRNAs (myomiRs), such as *miR-133a/b*, *miR-206*, *miR-499*, *miR-1* and *miR-27a*.

Moreover, 124 lncRNAs with detectable levels of expression were identified in gilthead sea bream myoblasts, with only 29 lncRNAs significantly modified in response to AA, and 11 lncRNAs in response to Igf-1. The majority of lncRNAs detected had relatively low expression, with one (*ENSSAUG00010015132*) having 10 times more transcription than the second most expressed. Eight lncRNAs appeared to have a strong negative correlation with several mRNAs, suggesting that they might regulate them directly, but with little impact on the global regulation. Also, 30 lncRNAs showed strong correlations and interactions with several miRNAs, suggesting their function as miRNA's sponges during the response of myoblasts to AA and Igf-1.

Conclusion

In summary, AA had a greater impact on the transcriptional profile of gilthead seabream myoblasts compared to Igf-1, and the correlations and prediction of interactions between mRNAs-miRNAs-lncRNAs pointed out the importance and complexity of the network controlling muscle development and growth in response to pro-growth signals.

Acknowledgements

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EARTHWORM VERMICOMPOST TO ENHANCE WHITE SHRIMP *Litopenaeus vannamei* GROWTH AND INHIBITE AHPND DISEASE IN A EXPERIMENTAL CULTURE

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Introduction

In Mexico the annual shrimp aquaculture rate production is growing, and thus demands more feeding inputs and diseases inhibitors. Among organic fertilizers, *Eisenia foetida* solid humus is considered as a great promoter for plankton growth, helping to develop these aquatic organisms. In other hand, the excessive use of antibiotics in aquaculture has negatively affected those species who are farmed (Marshall y Levy, 2011), and also has decreased shrimp growth (Bray et al., 2006) because bacteria are more resistant to antibiotics (Karunasagar et al., 1994). That's why it is important to find environmental alternatives to reduce shrimp diseases. The aim of this study was to evaluate different solid earthworm vermicompost doses as growth promoter and AHPND inhibitor.

Methodology

Five treatments with three replicates each one was seeded with 120 shrimp larvae in 120L tanks with zero water exchange. The tanks were fertilized with initial doses of control, 0.00mg·L^{-1} solid Vermicompost [VC], 275, 550, 825 and 1100 mg/L^{-1}. The doses were, thereafter, reduced by half at day fifteen. The experiment lasted 45 days. Growth, Food Conversion Ratio and survival were measured. After the growth experiment, mortality accumulation was evaluated for each treatment when shrimp were challenged against *Vibrio parahaemolyticus* in three liters bowls with three replicates with 10 shrimp of 1.5g each bowl.

Results

Water quality parameters were not constant during the experiment and had significant difference ($P>0.05$) between oxygen and pH treatments. We found that shrimp reared with the organic fertilizer had the best growth (Figure 1), and the lowest food conversion average.

On the other hand, we have that treatment over 550mg/l of vermicompost were effective against *V. parahaemolyticus*. Less than three shrimps died in these treatments in a period of 72h, contrary to treatments less than 550mg/l who had over 50% of the mortality in the first 18h of the challenge. Control had the highest mortality with 80% of the shrimp dead before the 24h trail.

Some studies suggest that vermicompost is an important food resource for juvenile shrimp (Chakrabarty, 2008). This organic fertilizer improves water quality and enhanced phytoplankton production (Bwala and Omoregie, 2009; Chakrabarty et al. 2009). We find that vermicompost is a real option to shrimp growth and can lower productions costs. It also can enhance shrimp survival and increase shrimp production when is used in the first stages of shrimp when it is more susceptible to diseases, but further investigation must be done to find what bacterium conglomerate in the vermicompost acts as inhibitor to AHPND.

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DIGESTIVE BIOCHEMISTRY OF LOBSTER: A JOURNEY FROM FUNDAMENTAL KNOWLEDGE TO THE AQUAFEED INDUSTRY

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Introduction

Spiny lobster aquaculture has increased during the past decades, attended to their high demand and commercial value. Fast-growing tropical species (e.g., *Panulirus argus*, *P. ornatus*) are the most suitable for this activity. Yet, the absence of cost-effective and nutritionally adequate formulated feeds remains as one of the drawbacks for the sustainable expansion of this activity. The reason is mainly due to the lack of information on digestibility and assimilation of formulated feeds by lobsters. Eighteen years have passed since we first measured the activity of a digestive enzyme in *P. argus*. Then we went into a series of studies covering the digestion of all major nutrients and now we have a clearer view, though still with many gaps, of the digestive capacity of this species. In this work, we integrated the knowledge gained on the digestive biochemistry of *P. argus* and provided important information on the digestion of carbohydrates (CH), protein and lipids, as well as key research directions to improve the performance of formulated feeds for this species and reach suitables comercial feed for this industry.

Digestion of proteins

Panulirus argus presents a wide battery of digestive proteases, whose activity and variations respond both to the requirements during the life cycle and during moulting. Protein digestion begins in the gastric chamber, where high trypsin and chymotrypsin activities are located. Three main trypsin isozyme phenotypes are present and differ in protein digestion efficiency. More frequent phenotype is the most efficient. Soybean meal is highly digestible due to the high number of protein fractions, though each fraction is poorly digested separately. In contrast, in spite of few soluble protein fractions in fish meals, these are individually more digested. Likewise, squid meal is highly digestible due to high amount of soluble low molecular weight proteins, also stimulating trypsin expression and guaranteeing the synthesis of new enzymes. Conversely, fish meal digestion is limited by the poor solubility of proteins. Secretion and expression of trypsins in *P. argus* are time-regulated processes. Food proteins stimulate trypsin secretion, while protein digestion end products (free amino acids) promote trypsin expression. These processes occur normally when the source of protein in the diet is of animal origin, but they are inhibited when there is an excess of soy protein (Perera and Simon, 2014).

Digestion of carbohydrates

The ability of spiny lobsters to use dietary CH as an energy source has been discussed in recent years. In general, crustaceans show a relatively slow incorporation of CH in the citric acid cycle. α-amylases present several isoforms (phenotypes) in *P. argus*, and the isoenzyme composition shapes the efficiency of CH digestion *in vitro*. Conversely to trypsins, the most frequent α-amylase phenotype was the one with the lowest digestion efficiency (Rodriguez-Viera et al 2016). The type A starches are more susceptible to lobster α-amylase, especially those from rice. Wheat has a high-moderate digestibility both *in vitro* and *in vivo*, therefore more appropriated for lobsters. Maize starch is poorly digested, and its inclusion in the diet affected protein digestibility (Rodriguez-Viera et al 2014). Lobsters regularly fed with increasing levels of wheat show a proportional increase in the activity of the glycolytic enzymes hexokinase and pyruvate kinase, corroborating that there is a stimulation of glycolysis. This metabolic modification is not a temporary response after feeding, but a global adjustment of the intermediary metabolism based on the CH in diet. However, this is only possible up to a certain level of CH inclusion (20%). Furthermore, the type of carbohydrate eaten presents a profound effect on the metabolism of other nutrients such as amino acids and fatty acids (Rodriguez-Viera et al 2021).

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Digestion of lipid

Despite lipids are important nutrients and energy sources for crustaceans, lipid digestion and the lipolytic enzymes remains poorly understood in this group. Recently, we experimentally demonstrate the presence of "true lipase" in the spiny lobster, and their capacity to hydrolyse different oils (Rodriguez-Viera et al., 2022). Fish oils with long-chain polyunsaturated fatty acids (lcPUFA) (e.g. EPA and DHA) are the most digestible. Yet, algae oils and plant derived oils such as rapeseed oil and olive oil showed high digestion rates and should be also considered in feed formulation for *P. argus*. Considering our results for rapeseed oil, this could be one of the vegetable oils suitable to be included in feeds for *P. argus*. Lipase/esterase activities are higher in the digestive gland with respect to the gastric juice, so there is a need for lipids to be properly emulsified by the gastric juice to reach the digestive gland in the right format for hydrolysis. Thus, we evaluated the effects of various emulsifiers (non-ionic surfactants and phospholipids). The highest final extent of lipid digestion was observed with Arabic gum (AG) and hydrolysed soy lecithin (HSL). However, the initial digestion rate was higher with HSL than with the rest of the emulsifiers tested, thus it presents a greater potential to be used as an emulsifier in lobsters' feeds.

Final remarks

The protein-sparing effect of lipids and carbohydrates on spiny lobster metabolism is of a particular significance as the spiny lobster metabolism is strongly directed towards the use of protein. Any effort to increase digestive efficiency in lobsters should include the optimization of the gastric digestion. Studies should focus on: i) improve protein solubility to increase digestibility, ii) select adequate sources and levels of CH to favour a gradual release of glucose and promote protein sparing effects, and iii) improve lipid emulsification to be better digested and used in the digestive gland. The knowledge gained on the digestive physiology of this lobster would aid in the design of aquafeeds that make the farming of this crustaceans more sustainable. However, the journey towards the correct dietary formulation for *P. argus* still continues.

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DEVELOPMENT OF NEXT-GENERATION PROBIOTICS TO COUNTERACT BACTERIAL DISEASES IN OYSTER AQUACULTURE

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Various *Vibrio* species are known to cause severe mortality events in oyster aquaculture, potentially leading to a shortage of bivalve seed for grow-out industries. In France, *Vibrio aestuarianus*, *V. coralliilyticus*, *V. harveyi*, *V. crassostreae*, *V. europaeus*, *V. neptunius*, and *V. tubiashii* have been identified as major pathogens causing disease in Pacific oysters (*Crassostrea gigas*). Probiotic bacteria are gaining momentum as a promising strategy for disease management in aquaculture, and previous studies have shown that the pretreatment of larval oysters with probiotics can reduce mortality when challenged with bacterial pathogens. This study aimed to isolate and select novel probiotics to increase the resistance of Pacific oysters to bacterial pathogens threatening aquaculture production. Oyster tissues and microalgae samples were used as sources of probiotics. 16S gene amplicon sequencing was employed to identify the bacterial community composition in the samples, to ameliorate the *in vitro* experiments. The samples were then plated into eight different agar media to allow for the growth of a diverse range of bacterial species. The isolated bacteria were then tested *in vitro* against pathogenic *Vibrio* species using the replica plating technique. Strains that suppressed the pathogens' growth were screened for safety and physiologic criteria. Safety criteria included hemolysis test, susceptibility to antibiotics, and molecular identification. Physiological criteria included microbial adhesion, autoaggregation and coaggregation, production of siderophore, hydrogen peroxide, organic acid, halogenated furanones, and bacteriocins. The isolated strains identified as *Shewanella* sp. and *Vibrio* sp. met the targeted selection criteria, making them suitable as candidate probiotics. Follow-up research will include *in vivo* experiments to validate the efficacy of the candidate probiotics.

THE EFFECTS OF PRE-SACRIFICE AND CROWDING ON THE HEART ACTIVITY OF COMMERCIAL SEABASS (*Dicentrarchus labrax*)

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The pre-slaughter period is one of the most critical for fish and can affect welfare and influence the quality of the final product during storage. The procedures that are carried out in this period are: fasting, handling and crowding, defishing or pumping, capture and finally death. All of these steps, regardless of how well done, can cause adverse effects on your fish. Stress during the antemortem period can interact with and influence the physiology of the animal, worsening their performance. Additionally, in the postmortem period, biochemical processes generated by the antemortem stress act on the muscle affecting the quality and durability of the final product. There are often unavoidable situations where multiple stress factors interact and affect the final condition. Aquaculture production involves the management of relatively small animals (less than 1 kg) with many thousands of individuals in small spaces (between 37 and 120 million farmed fish were killed for human consumption in 2010). Therefore, from an individual point of view, animal welfare is an important issue in aquaculture. In this sense, it is crucial to obtain information on how the handling of fish during pre-slaughter and slaughter periods can affect their welfare and the quality of the final product. This knowledge will help to propose changes in current handling methods during the final phase of the production process, being one of the critical points that most affect the welfare of the fish.

Crowding is the first stage in harvest and transportation operations. Wall (2001) comments that crowding is one of the main causes of discomfort during harvest, being shortage of oxygen the most common problem associated with crowding . However, even maintaining high oxygen levels, crowding changes many other aspects of fish physiology, the effects of which can be observed up to several days later (Ortuño et al., 2001). Different species respond to this intervention in different ways. Under commercial conditions, the density of fish used for seabass crowding is approximately 250 kg/m^3. In sea bass, there are few studies on the effects of crowding and increased physical activity during pre-slaughter operations.

Technology now make possible to monitor the behaviour of small groups of individual fish as bioindicators of wellbeing. Bio-loggers may detect unusual patterns in fish heart rate, which could serve as an early indicator of whether fish health or welfare is becoming compromised. The use of heart rate bio-loggers to monitor fish heart activity, has shown to provide unique insights into the physiological and behavioural state of fish over time (Brijs et al., 2019; Hvas et al., 2020).

The objective of the present study was to assess the effects of confinement in the heart activity of seabass during the period of crowding using individually implanted bio-loggers, which can detect seabass stress levels during crowding events. Commercial size seabass were held in a flow-through system with full strength seawater for a whole year. Dissolved oxygen was maintained close to saturation. Fish were held in a rectangular tank of 10000 L capacity. Stocking density was 11 kg/m^3.

Ten days prior to the experiment , nine commercial-size seabass were implanted with DST milli-HRT loggers (StarOddi®, size: 13.0 × 39.5 mm, weight: 11.8 g) wich monitor heart rate (fH), electrocardiogram (ECG) and internal temperature (range 5 °C to +45 °C). These loggers are also equipped with real-time clock with accuracy of ±1 min/month.

These loggers recorded heart and acceleration hourly for that particular fish for the 36 h prior to crowding and then every 2 minutes for the 180 minutes which was the crowding period. After this period, fish were sacrificed and the loggers recovered.

During crowding, the level of water was lowered (mimicking bringing the fish to the surface) and fish were cornered at one end of the tank with a net which was place across the tank preventing them from moving. During the crowding exposure period fish were held at an estimated density of 118 kg/m^3.

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This experiment was performed in the summer with a water temperature of 28ºC and in the winter with a water temperature of 9.5ºC.

Fish used in this experiment had an average standard length of 32.48 ± 3.25 cm in the summer and 27.66±2.1 cm in the winter and an average wet weight of 555.20 ± 141.97 g in the summer and 389 ± 68.14 g in the winter.

Baseline values of heart rate were 57 ± 2 beats per minute (bpm) in the winter and 69 ± 2 bpm in the summer, showing a strong relationship with water temperature. Averaged heart rates and accelerations showed slightly variations along the 180 minutes that lasted crowding, but no significant effects were observed by crowding procedure during winter. In the summer, however, the heart rate of fish increased significantly at the beginning of the crowding period and slowly decreased over time, with great variation among individuals.

Therefore, the effects of crowding on heart rate seem to depend on the seasonal period when it is performed at the farms.

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LAND BASED, HIGH DENSITY CULTIVATION AND CO_2 UPTAKE BY PANEL ARRAYS OF THE RED SEAWEED *Gracilaria vermiculophylla*

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New approaches are needed for land-based cultivation of macrophytic red algae that reduce costly aeration requirements for biomass suspension and enable process intensification. The goal of this study was to demonstrate the high-density cultivation of the carbohydrate-rich macrophytic red alga *Gracilaria vermiculophylla* on vertical arrays of panels deployed in an open channel raceway configuration similar to raceway ponds that have been developed for mass cultivation of microalgae.

A clonal culture of *G. vermiculophylla*, consisting of branched, cylindrical thallus tissues of 8-10 cm length, was mechanically blended into 2-3 cm fragments and then fluidically injected onto a 3 mm polypropylene mesh support. Immobilized *G. vermiculophylla* mesh panels were aligned parallel to flowing seawater medium at nominal bulk velocities ranging from 5 to of 50 cm s^{-1} in a 100 L raceway pond of 20 cm liquid depth (FIG. 1). This raceway was equipped with real-time measurement of CO_2 concentration in the inlet and outlet gas for determination of CO_2 uptake dynamics. Specific rates for CO_2 uptake became saturated at 8000 ppm CO_2.

To achieve process intensification under nutrient-replete conditions at 22 °C, row spacing was minimized at 6.5 cm, and the inlet gas CO_2 was increased from 1000 to 4000 ppm (day 7-14), and then to 8000 ppm (day 14-23) at 0.010 L gas L^{-1} liquid min^{-1} gas flow. Over the 23 day cultivation, biomass on the panel increased by a factor of 48, with final biomass loading exceeding 10 kg FW m^{-2} panel area, and cumulative CO_2 capture of 65%. The cumulative average areal productivity within the panel zone of the raceway exceeded 60 g AFDW m^{-2} day^{-1}, and final biomass density nearing 7.2 g AFDW L^{-1} (47 g FW L^{-1}) was achieved after 23 days (FIG. 2). Overall, these outcomes demonstrate the potential for land-based raceway cultivation of clonal red macroalgae of present and future commercial significance.

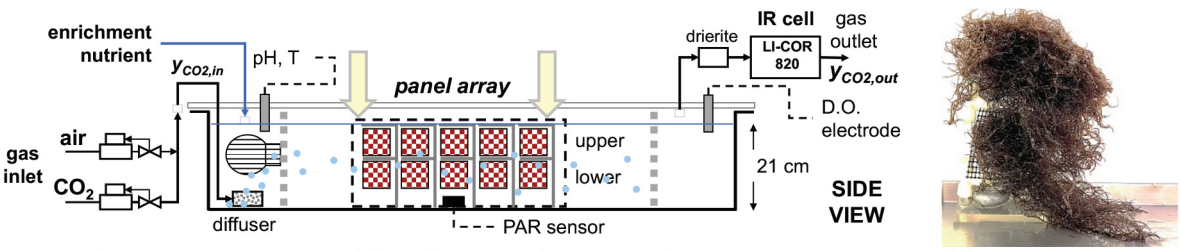

FIG 1. (a) 100 L raceway with red macroalgae panels. (b) Panel after 21 days

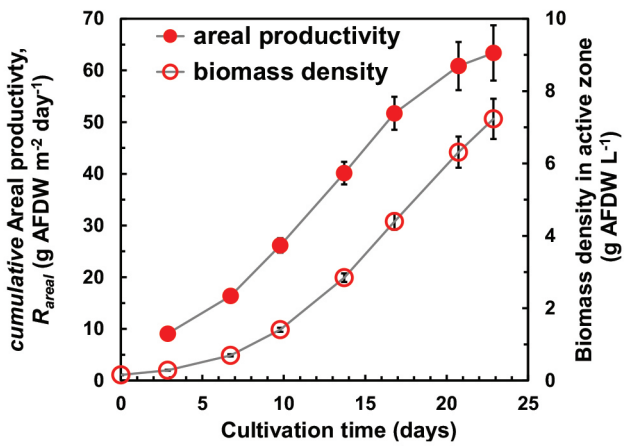

FIG. 2. Biomass productivity.

REAL-TIME SEGMENTATION AND WEIGHT ESTIMATION OF FLATFISH USING IMAGE PROCESSING AND DEEP LEARNING IN AQUICULTURE

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Introduction

Advanced image processing combined with the development of artificial intelligence algorithms has impacted all industrial sectors, including aquaculture (Zhao et al, 2021). The usual harsh surrounding circumstances, salinity, humidity or ubiquity, among others, make it difficult, but not impossible, to increasingly integrate information and communication technologies (ICT) and AI in this sector. In hatcheries, knowing the biomass of the plant is a key parameter. In many use cases, the initial challenge faced by automation begins with the automatic identification of specimens (Fernandes et al, 2020). This is carried out by a surveillance system and a previously constructed database from which information can be inferred, thus providing an augmented reality-based industrial model. In this work, an autonomous, real-time processing, and low-complexity system for turbot (*scophthalmus maxima*) segmentation and weight estimation is presented. To train the segmentation model and the weight estimation model, a database of more than 6000 turbot images has been created.

Materials and Methods

Specifically, the developed system has been assembled and verified by installing it above a weight classification belt sorting machine used in a turbot breeding plant. The operators transfer the fish one by one from a hopper to a conveyor belt, where they are weighed and distributed into weight-balanced bins. On that belt, the speed is up to one fish per second.

The system is equipped with a camera that records high-resolution video of the fish on the conveyor belt (Fig. 1 left). There is a second camera that records the weight information displayed by belt balance. This recording process is automated by means of OCR identification of the images. This has allowed for the semiautomatic construction of a database that contains for each image of a fish its real weight. Once an image acquisition and processing system was available, a model was developed to detect and segment the fish within the images (Fig. 2). For this purpose, YOLOv5 (Ultralytics) was trained with a database of various species of fish (Roboflow) to self-detect and segment tasks. Then, the hue, saturation and value (HSV) parameters were extracted to perform and umbralisation of the images. The last step consists of applying an opening/closing shift of the pixels. With this post-processing, an accurate binary image of the shape of the fish can be extracted. Later, both the area and the length of the fish are obtained, measured as the total number of white pixels or the number of pixels between the head and the tail, respectively. To do this, the extrinsic parameters of the camera were considered.

Finally, six machine learning models have been compared in order to obtain the best relationship between weight and area or length. For this purpose, a total of 6095 images of fish between 250g and 2kg were used; Fig. 3 presents a graph were each dot corresponds to a fish, its real weight, and calculated length. Finally, each of the six models has been validated using the K-fold technique (Gufosowa).

Results

In fish detection and segmentation tasks, YOLOv5 trained with the aforementioned database was able to detect fish with a measured precision of 0.90 and a recall of 0.92. Table 1 shows the root mean square error (RMSE) obtained inferring the weight from both length and area with 6 different algorithms trained with the collected images. As it can be seen, the 2nd and 3rd grade polynomic regressions and KNV outperforms the rest of algorithms in both cases, length and area. Considering the limitation of processing images at a high rate (less than 0.1s per frame), 2nd degree polynomial regression is chosen as the best option. Thus, the average processing time of the whole system inferring weight from length is 0.065ms using an ORIN NVIDIA platform. Using area-based models this time is up to 5 times higher. Finally, it is work mentioning that although this work focused on turbot as a use case, the technique could be easily transferable to any other flatfish species.

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BENEATH THE SURFACE: HOW SUB-REGULATORY LEVELS OF FUSARIOTOXINS IN AQUACULTURE FEEDS IMPACT THE HEALTH OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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The main driver of the Blue Revolution is the potential of modern aquaculture to provide healthy and sustainable protein for the increasing human population. As in the farming of terrestrial animals, the feed used in aquaculture has great bioeconomic importance. Aquaculture feeds in particular are constantly evolving, driven by economic and environmental factors. A major development is the reduction in reliance on raw materials from marine origin which has led to an increased use of globally sourced plant-based raw ingredients, this in turns inherently increased the risk of mycotoxin exposure.

Fusariotoxins are a group of mycotoxins produced by various fungal species of the genus *Fusarium*. These toxins are among the most prevalent mycotoxins detected globally. The group includes, among others, fumonisins and zearalenone, which are known for their negative effects on sphingolipid metabolism and reproductive-endocrine disturbance, respectively.

European legislation recommends guidance values for certain mycotoxins to ensure food and feed safety and the maintenance of animal welfare. For fumonisins, a 10 mg/kg limit is defined for fish feeds. However, no specific value for zearalenone is defined for this matrix, and a general maximal value of 2 mg/kg (defined for cereal feed materials) is being used de facto as an upper limit.

Unlike other species, few studies describe the effects of sub-regulatory levels of fusariotoxins on salmonids. Two recent studies comprised of three independent *in vivo* trials, were aimed at addressing this knowledge gap. We assessed the impact of oral exposure to the fusariotoxins fumonisins and zearalenone, below EU guidance levels, on rainbow trout at different developmental stages. The study assessed biomarkers such as metabolites in gut content (in all trials), the sphinganine-to-sphingosine (Sa:So) ratio (in the fumonisins studies), and plasma vitellogenin (VTG) and tissue metabolites (in the zearalenone study). These biomarkers were used as endpoints to evaluate the toxic effects of fusariotoxins and the detoxification efficacy of fumonisins by FumD and zearalenone by ZenA, both of which are commercial feed enzymes designed for specific detoxification.

The results of this study indicate that short-term oral exposure to sub-regulatory levels of fusariotoxins has negative effects on rainbow trout. Commercially available fish feeds that comply with the European regulatory levels may still pose a risk to fish health and farmers' operations. Thus, the guidance levels should be revised, and further studies should investigate these findings. However, the inactivation of the mycotoxins by feed enzymes FumD and ZenA proved to be an effective strategy to significantly reduce the observed effects in the selected endpoints.

ALTERNATIVE DIETS FOR IMTA PRODUCTION OF GILTHEAD SEA BREAM (*Sparus aurata*): GROWTH PERFORMANCES AND FILLETS QUALITY

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Introduction

Worldwide aquaculture production has grown strongly in recent years [1] primarily due to the greater fish demand, which increased from 14.3 kg in the 1990s to 20.2 kg per capita in 2020 [1]. Fish is an excellent protein source for human nutrition and aquaculture is gaining importance, especially in developing countries [2]. However, there is a large environmental concern about "aquafeed" and the capture-based production of fish meal and fish oil [3]. For these reasons, several alternatives have been investigated such as processed-animal by-products, insect meals, crop-based ingredients, and algae [3]. Nevertheless, crop-based meals and oils (e.g., soybean, corn, canola, palm, and sunflower) that are currently largely adopted in aquaculture, have been noted to impair growth performances and n-3 fatty acids levels of farmed seafood [2]. Moreover, the environmental impact of feed production should be also considered (e.g., in terms of land use, soil exploitation, and overseas transportation). Based on the production concept of the SIMTAP project, low-trophic organisms potentially producible in integrated multi-trophic aquaculture systems (IMTA) can be exploited as feed for farmed fish [4]. The aim was to investigate the effect of an alternative fresh diet formulated with mussels, clams, and polychaetes on gilthead sea bream (GSB, *Sparus aurata*) growth performance and fillet nutritional profile, against the commercial feed.

Materials and Methods

All the experimental procedures were approved by the Animal Welfare Board of the University of Pisa and the Italian Ministry of Health (B290E.N.AHZ). The experiments were carried-out in the IMTA system built at the Department of Agriculture Food and Environment of the University of Pisa. A total of 256 GSB individuals (initial body weight, BW 249.17±30.06 g) were pit-tagged and distributed in six 450-L tanks and fed two diets which lasted 64 days. Treatment F100 received a commercial feed containing vegetable ingredients (NaturAlleva® ForeSea 6.5 mm, VRM©, Italy) while treatment M100+ a mixture (on a dry matter basis) consisting of 49% frozen cooked mussels (*Mytilus platensis*), 30% frozen cooked clams (*Paphia textile*), 20% frozen marine polychaetes (*Nereis virens*), and 1% of mineral premix (VRM©, Italy), this latter equal to that used in the commercial feed. Fish BW, length, and feed consumption were measured. Hence, specific growth rate (SGR) was also calculated. Moreover, at the end of the experiment 15 individuals from each tank were slaughtered and dissected for the determination of viscera and liver weight, thus viscera-somatic index (VSI) and hepato-somatic index (HSI). Also, fish fillets from five fish from each tank and feeds were sampled for proximate composition, fatty acid (FA) and amino acid profile, and mineral content. Data were analyzed using ANOVA and the least significance means were compared by the Tukey test, while non-parametric tests was performed for SGR. Mean values of measurements were considered statistically different per $P<0.05$.

Results and discussion

At the end of the experiment, the individual BW gain was significantly lower in F100 fish (84.2±2.48 g) than M100+ (101.5±2.02 g). Significant differences were also observed for SGR: 0.4±0.12 and 0.6±0.01 % d^{-1} for F100 and M100+ treatment, respectively. Moreover, VSI and HSI values were significantly higher in F100 group (VSI: 6.4±0.24 vs 5.8±0.15 %; HSI 2.4±0.09 % vs 1.9±0.05 % in F100 and M100+ respectively). These results might be explained by a better digestibility and the high protein to lipid ratio in M100+ diet (9.04 and 2.28 in M100+ and F100, respectively). The latter may have stimulated more the growth rather than fat deposition in viscera. With regards to fish fillet proximate composition, no differences were observed, except for ash content. Unsaturated FA was the main fatty acid class in all fillets, with a significantly higher content in F100 than in M100+ (79.55±0.187 vs 73.52±0.433 % of total FA) due to the significantly higher mono-unsaturated fatty acids (49.97±0.400 vs 44.56±0.541 % of total FA in F100 and M100+ respectively). No differences were observed for poly-unsaturated fatty acids (29.58±0.329and 28.95±0.460 % of total FA in F100 and M100+ respectively). However, some significant differences were observed in individual essential FA such as Arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) which were significantly higher in in M100+. (ARA: 0.21±0.037 vs 0.557±0.091, EPA 1.19±0.088 vs 3.653±0.192, DHA: 3.22±0.172 vs 6.005±0.255 % of total FA in F100 and M100+ respectively). On the other hand, C18:2-n6 was higher in F100 (18.21±0.224 vs 12.58±0.372

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% of total FA in in F100 and M100+ respectively). These differences can be explained by the FA composition in the two diets (data not shown). It is worthy to notice that the composition of M100+ fillets showed relevant similarities to wild GSB [5]. No significant differences were detected in amino acid profile and mineral content of fish, except for potassium content (19.30±0.440 vs 17.82±0.320 g kg^{-1} DM in F100 and M100+, respectively).

Conclusion

In conclusion, the alternative diets based on mussels, clams, and polychaetes potentially obtained from IMTA systems, can be successfully used for sea bream production in substitution of a commercial diet. The alternative diet performed slightly better than commercial feed, in terms of growth performances as well as for the fatty acid profile of fillet, which was more similar to wild GSB in terms of essential fatty acids profile**.** Further research is needed regarding the exploitation of IMTA-obtained organisms in GSB production in terms of environmental impact.

Acknowledgments

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STATE OF THE ART OF PRIVATELY FUNDED RESEARCH ON ARTIFICIAL REPRODUCTION OF EUROPEAN EEL (*A. anguilla*), WITH FOCUS ON LARVAL REARING

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Background

Glasaal Volendam is a private research company founded in 2012 in Volendam, a former eel-fishing city in the north of the Netherlands. The company aims to close the eel life cycle in captivity and set up a large-scale production facility to farm glass eels (juvenile European eel). Glass eels can be sold to eel grow-out companies, which are now solely dependent on wild caught glass eels. Replacing these wild glass eels with farmed ones could relieve the legal and illegal fishing pressure on this already endangered species.

The life cycle of European eel *A. anguilla* begins with hatching in the Sargasso Sea. From here larvae drift as leptocephali back to the coasts of Europe and then transform into glass-eels as they enter freshwater rivers in Europe. They migrate upstream, maturing into elvers and developing pigmentation in freshwater. Over several years, they become sexually mature adult eels and undergo a final transformation into silver eels before migrating back to the Sargasso Sea to spawn, completing the cycle.

The life cycle of European eel has not yet been closed in captivity, unlike the one of Japanese eel *A. japonica*, which was completed in 2003 (Tanaka et al. 2003). However, considerable progress has been made in recent years towards closing the life cycle of European eel in captivity. This translates in the production of high-quality gametes, embryos, yolk-sac larvae and pre-leptocephali feeding larvae.

Materials and methods

To conduct our research, we obtain the broodstock from the wild through local fishermen. Eels are housed in 500l tanks with a closed re-circulation system. During the maturation period, they are kept at low density (20 kg/m^3), with 36‰ salinity and 18 °C of temperature. Eels are maintained under dimmed light conditions to mimic daily photoperiod. No feed is provided during maturation. To induce vitellogenesis, females receive weekly injections of Carp Pituitary Extract (CPE) for 15-20 weeks (Kagawa et al., 2005) and weekly biopsies are taken to evaluate oocyte development (Paalstra et al., 2005). To induce ovulation, females are injected 12 hours before spawning with DHP (7α,20ß-dihydroxy4-pregnen-3-one; 2 mg per/kg body weight) according to Ohta et al. (1996). Male broodstock is obtained from commercial eel farms and receives weekly injections of recombinant human chorionic gonadotropin (Perez et al. 2005). Milt is collected after 10 weeks of hormonal treatment, evaluated for quality under the microscope and mixed with eggs at the time of spawning. Fertilized eggs are incubated for 48 hours in 200 l tanks and then hatching larvae are moved to 500 l tanks. On day 14 after hatching, when mouth, primitive digestive system and eyes are formed (Sørensen et al. 2016), larvae are moved to feeding systems where experimental diets of different compositions are being tested. Larvae are fed five times a day a slurry-like diet (Tanaka et al. 2003). Different diet compositions are tested in order to improve survival and growth rates, to reach leptocephalus stage first and glass eel stage ultimately. Through pictures larval growth is measured and developmental changes in larval morphology are monitored. Ingestion is evaluated by direct observation after each feeding session.

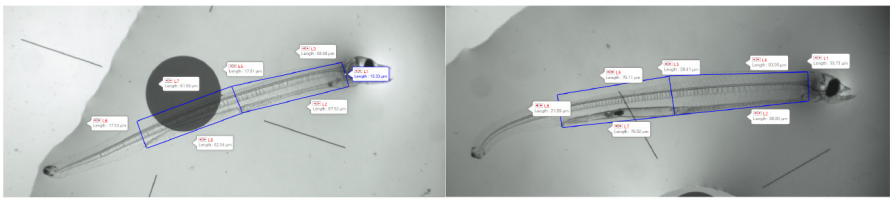

Fig.1: Example of pictures used to measure length and width of 14 days post hatching (left) and 35 days post hatching (right) larvae, during early feeding period.

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Results and future steps

At Glasaal Voendam we made significant progress towards closing the life cycle of the European eel. At first, the research was primarily focused on the artificial maturation of the broodstock. Currently we can produce vast numbers of fertilized eggs weekly. From 2018 onwards, the focus shifted to larval feeding. We improved larval survival rates at first feeding (14 days post-hatching) from an average of 0.3% in 2018 to 3.8% in 2021 and 21.7% in 2022. This year, we have observed trials with over 50% of the larval batch surviving until first feeding. These improved survival rates allowed us to focus on the larval feeding. After obtaining the first signs of food ingestion by larvae in 2018, we gradually improved feeding technique and larval diets, to enhance survival and growth rates. Being the average length at first feeding 0.7 cm and the maximal survival 30 days, in 2022 we successfully grew our first batch of larvae up to 1.4 cm. The oldest larvae of the batch survived for 94 days. We then focused on nutritional values of the larval diets, to enhance growth rates and reach the leptocephalus and the glass eel stage.

Alongside, to obtain growing larvae, we conducted experiments on parameters such as temperature, light, salinity, water quality, type of flow and tank design, to identify optimal rearing conditions. We also developed a method to analyze larvae pictures and we weekly process measurement data to extrapolate growth curves, to monitor larval growth rate and to compare it with the ones from past experiments.

In the first quarter of 2023 the larval growth rate was improved by 60%. The primary and most compelling goal for the next years is to reach the glass eel stage. The second phase of our company will then involve upscaling to commercial size.

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EVALUATION OF DEFATTED BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE MEAL AS AN ALTERNATIVE PROTEIN SOURCE IN THE DIETS OF EUROPEAN SEA BASS JUVENILES (*Dicentrarchus labrax*): EFFECTS ON GROWTH PERFORMANCE

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Introduction

Fish meal (FM) has been considered as the ideal protein source for fish production, due to its overall balanced nutritional composition, high digestibility, and palatability. However, due to shortages in its worldwide availability along with its negative environmental impact, interest has shifted towards the evaluation of sustainable alternatives to reduce the dependence on FM (Nogales-Mérida et al., 2018). The use of insect meals in feed for farmed fish was recently approved by the European Commission. Insects are fast-growing, reproduce easily and efficiently, and have an excellent ability to utilize low-quality organic material and convert it into a high-value protein (Barroso et al., 2014). The aim of this study was to evaluate the potential of Black soldier fly (BSF) larvae to replace fishmeal in terms of growth performance, feed utilization, and the health of European seabass (*Dicentrarchus labrax*).

Materials and Methods

A total of nine different substrates were evaluated as potential feeding mediums for the production of BSF larvae in terms of nutritional profile and growth performance. The HI6 insect meal was chosen as the most promising for use in aquafeeds as it presented the highest protein levels in the shortest possible growth period. However, its inclusion in the aquafeeds proved to be a challenging task as its high lipid content (32%) hampered the homogenous grinding. To resolve this issue, a laboratory defatting method was developed using hexane as an organic solvent to extract the fat, The choice of the solvent was carefully considered and meticulously tested to evaluate its effects on the nutritional profile of the alternative novel feed ingredient. Using the defatted insect meal (12% fat), six isonitrogenous (45% crude protein) and isolipidic (16% crude lipid) diets were formulated: a control diet with a high percentage of fish meal incorporation (30%) was used as well as five experimental diets in which FM was replaced by 20, 30, 40, 50, and 60% with defatted BSF larvae meal, respectively. Differences in the crude protein content between fish meal (67%) and insect meal (53%) were compensated by the simultaneous sequential increase of soy protein concentrate (SPC) and sunflower meal in parallel with the gradual reduction of the wheat flour. All diets (3.5 mm pellets) were produced by cooking extrusion.

European sea bass juveniles were obtained from a commercial fish farm located in Megara, Attiki and transferred to the Hellenic Center for Marine Research (HCMR) facility located in the campus of Agricultural University of Athens, Greece. The fish were distributed among 18 cylindroconical experimental tanks with a volume of 1m^3 in a PLC controlled recirculating aquaculture system (RAS), 32 fish per tank, with 3 replicates for each feed. The average sea bass initial weight among the tanks was 33.85 ± 0.40g (SD). The trial lasted for approximately two and half months, fish were hand-fed to apparent satiation, twice per day, and uneaten dry feed that was collected in each tank's waste collection system was monitored and taken into account before calculating the daily feed consumption. The average seawater temperature was 22.71 ± 3.3 ^{o}C.

Results

The trial was carried out smoothly without any problems or significant mortalities among the treatments. For growth performance parameters, tanks were considered as the experimental units and fish represented the sample units. The data from the individual observations were tested for normality and homogeneity of variance prior to be subjected to one-way ANOVA using Kolmogorov–Smirnov and Levene's tests, respectively. Significant differences between means were determined by Tukey's test (Statistica version 13.0). The evaluation of the growth performance indices showed no statistically significant differences among the experimental diets. Although, it is worth noting that the highest individual body weight was observed in both the control and HI6% diets (104.97 g and 105.11 g respectively) and the lowest in the feed HI6 30% (99.02 g). Total feed consumption (TFI), Specific growth rate (SGR) and daily growth index (DGI) followed

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the same trend. The thermal growth coefficient (TGC) was found similar among the different dietary treatments. In terms of feed utilization, the lowest feed conversion ratio (FCR) was found in the HI6 30% and the Control (1.28 & 1.26) diets along with the highest values of the protein efficiency ratio (PER, 1.73 & 1.75). The somatometric indices did not reveal any statistically significant variations. However, its is of interest that the perivisceral fat index presented its lowest value in the fish fed with the HI6 40% (5.19) diet and the highest in the HI6 60% (7.16). Finally, whole body proximate composition did not present any statistically significant differences among the diets.

Overall, the acquired data were quite promising as the fish growth and health were satisfactory in all experimental diets and zootechnical indices were representative of fish size and species. Furthermore, the results of this study clearly demonstrated the potential of the defatted BSF as sustainable alternative ingredient to fish meal in the feeds of the European seabass. However, additional analyses are in progress to further evaluate the results of the substitution.

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STUDIES ON THE USE OF LOCALLY AVAILABLE (COXS BAZAR AND SAINT MARTIN) ALTERNATIVE RENEWABLE SEAWEED WASTES AS COMPOST ORGANIC FERTILIZER RESOURCES

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Marine red algae from the Bangladesh Bay of Bengal Hypnea Sp have been used as organic materials due to the presence of a number of plant growth-stimulating compounds. The effect of various seaweed species on plant growth and development with an emphasis on the use of this renewable bio-resource in sustainable agriculture of northern fertilizers raw materials system. Organically made fertilizers play an important role in increasing crop yield and the quality of crops promises improvements considering climate adaptation. Seaweed wastes compost was put in evaluation trials at Sreemangal, Bangladesh to evaluate its efficacy and find out the optimum dose for profitable Betel leaf production. This part of the study is directed toward the analysis of the future trend and performances of composting seaweed wastes. The science of seaweeds explores, how analysis of the future trend and performances of composting seaweed wastes. A field study was conducted at three sites at Khasia farmers of Sreemangal Khasia betel leaf cultivation community area of Bangladesh. Seaweed wastes mixed with compost organic fertilizer dose of 50g per support tree . The highest betel leaf yield was obtained from seaweed wastes mixed with compost organic fertilizer applied to plants. Table 1. (2880 leaf). Two people have not used it, but the one who has used it has had good results. This study suggests that seaweed wastes mixed with organic fertilizer are suitable for betel leaf cultivation. Area-based conservation is a key tool for delivering the SDG goal of responsible production and consumption.

INTRODUCTION

Providing a safe alternative to chemical fertilizers is a crying need of the present time. Marine red algae from the Bangladesh Bay of Bengal Hypnea Sp are often regarded as an underutilized bio-resource seaweed and have been used as organic materials due to the presence of a number of plant growth-stimulating compounds.The effect of various seaweed species on plant growth and development with an emphasis on the use of this renewable bio-resource in sustainable agriculture of northern fertilizers raw materials system. Organically made fertilizers play an important role in increasing crop yield and the quality of crops promises improvements considering climate adaptation. (www.northernfertilizer.com). Although chemical crop fertilizers boost crop yield, they are also responsible for environmental pollution all around the world. Northern organic and balanced fertilizers provide a safe alternative to chemical fertilizers while having more agricultural output and reducing chemical fertilizer usage. Technology-based circular economy model. Seaweeds or marine macroalgae are rich in diverse compounds like lipids, proteins, carbohydrates, phytohormones, amino acids, osmoprotectants, antimicrobial compounds and minerals. Their potential for agricultural applications has been used since antiquity, but recent demands of organic farming and organic food stimulated much the application of organic treatments like seaweed extracts in agriculture. Md. Mohidul Islam, Bangladesh Fisheries Research Institute (BFRI), Md. Shahzad Kuli Khan, Marine Fisheries & Technology Station. Bangladesh Fisheries Research Institute, Jakia Hasan, Bangladesh Fisheries Research Institute, Debbrota Mallick, Dauphin Island Sea Lab,Seaweed Hypnea sp. culture in Cox's Bazar coast, Bangladesh October 2017,Bangladesh Journal of Zoology 45(1):37-46 DOI:10.3329/bjz.v45i1.34192. Project: Development of culture of seaweeds in south-eastern coast of Bangladesh.

Table 1. Yield of betel leaf as influenced by seaweed wastes based organic fertilizer

Demo tree	Farmers Name & Address	Betel Leaf Plucking per day
1	Harun,Aliachhara, Habiganj, Bangladesh.	2780
2	Sonet,LongliaPunji , Sreemangal, Bangladesh.	2880
3	Victor Somer, Dubblechhara Punji, Moulvibazar, Bangladesh.	2780

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Aims of The Study

Seaweed wastes compost was put in evaluation trials at Sreemangal, Bangladesh to evaluate its efficacy and find out the optimum dose for profitable Betel leaf production.

Objectives of the study

The objectives are as follows:

Main Research objectives: This part of the study is directed toward the analysis of the future trend and performances of composting seaweeds wastes and Seaweeds can sequester carbon and reduce atmospheric carbon dioxide levels, thereby mitigating the effects of global warming.

Materials and Methods:

A field study was conducted at three sites at Khasia farmers of Sreemangal Khasia betel leaf cultivation community area of Bangladesh. Seaweed wastes mixed with compost organic fertilizer dose of 50g per support tree .

The present study proceeds to examine the following research questions:

1. Main research question-The science of seaweeds explores how analysis of the future trend and performances of Composting Seaweed wastes ?
2. How do the environmental settings of the rich Khasia betel leaf farmers of Sreemangal provide bases for evolving varied seaweed based compost organic fertilizer farming in different parts of the community?
3. In what ways do the traditional values and indigenous knowledge of the farmers contribute towards diversity and sustainability of the community seaweed based compost organic fertilizer farming?
4. How far and in what ways the seaweed based compost organic fertilizer farming in community areas are getting modfied overtime and changing socio-economic context?
5. Is seaweed based compost organic fertilizer agriculture currently prevalent in the community sustainable? If not, what are the probable measures to be adopted to make the seaweed based compost organic fertilizer farming economically more viable and ecologically more acceptable?
6. What kinds of difficulties are encountered by the farmers during their cultivation of crops through seaweed based compost organic fertilizer farming?

Sample rich Khasia farmers of Sreemangal khasia betel leaf cultivation community area map selected for meticulous study. Seaweeds wastes mixed compost organic fertilizer dose 50g per support tree for farmer Sonet, farmer Harun, own conventional practice dose and farmer Victor own conventional practice dose per support tree .

1. Harun, Aliachhara, Habiganj, Betel leaf plucking 2780 (own conventional practice dose per support tree application)
2. Sonet, LongliaPunji , Sreemangal. Betel leaf plucking 2880 (50g seaweed wastes mixed organic fertilizer per support tree application
3. Victor Somer, Dubblechhara Punji, Moulvibazar. Betel leaf plucking 2780 (own conventional practice dose per support tree application)

Two people have not used it, the one who has used it has good results.

After the fieldwork primary and secondary data collected from different areas.

Results:

The highest betel leaf yield was obtained from seaweed wastes mixed with compost organic fertilizer applied to plants. Table 1. (2880 leaf). Two people have not used it, but the one who has used it has had good results. This study suggests that seaweed wastes mixed with organic fertilizer are suitable for betel leaf cultivation.

Conclusion:

Khasia community people in Sreemangal Upazila, Bangladesh are used to cultivating betel leaf as their main livelihood. It is their only living means. Their families live on sale of several Kuri (20 kanta or 2880 pieces) of betel leaves daily. Most of the people of this area are involved in agriculture, especially betel leaf. This study suggests that seaweed wastes mixed with organic fertilizer is suitable for betel leaf cultivation. Area-based conservation is a key tool for delivering the SDG goal of responsible production and consumption

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NUTRIENT FOR FISH OR NUTRIENT FROM FISH: THE RETENTION AND DISCARDING OF DIGESTED PHOSPHORUS BY CARP IN FISHPONDS

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Introduction

Phosphorus (P) is directly linked to eutrophication in shallow-lake systems, either alone (absolutely) or in tandem (relatively) with nitrogen (N). P in aquatic ecosystem can occur bound to sediment (legacy P), seston and nekton (organically bound P), and in free algal-reactive state (orthophosphate-P). The last one is of concern for standing freshwaters, which trigger an overshooting of primary productivity (algal blooms); under equally strong dissolved inorganic nitrogen fluxes (DIN) [1]. Already, the primary excretory product of fish is DIN (ammoniacal-N). In this context, the branchial-urinary losses of digested P by fish as dissolved reactive phosphorus (DRP) is likely the worst situation. Fish can act as pumps in fishponds converting organically (food) bound P to algal-reactive orthophosphate-P [2]. Present study aimed to provide a mechanistic understanding when the fish are source or sink of P, *i.e.,* drive or mitigate dissolved reactive phosphorus (DRP) in fishponds. By doing so, regulating the eutrophication.

Materials and Methods

We hypothesize the more suppressed DRP excretion could be, the more efficient ecosystem P use would be. More P would stay locked in fish biomass (secondary consumers) or zooplankton (primary consumers) and less available to algal biomass (primary consumers). Fish biomass can act as source or sink of P; sometimes related to food's digestible N: P stoichiometry and many times not. For example, at a constant N or P intake, DRP excretion might depend on supply of one or two indispensable amino acids, and digestible non-protein energy per unit of food intake. Other biological factors, such as presence or absence of scales or switching to basal metabolism might impart control on "phosphorus use efficiency" (PUE) and DRP excretion. Through isogenous but objectively formulated (WinFeed®) semi-purified research diets (fed 4% of body weight; split into 2 doses daily), PUE and DRP losses in a cyprinid model (*Cyprinus carpio*; age 1+; 22 g to 50.4 g) were investigated.

In a series of five experiments (twelve 120 L tank Guelph-RAS; 4 group x 3 replicate design; ~30 days each; ~250 g biomass tank^{-1}), following objectives were investigated: (a) differences in PUE and DRP losses between scaleless and scaly fish under a P-deficient diet (0.21% P); (b) effect of graded levels of bioavailable P (0.21%, 0.83%, 1.39%, 1.92%) on PUE and DRP losses, at isonitrogenous (38% digestible protein, DP) and isoenergetic diets (185 kcal 100 g^{-1} digestible non-protein energy, NPE); (c) effect of graded levels of bioavailable lysine (1.43%, 1.71%, 2.05%, 2.39%) and methionine (0.21%, 0.49%, 0.69%, 0.89%) on PUE and DRP losses, at isonitrogenous (26% DP), iso-phosphorus (0.6%) and isoenergetic diets (185 kcal NPE 100 g^{-1}); (d) effect of graded levels of dietary non-protein energy (168 kcal, 181 kcal, 183 kcal NPE 100 g^{-1}) on PUE and DRP losses, at isonitrogenous (37% DP), iso-phosphorus (0.9%); (e) lastly, PUE and DRP losses at basal metabolism conditions (7°C, feed deprivation, 30 days). The entire series of laboratory experiments lasted 40 weeks. The analysis had a mass balance or nutritional bioenergetics approach between fed nutrients (with known and standard digestibility) and carcass/ biomass gain of nutrients (growth trial and carcass analysis) to compute N, P retentions and non-faecal losses [2].

CURRENT STATE-OF-THE-ART IN eDNA-BASED BENTHIC BIOMONITORING OF SALMON AQUACULTURE INSTALLATIONS

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Introduction

In the quest to safeguard nature and its crucial ecosystem services, the monitoring of aquaculture installations demands a regular assessment of the surrounding environment. The traditional approach involves labor-intensive sampling, taxonomic identification of macrofauna, and inference of ecological status. Seeking alternatives, the scientific community is turning to bacteria-based environmental DNA (eDNA) metabarcoding as a promising monitoring tool. By harnessing the power of eDNA monitoring in aquaculture environments, it becomes possible to detect and identify species, as well as monitor environmental impacts. Through the analysis of benthic bacterial communities, eDNA metabarcoding can effectively infer the ecological status of investigated samples in a timely and resource efficient manner. Monitoring based on eDNA links the advantage of bacteria's rapid response to environmental influences and eliminates the need for laborious enumeration and identification of organisms. Integrating eDNA into the monitoring process not only serves regulatory compliance but also proves to be a valuable farm management tool, as farmers can optimize sustainable production within the limits of the legislature. Real-time eDNA-based monitoring provides farmers with the ability to promptly react to environmental changes and potential challenges, ultimately enhancing their decision-making process. As a result, the eDNA-based monitoring approach is gaining increasing significance in the field of aquaculture.

Current progress in eDNA-based monitoring

Building upon recent scientific studies, significant progress has been made in leveraging eDNA analysis of benthic sediment samples for environmental impact assessments in the aquaculture industry. These studies have demonstrated that eDNA metabarcoding holds tremendous potential in providing valuable insights into the ecological status of salmon aquaculture installations. In line with these advancements, our research endeavors have focused on addressing previously unresolved sub-steps in the eDNA metabarcoding process. This includes crucial areas such as sample preservation during transport, ensuring reproducibility across laboratories, identifying appropriate bioinformatic methods, and determining minimum sequencing depth requirements. We found that the use of common preservation methods during sampling had no discernible effect on the final ecosystem assessment. This insight helps to simplify the sample collection process and enables to adapt

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it more easily to individual needs. Moreover, our research highlights the reproducibility of the eDNA metabarcoding-based method. Multiple independent laboratories, when employing standardized protocols, have consistently derived coherent ecological interpretations, further underscoring the reliability and robustness of the approach. In our pursuit of refining the eDNA metabarcoding process, we have explored innovative approaches such as machine learning. This exciting avenue of research has showcased the tremendous potential of machine learning algorithms in reliably predicting the environmental status. In fact, our findings reveal that machine learning algorithms, particularly the employment of the Random Forest algorithm, outperform traditional statistical methods (e.g., Indicator Value-approach) and novel abundance-based models (e.g., Quantile Regression Splines) in terms of accuracy and/or automatability. Furthermore, we have demonstrated that sequencing efforts and associated costs are often overestimated. Surprisingly, even with shallow sequencing depths, our research has shown that machine learning-based prediction of ecological status and accurate biomonitoring can be achieved. This revelation not only underscores the efficiency of eDNA-based monitoring but also positions it as a cost-effective solution that exceeds previous expectations. Additionally, we have successfully identified universal bacterial core taxa that serve as reliable indicators of varying levels of aquaculture impact. These core taxa exhibit distinct patterns that allow for the aquaculture impact, irrespective of factors such as sampling season, sampled country, seafloor substrate type, or local farming and environmental conditions. This remarkable finding highlights the robustness and universality of these bacterial indicators in assessing the ecological status of aquaculture installations, regardless of the specific contextual variables at play.

Conclusion

The use of eDNA-based monitoring in aquaculture can improve efficiency, accuracy, and cost-effectiveness compared to traditional methods. By embracing and integrating eDNA metabarcoding into routine monitoring programs, the aquaculture industry stands to gain substantial benefits. By establishing standard protocols and integrating eDNA metabarcoding into legislative regulations, aquaculture companies can replace or supplement existing monitoring approaches, resulting in cost-effective and efficient compliance monitoring. With the multifaceted application of eDNA technology, aquaculture managers and farmers can now not only comply with regulations but also optimize their practices, ensuring sustainable production and responsible management of aquaculture installations while preserving the surrounding ecosystems.

The provided poster serves as a visual representation of our collective efforts in advancing eDNA metabarcoding for benthic monitoring of aquaculture installations. Moreover, the poster will facilitate engaging discussions about the prospects of eDNA metabarcoding, including the forthcoming challenges that need to be addressed in the future.

BACTERIAL SINGLE CELL PROTEINS: NOVEL ALTERNATIVES TO THE USE OF FISH MEAL IN THE DIETS OF FARMED WHITELEG SHRIMP *Penaeus vannamei*

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Introduction

A potential solution to the increasing demand of fish meal (FM) in aquafeeds would be the replacement of FM in the diets by alternative protein sources, but they do not normally meet with the nutritional requirements of shrimp and can lead to nutritional imbalances (Malcorps et al., 2019). Moreover, shrimp farming is usually affected by bacterial diseases, such as these known as "Bright-red syndrome" and "bacterial white tail disease" in whiteleg shrimp (*Penaeus vannamei*) caused by *Vibrio harveyi*, which is one of its most pathogenic agents in terms of mortality rates (Soto-Rodriguez et al., 2012; Zhou et al., 2012). Under this backdrop of threats to shrimp aquaculture, the use of single cell proteins (SCPs) in shrimp diets may be a potential nutritional alternative to FM. In particular, bacterial single cell proteins (BSCP) are composed of up to 80% of protein content and have a high proportion of essential amino acids, vitamins, phospholipids, and other functional molecules (Pereira et al., 2022). Further, some studies testing the replacement of FM by BCSP coming from *Methylococcus capsulatus* in whiteleg shrimp have reported promising results for improvement of immune status, disease resistance and gut microbiota (Chen et al., 2021; Jintasataporn et al., 2021; Felix et al., 2023). Thus, this work aimed to evaluate the suitability of a BSCP from *M. capsulatus* as an alternative to FM in *Pennaus vannamei*.

Materials and Methods

A 140 days-nutritional trial was carried out in whiteleg shrimp fed five isonitrogenous (36% crude protein) and isolipidic (8% crude fat) experimental diets. One was formulated based on a commercial feed for whiteleg shrimp and was used as a control diet (D1), and the others were the same diet but with graded levels of FM replacement by the BSCP (Uniprotein® Aqua, Unibio, Roskilde, Denmark): 75% FM, 25% BSCP (D2); 50% FM, 50% BSCP (D3); 25% FM, 75% BSCP (D4); and 0% FM, 100% BSCP (D5). At the end of the trial, survival, growth in terms of final body weight (BW_f) and specific growth rate (SGR), and feed conversion ratio (FCR) were measured. Body proximate composition was also evaluated, as well as the body profile of fatty acids and of amino acids. The overall condition of the intestine was histologically evaluated. In addition, DNA was extracted from the shrimp intestines, and 16S rRNA gene library sequencing (Illumina - MiSeq platform) was conducted using specific primers for the V3-V4 hypervariable regions. After the trial, a bacterial challenge of 10 days was also performed, in which whiteleg shrimp was injected with the pathogen *V. harveyi* in order to measure the survival rates for each dietary group.

Results and Discussion

Whiteleg shrimp fed the control diet showed a survival rate of 47.7 ± 2.9%, while substituting all the FM by the BSCP it increased to 65.3 ± 2.5%, an improvement of 75% ($P < 0.05$). On the other hand, the BW_f decreased in shrimp fed D4 and D5 (13.1 ± 0.72%; 11.5 ± 0.72%, respectively) with respect to those fed D1 (16.0 ± 0.34%; $P < 0.05$), while the rest of diets did not compromise the BW ($P > 0.05$). Similarly, D4 and D5 reduced the SGR from 4.89 ± 0.02% (D1) to 4.74 ± 0.04% and 4.65 ± 0.05% BW/day, respectively ($P < 0.05$). Considering that there were not differences in apparent FCR values among any dietary group ($P > 0.05$), probably the decreased BW_f and SGR with the higher inclusions of BSCP were attributed to the higher stocking density, in line with the observed negative correlation between survival and BW (Pearson Product Correlation; r = -0.90, $P < 0.001$). In fact, many aquaculture studies in shrimps and fishes have reported a dependency between growth and stocking density, which are usually inversely correlated (Schram et al., 2006; Rodríguez-Olague et al., 2021). Regarding gut microbial communities, shrimp fed D2 displayed a reduction in richness (ACE index = 156.81 ± 26.39) with respect to those fed with the control diet (201.02 ± 68.24; $P < 0.05$). Similarly, shrimp fed D2 showed a different beta diversity based on Weighted UniFrac distances with respect to the control group (as well as with respect to those fed the D4 and D5) (PERMANOVA; $P < 0.05$). However, all the dietary groups showed differential phylogenetic features among them (Unweighted UniFrac distances; $P < 0.05$). These results were reflected in the differential relative abundances of the genera *Albimonas*, *Nautella*, *Aurantivirga*, *Tropicibacter, Pseudoalteromonas*, *Aliiroseovarius* and *Alkalimarinus* among others ($P < 0.05$), as will be discussed. There were no significant differences in survival when whiteleg shrimp was challenged with *V. harveyi* ($P > 0.05$). To sum up, the use of Uniprotein® as a protein source in whiteleg shrimp diets is a sustainable and feasible strategy for improving its production.

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EFFECTS OF REPEATED ACUTE HYPOXIC STRESS ON HAEMATOLOGICAL, PHYSIOLOGICAL AND GENE EXPRESSION RESPONSE IN RAINBOW TROUT *Oncorhynchus mykiss*

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Introduction

Oxygen is a limiting factor both in the environment and production systems, so reduction may become a stressor. Diel cyclic hypoxia may occur with varying frequency and duration in freshwater habitats and in the context of climate change, the frequency of this situations will increase. Under a stressful situation fish activate the hypothalamic-pituitary-interrenal axis (HPI) which triggers the release of cortisol that induces secondary and tertiary responses. The level of activation of this response will depend on the intensity and the duration of the stressor Also, the recovery of individuals subjected to such stressors depends on their ability to modulate physiological, biochemical, and behavioural responses to maintain metabolic functions and homeostasis. Since episodes of repeated hypoxic stress have been less studied, the aim of this study is to determine the haematological, physiological and molecular response of rainbow trout under repeated hypoxia observe whether there is a habituation response.

Materials and methods

Rainbow trout juveniles were acclimated to AQUAB fish facilities (Universitat Autònoma de Barcelona, UAB), After the acclimation period, fish were randomly divided in 5 different treatment groups, 2 control groups (absolute control (AC) and manipulated control (MC)) and 3 hypoxia groups: H1 that only received 1 hypoxic exposure, group H2 which received 2 hypoxic exposures and group H3 which received 3 hypoxic exposures.

Every exposure to hypoxia consisted in reducing the water oxygen level in the tanks from 8-9 mg/L to 2 mg/L by removing the aeration pumps and bubbling N_2 into the system. Then fish were left in the tanks for 1 hour and sampled subsequently at 1,6 and 24 post exposure. Water oxygen levels were continuously monitored during the experiment. After the challenge, 9 fish per treatment, and time-point were euthanized with an overdose of MS-222.Samples of blood, skin, gills, and intestine were taken.

Table 1. Table 1. Red blood cells number (RBC), Haemoglobin (HGB) in rainbow trout

Group	RBC ($10^3/\mu L$)	HGB (g/dL)
AC	0.961 ± 0.0224 [b]	5.57 ± 0.108 [ab]
MC	1.058 ± 0.0229 [a]	5.78 ± 0.110 [a]
H1	0.939 ± 0.0224 [b]	5.61 ± 0.108[ab]
H2	1.031 ± 0.0229 [a]	5.77 ± 0.123 [ab]
H3	0.975 ± 0.0224 [ab]	5.35 ± 0.108 [b]

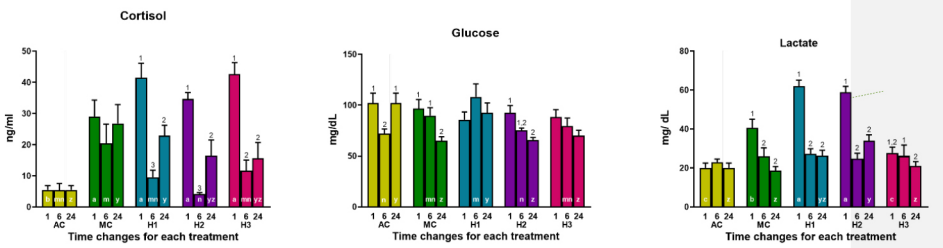

Figure 1. Plasma cortisol, lactate and glucose in rainbow trout blood 1, 6, and 24h after treatment. Data are represented as mean ± SEM (n=9 per sampling time-point). Significant differences ($p < 0.05$) between treatments at the same sampling time are shown with different letters (from a to c at 1h, from m to n at 6h and from y to z at 24h after treatment). Significant differences in sampling time among the same treatment are shown by numbers ($p < 0.05$).

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Results

Haematological parameters as red blood cells number or haemoglobin show that manipulation increase these levels, while hypoxia decreases them. Interestingly, fish are able to recover the control level of haematological parameters after 3 shocks (Table 1).

Respect to the physiological response we observe that cortisol level show and increase at 1 hour post exposure. Also, looking at lactate levels we observe that there is an increase in H1 and H2 at 1 hour, but not in H3 suggesting a metabolic habituation to hypoxic exposures. Finally, regarding glucose levels there is an increase at 1 hour, and a later recovering of control levels at 24 hours.

Finally changes in gene expression were also observed in *crh and gr1* stress response genes and in *l1b, il10* immune response genes.

Discussion

The results of our experiment on induced hypoxia suggest that trout subjected to repeated acute hypoxia could cope with oxygen levels down to 2mg/L of oxygen in water up to 24 hours after the stressor, and that subjecting the fish to the same stressor, trout shows a trend for habituation. Thus, among the hematological variables, red blood cells (RBC) and hemoglobin (HGB) show an increase in the CM group which may be due to the handling of the animals as observed in Acerete et al (2004). On the contrary, as observed in H1, hypoxia produces a decrease in RBCs, probably because the oxygen delivery system is excessively altered and then the overall metabolism and activity decreases (Pichanvart et al 2002). The increase of hematocrit levels after 1 hour would be associated to balancing out the extra need for oxygen (Muszee et al 1998).

Glucose is used as a substrate for glycolytic activity leading to the release of cell energy so that as time progresses these reserves are used. Nevertheless, in most cases an acute stress induces an increase in plasma glucose as a result of the energetic needs derived from the stress situation (Abdel-Twaab et al 2019). Lactate levels increase facing a situation of hypoxia, as to maintain the cellular energy balance. This may be explained by the fact that oxygen-independent mechanisms are needed since oxygen availability is 15 times lower under hypoxic conditions (Richard J.G, 2011).

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SINGLE CELL AND SPATIAL TRANSCRIPTOMICS OFFER INSIGHTS INTO HEALING AND IMMUNITY IN THE SKIN OF ATLANTIC SALMON

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Introduction

Atlantic salmon is one of the most important aquaculture species worldwide, accounting for 4.5% of global finfish trade, and demand is steadily increasing (FAO 2020). However, production is hindered by skin diseases such as sea lice and winter ulcerating bacterial infections, which cause substantial welfare issues as well as economic losses (Fish Health report 2022). With both diseases on the rise, there has been a growing interest on salmon skin biology. One of the main characteristics of skin is its ability to heal, enabled by the presence of specialized cells that work together to repair damage and restore the integrity of the tissue (*Hou et al*. 2020). Knowledge of the cellular composition and genetic response to infection, wounding and healing of Atlantic salmon skin is needed in order to gain a better understanding of how these pathogens are bypassing and suppressing the healing capabilities of skin.

Materials and Methods

Samples for single cell sequencing and spatial transcriptomics were collected from two healthy skin samples and four mechanically wounded skin samples at 2 and 14 days post wounding (two samples each time point), representing the early acute inflammatory phase and the tissue repair phase, respectively. Mechanical wounds were introduced by a sterile punch biopsy tool as described by (Sveen *et al*. 2018).Single-nuclei RNA-seq libraries were generated for all samples using a 10x Chromium and following standard 10x protocols. Spatial transcriptomics libraries where prepared using the 10x visium protocol. Library mapping was performed with STAR (Kaminow et al. 2021) with v3.1 of the genome of Atlantic salmon as reference. Results were analysed using Seurat (Stuart et al. 2019).

Results

We generated single cell atlases across a wound healing time course. We identified pluripotent mesenchymal stem cells that differentiate into diverse cell types including adipose, bone and vascular cells and appear to be crucial in the wound healing process. We then used spatial transcriptomics techniques to locate these cell types in the tissue, and demonstrate their proximity to the wound and importance to the healing process (Figure 1). We further interrogate the role of these cells in the healing process, including their interactions with keratinocytes and immune cells

Conclusion

This work represents a major enhancement in our understanding of the wound healing process in Atlantic salmon, identifying and characterising the key role of pluripotent mesenchymal stem cells. Importantly, it provides a framework for comparison of healthy wound healing and healing of pathogen-afflicted wounds, such as those caused by sea lice and winter ulcerating bacteria.

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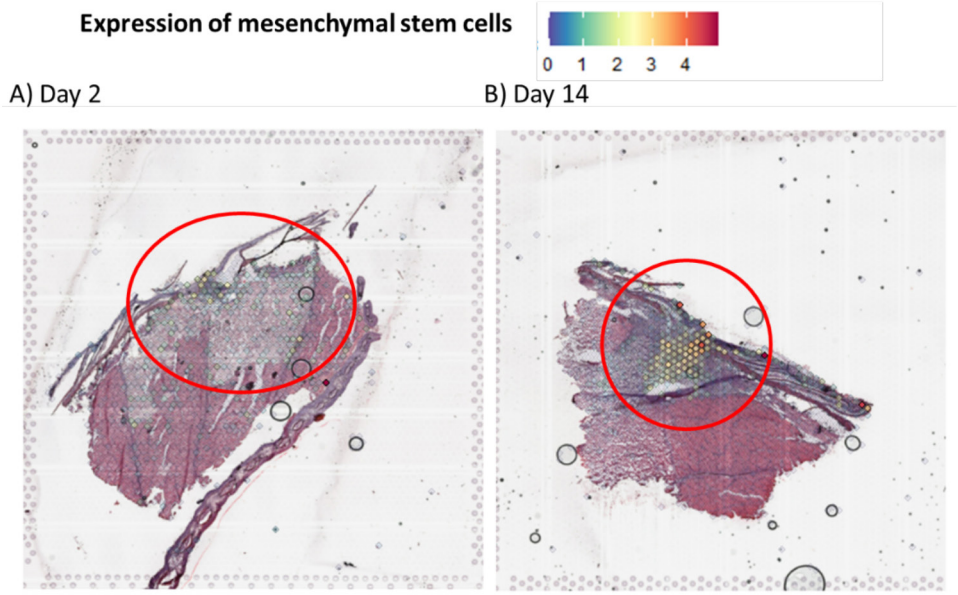

Figure 1. Mesenchymal stem cell (MSC) populations in the skin of Atlantic salmon during wound healing. The site of mechanical induced wound is marked with a red circle. (A) Day 2 post injury, during the acute inflammation phase not many MSCs are present. (B) Day 14 post injury, during the remodelling stage MSCs are abundant and likely proliferating in the wound site.

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CENTESIMAL COMPOSITION OF PICKLED AMAZONIAN-SHRIMP *Macrobrachium amazonicum* IN DIFFERENT SAUCES

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Introduction

Fish is considered a privileged meat product from a nutritional point of view, as it is rich in polyunsaturated fatty acids (especially omega 3), has high quality protein, digestibility, biological value, and is also a source of vitamins and minerals (DIAS and SILVA, 2021).

In the Amazon region, fishing is considered the basis of the economy of some cities and also of traditional communities, which stands out for the richness of fish that are exploited and also for the amount of fish and shrimp captured annually (COSTA *et al.* 2018).

Thus, thinking about the technologies related to value-added preserves (new cuts, new sauces, new ingredients, etc.), intended for human consumption and nutrition, and also the importance of obtaining information on the centesimal composition of these processed foods , was that efforts were directed towards the characterization of the nutritional components of canned amazonian-shrimp (*Macrobrachium amazonicum*) in two different topping sauces, with a focus on evaluating the dietary patterns of these foods.

Material and methods

Amazonian-shrimp (*Macrobrachium amazonicum*) were acquired in the municipality of Macapá, state of Amapá, Eastern Amazon, through donations made by artisanal fishermen from traditional communities in this municipality.

The shrimp used for the preparation of preserves were processed, where they were first peeled and gutted and then washed in chlorinated water with 20 ppm of free chlorine. The preparation of the artisanal preserves was carried out as follows: first, the shrimp were inspected, after which they were placed in glass jars, and then the topping sauce (tomato sauce (CCMT) and spice sauce was added regional (CCMER)), where then the pots were covered and then submitted to the cooking process. The pots containing the ingredients were subjected to a heat treatment (cooking) in a pressure cooker for 50 minutes. In order to avoid bulging lids, as well as the correct destruction of microorganisms, opening the pan and removing the pots was done after the pan had cooled down.

Results and discussion

Table 1. Proximate composition of artisanal preserved amazonian-shrimp (*Macrobrachium amazonicum*) in tomato sauce and regional spice sauce.

Parameter	Product	
	CCMT[1]	CCMER[2]
Humidity (g/100g)	81,29±1,18	65,60±3,37
Mineral waste (g/100g)	0,20±0,11	0,53±0,45
Total lipids (g/100g)	2,39±0,56	13,85±3,00
Total proteins (g/100g)	10,54±1,01	17,00±0,97
Carbohydrates (g/100g)	6,46±0,40	5,14±0,80
Calorific value (Kcal/100g)	91,39±1,35	198,56±31,57

[1]Canned amazonian-shrimp in tomato sauce.
[2]Canned amazonian-shrimp with regional spice sauce.

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The centesimal analyzes were carried out from the preserves prepared in an artisanal way, in which moisture, mineral residues, total lipids, total proteins, carbohydrates and caloric value were determined, according to the official international methods of food analysis (AOAC, 1990). The centesimal composition analyzes were performed in six replicates, per sample.

Results and discussion

After comparison with the literature (DIAS and SILVA, 2021; COLEMBERGUE, GULARTE and ESPÍRITO SANTO, 2011), it was possible to conclude that the CCMT presented low levels of proteins, total lipids and mineral residues, high moisture contents, moderate amounts of carbohydrates, as well as low calorie values. As for the CCMER, they presented moderate levels of moisture, carbohydrates and lipids, high values of total proteins and calories, as well as low levels of mineral residues.

Only the humidity value for CCMER is in accordance with the literature, as it matches a cooked product. Regarding the mineral residue and the amount of lipids, the values found by Colembergue, Gularte and Espírito Santo (2011) for sardines in tomato sauce were higher when compared to this work. As for the protein portion, the values normally found in fresh shrimp (*Macrobrachium amazonicum*) (DIAS and SILVA, 2021) is around 22 g/100g, above that found by the present study for both preserves, 10 g/100g (CCMT) and 17 g/100g (CCMER). In the literature, canned sardines in tomato sauce show higher values for mineral residues, proteins and fats and lower values for moisture and carbohydrates, when compared to this work.

Conclusion

Thus, the preserve prepared by this work is easy to prepare and handle, and can be replicated without much cost, since materials and ingredients are used that can be easily found in markets and fairs. Both preserves have shown to have good dietary standards, as they have nutritional components that can be used in diets that require high levels of proteins and lipids, and low levels of minerals, which nutritionally justifies a stimulus to the consumption of artisanal canned fish.

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SHOCKING RESEARCH LEAVING EVERYONE STUNNED: USING ELECTRICAL STUNNING AS A METHOD OF EUTHANIZATION OF ZEBRAFISH (*Danio rerio*) IN LABORATORY CONDITIONS

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Background

The zebrafish (*Danio rerio*) accounts for approximately 50% of fishes used in research and its adoption has grown rapidly since the 1990's (Kinth *et al.* 2013). The high number of individuals used in research warrants for an accessible, effective, and humane manner to stun and kill zebrafish in the laboratory. Common methods adopted in research today include overdose of anaesthetics, rapid cooling followed by brain destruction or exsanguination. However, there is evidence that these methods are aversive (*e.g.,* many anaesthetics) and require capture, handling and transferring to another tank, thus causing stress (Wong *et al.* 2014). Electrical stunning could be a more effective and less stressful euthanasia method but although it is extensively used in aquaculture, very little is known about the use of electrical stunning for zebrafish (Mocho & von Krogh 2022). One study has demonstrated that euthanizing zebrafish embryos and larvae with electricity is rapid and reliable (Mocho *et al.* 2022). Here, we have developed a suitable electrical stunning method for euthanizing zebrafish in laboratory settings. Our aim is to investigate i) whether adult zebrafish can be euthanized humanely and effectively using electricity, and ii) what is the minimal, electrical field required to achieve a humane and effective euthanasia without using unnecessarily high voltage. Furthermore, we have developed portable electrode panels that can be placed in a common zebrafish housing tank to reduce the amount of handling and therefore, minimize the unnecessary stress that zebrafish may experience both before and during the procedure of euthanasia.

Material and methods

In all of the trials, water temperature, conductivity, and frequency were kept constant at 28±0.3 °C, 800±5 µS/cm and 50 Hz, respectively, whereas the voltage was manipulated to achieve different electrical field strengths. The first trials focused on finding a field strength that is sufficient to stun the zebrafish immediately without the fish showing any adverse reaction. This was done to verify that the fish experienced an immediate loss of consciousness, and therefore would not endure extreme stress and pain during the stunning. In these trials, the electrical stun duration was 1 s. A behavioural assessment was done by visually inspecting the fish during and after the stun. Video footage was used to revise the reaction and to confirm all the trials. After the appropriate minimal field strength was verified, single zebrafish where stunned for 30 s to demonstrate the efficacy of the field strength to eliminate the subject. The fish were inspected for 30 min after the stun to verify that the stun was irreversible. Zebrafish where then stunned in groups of 5 fish per tank and later in groups of 10 fish per tank for 30 s. After a sufficient field strength was concluded, portable and adjustable electrode panels were constructed. The final design was made to be suitable for an 8 L standardized zebrafish tank (Tecniplast S.p.A, Italy), which is commonly used for zebrafish housing. The electrode panels were designed to have adjustable handles and supporting

Table 1. **The conducted trials separated by tank size, group size, and average field strength (FS) and average current density (CD).** The recovery rate was calculated by dividing the recovered fish in the same trial group by the total number of fish in the same trial group. S indicates that the trial group included only successful trials and U that one or more trials were unsuccessful. It is to be noted that more trials are yet to be conducted, and the results may therefore change later.

Tank (L)	Group size	No. of trials	T (°C)	Conductivity (µS/cm)	Av. FS (V_{rms}/cm)	Av. CD (A_{rms}/dm^2)	Recovery rate	Status
3.5	10	11	28.1	803	5.04	0.06	0.0 %	S
3.5	15	1	28.0	808	5.31	0.06	0.7 %	U
3.5	15	5	28.2	803	6.70	0.08	0.0 %	S
8	10	4	28.1	800	4.99	0.05	1.0 %	U
8	10	6	28.0	800	6.04	0.06	0.0 %	S
8	40	2	28.2	798	6.04	0.06	0.03%	U
8	40	2	28.4	806	7.1	0.08	0.0%	S

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staffs to be able to adjust the electrode separation to be fitted also into the smaller, 3.5 L standard tank (Tecniplast S.p.A, Italy). The portable electrodes where then tested using both 8- and 3.5 L tanks by stunning groups of 10 fish for 30 s as well as stunning the number of fish equal to the maximum capacity for each tank, i.e., 40 fish in the large tank and 15 fish in the small tank for 30 s. Only trials where all fish were stunned irreversibly were determined successful. If the trial was deemed unsuccessful, the voltage was adjusted so that the electric field strength was 1.0 V/cm higher.

Results

Zebrafish that that were stunned irreversibly showed no aversive nor aggravated behaviour during the stun. It was conducted that 5±0.3 V/cm was sufficient to euthanize 1, 5 or 10 fish per trial in a 3.5 L tank, but stunning the maximum capacity of fish in the same tank required a 6.7±0.1 V/cm. For the larger, 8 L tank, 6.0±4 V/cm was sufficient to euthanize 10 fish at once. The trials conducted with the standard housing tanks are presented in Table 1.

Conclusions

Electrical stunning was highly effective in causing immediate loss of conscious and death without recovery in zebrafish. Many large facilities are killing hundreds of zebrafish every week thus this technique represents a more refined method without the use of chemicals, cooling stress or handling of the animals. Depending on the number of zebrafish and tank size, the sufficient field strength does need to be adjusted. Our results comply with the previous assumptions regarding electrical stunning of young forms of zebrafish (Mocho *et al.* 2022), and therefore electrical stunning could contribute to a more humane euthanization and better welfare of adult zebrafish in the future. This approach could be extrapolated to other species of fish used in research including aquaculture species where killing large numbers of fish humanely remains a challenge.

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HOLOTHURIA FORSKALI REARED UNDER FLAT OYSTERS: POLYCULTURE OR INTEGRATED MULTITROPHIC AQUACULTURE?

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Integrated multitrophic aquaculture (IMTA) allows to diversify production while reducing the footprint of a monoculture and is now widely recognized as a tool to increase the sustainability of aquaculture systems (Chopin et al., 2012). Holothurians, or sea cucumbers, are excellent candidates for integration in IMTA (Zamora et al., 2018), but European demonstrators are still scarce, mostly because of our limited capacity to reproduce European Holothurian species.

This presentation shares the latest results of a 15 months study testing the potential of growing *Holothuria forskali* underneath flat oysters, *Ostrea edulis.* Juveniles specimen of *H. foskali*, fertilized and reared in controlled conditions (Laguerre et al., 2020) up to 5g, have been stocked at different densities: 9, 18, 27 and 36 individuals/m^2 under 3 rows of flat oysters in mesh bags placed above the intermediate shoreface. Growth and survival have been monitored and showed exceptional results, with a 95% survival and body masses multiplied by more than 20 for the lowest density. Despite slower growth observed for higher densities, total biomass was multiplied by 6 in the highest density, which translated in a 1035 g/m^2 gain. These results demonstrate the great potential of growing *H. forskali* in combination with the studied oyster production system, in order to diversify production and income with neglectable extra efforts, time and investment.

Yet, to qualify an aquaculture production system as IMTA, trophic links need to be demonstrated in order to prove that holothurians benefit from the waste of the other species. A supplemental condition was therefore added and consisted in juvenile *H. forskali* stocked in similar conditions but outside the waste plume of the oysters. Surprisingly, growth was identical as below the oysters indicating that *H. forskali* can benefit from suitable food independently from oysters' waste. Isotopic analyses and fatty acid compositions of the different conditions help better understanding trophic relationships in the system.

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OPTIMALIZATION OF THE MACRONUTRIENT COMPOSITION IN THE ATLANTIC HALIBUT DIET

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Farming of Atlantic halibut had its modest start in the 1980s and has slowly grown into an industry that sold 1900 metric tons in 2020. This has been a challenging endeavor where one bottle neck has replaced the next, and the first decades were largely dedicated to solving difficulties related to larval development. However, the industry has endured, and the current focus has moved from early development to optimalization growth and welfare to market size. We have therefore investigated the effect of varying levels of the macronutrients (protein, lipids and carbohydrates) on the grow out phase. Twelve diets were designed ranging in protein from 45 to 77 %, lipids ranging from 5 to 30 % and carbohydrates from 5 to 25 % of the diet. This represents a three-component mixture design, allowing analysis of the mixed effect of the factors on the fish. Atlantic halibut juveniles (mean weight, 300 g; 120 in each tank) were randomly distributed to 15 tanks for one year.

The effect of macronutrients on growth changed during the growth period. In the start of the trial both high levels of carbohydrate and lipid had a negative effect on SGR, whereas the negative effect of high carbohydrate was less important toward the end of the trial. There was no effect of diet composition on digestibility of lipids and proteins. Muscle composition was not affected by diet composition, but the liver composition reflected dietary variations. Effects on appetite and welfare will be presented and discussed.

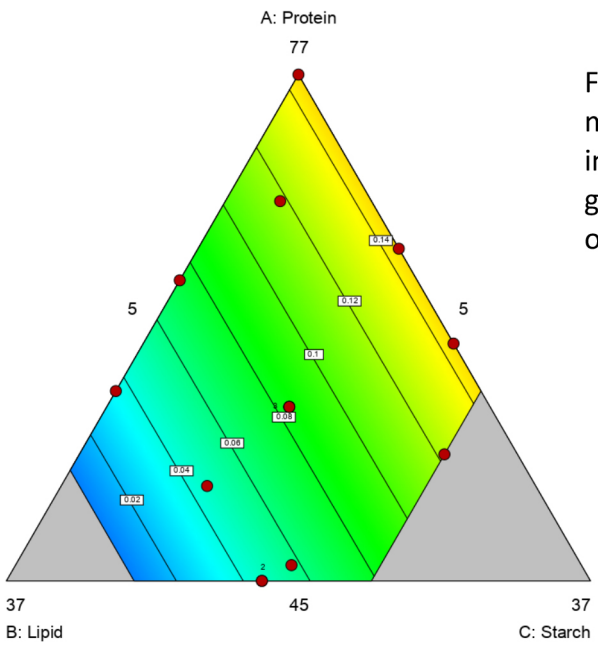

Fig. Specific growth rate (SGR) the last two months of the feeding trial. The colour scale indicates SGR between -0.0185055 0.19008 . This growth period showed a linear negative effect of lipid on SGR.

EFFECT OF DIET COMPOSITION AND STARVATION ON THE EXPRESSION OF AKT/mTOR SIGNALING PATHWAYS IN SKELETAL MUSCLE OF GILTHEAD SEA BREAM (*Sparus aurata*)

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Introduction

White skeletal muscle contributes to 40-60 % of fish body mass and to overall growth in vertebrates. Development of this tissue has a well ordered and adaptative structure. The process of myogenesis shows significant plasticity, which is essential for the proliferation, differentiation, migration, and fusion of new myoblasts. This process is primarily controlled by myogenic regulatory factors (MRFs), a large group of proteins containing a basic DNA-binding motif and a helix-loop-helix dimerization domain (Braun & Gautel, 2011). Moreover, somatotropic components such as insulin-like growth factor 1 (IGF-1) exert an important role in nutritional regulation of metabolism, increasing muscle mass and decreases muscle atrophy (Vélez et al., 2014). IGF-1 and amino acids activate the phosphatidylinositol 3-kinase (Pi3k)-Akt pathway, which leads to phosphorylation of the serine/threonine protein kinase mechanistic target of rapamycin mTOR and activation of a critical pathway involved in cellular processes such as apoptosis, protein synthesis, gene transcription and cell proliferation (Vélez et al., 2014). Indeed, mTOR is an essential sensor of nutrient and amino acid availability through phosphorylation of 40S ribosomal protein S6 (S6). With the aim to increase our current knowledge about the nutritional regulation of skeletal muscle growth and development in fish, we addressed the effect of dietary macronutrient composition on the expression of genes involved in Akt/mTOR pathway, MRFs, and IGF-1 in white skeletal muscle of gilthead sea bream (*Sparus aurata*) juveniles maintained in different conditions (fed and fasted).

Materials and Methods

S. aurata juveniles were obtained from Piscicultura Marina Mediterranea (Burriana, Castellón, Spain). A total of 330 fish (8.22 g ± 0.26 body weight) were transported to the laboratory and distributed in 12 aquaria of 260 l supplied with running seawater at 21 ◦C in a closed system with active pump filter and UV lamps. Three diets were formulated with gross energy at 20–22 kJ/g and macronutrient composition at levels above and below those in commercially available aquafeeds: HLL (high protein, low lipid, low carbohydrate), MHL (medium protein, high lipid, low carbohydrate) and LLH (low protein, low lipid, high carbohydrate). Fish were fed for 37 days twice daily to satiety. On the other hand, a group of fish was maintained in fasting conditions for the same period of time. Total RNA was extracted from skeletal muscle and gene expression was analyzed by quantitative real-time RT-PCR (qRT-PCR).

Results and Discussion

Among fed fish, the mRNA levels of mTOR and S6 protein showed a significant dependence on the protein/carbohydrate ratio in the diet. For mTOR, diet HLL promoted the highest expression values, which were 1.7-fold and 2.4-fold greater than in fish fed diets MHL and LLH, respectively (Fig. 1a). Likewise, fish fed HLL presented 1.3-fold and 1.6-fold higher S6 mRNA levels than fish supplied with MHL and LLH, respectively. The expression levels of mTOR and S6 in starved fish were similar to those observed in the skeletal muscle of fish fed diet LLH (Fig. 1a-b). On the other hand, the expression of Akt showed a behavior similar to that of mTOR and S6 (Fig. 1c). In agreement with results in *S. aurata* (Lavajoo et al., 2020), muscle mRNA levels of mTOR and S6 decreased upon starvation. However, no significant differences in Akt expression were found between fed and fasted fish. Starvation for 37 days significantly downregulated 2.9–6.0 fold IGF-I expression depending on the diet supplied. Among fed fish, significant upregulation of IGF-I (1.8–2.0 fold) was found in fish fed diets with improved growth parameters (MHL and HLL) (Fig. 1d). Consistently, the combined action of Igf1 and amino acids stimulated mTOR and Akt activity in *S. aurata* myocytes (Vélez et al., 2014), and low protein diets diminished growth performance and mTOR signaling pathway in yellow catfish (*Pelteobagrus fulvidraco*) by downregulating the mRNA levels of key genes such as IGF-I, mTOR and Akt (Qin et al., 2019). Dietary macronutrient composition and food

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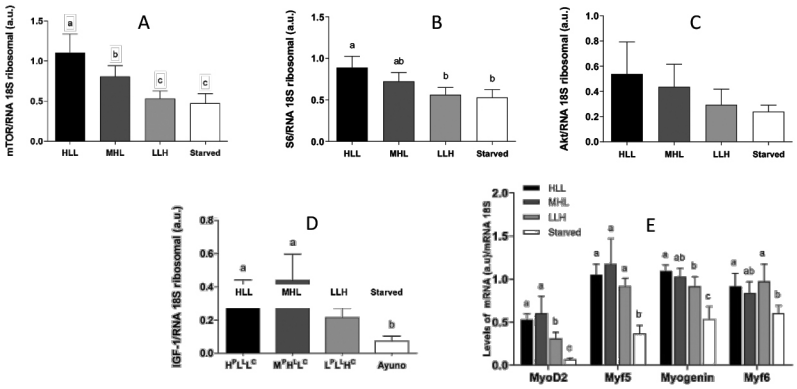

Fig. 2. Effect of diet composition and starvation on the expression of mTOR (a), S6 (b), Akt (c), IGF-I (d), and MRFs (e). The mRNA levels for each gene were normalised with the geometrical mean of S. aurata 18s and ef1α, which were used as housekeeping genes. Results are presented as mean ±SEM (n =4–6 fish). Different letters above deviation bars indicate significant differences (P <0.05) among conditions.

deprivation significantly affected the expression of MRFs in the white skeletal muscle of *S. aurata*. Starvation for 37 days significantly downregulated the mRNA levels of all MRFs assayed. Concerning fed animals, the supply of HLL and MHL significantly enhanced 1.7–1.9 fold the expression levels of myod2 compared to fish fed with diet LLH (Fig. 1e). In a significant manner but to a lesser extent than myod2, feeding fish with the diet containing the lowest protein/carbohydrate ratio (LLH) decreased myf5 and myogenin mRNA levels when compared to fish fed with MHL and HLL diets, respectively. The only MRF assayed whose mRNA levels did not significantly differ as a result of diet composition was Myf6 (Fig. 1e).

Conclusion

The results of the present study show that the expression of genes involved in Akt/mTOR pathway, MRFs, and IGF-1 in white skeletal muscle of gilthead sea bream juveniles maintained in different conditions was markedly affected by nutritional status and dietary macronutrient composition.

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REPRODUCTIVE PROCESS AND THE IMMUNE SYSTEM IN FRESHWATER FAMALES PRAWN *Macrobrachium americanum*

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Introduction

The prawn *Macrobrachium americanum* is a native crustacean in rivers of northwest part of México. CIIDIR-Sinaloa, is working in establishing conditions for reproducing *M. americanum*. Actually, several variables have been controlled for management of mate, egg and larvae development. During these studies, mortality of berried females was common and deserved our attention. The goal of this study was to analyze the pro-Phenol-Oxidase system, the total hemocytes count and coagulation in reproductive females.

Materials and methods

Four groups of 10 organisms were arranged in four groups: 1. Females with immature gonad, 2. Females after five days of ovulation, 3. females before five days of harvest and 4. Male. Organisms were obtained from Sinaloa River with a mean weight of 74g, they were reared during 2 months feeding tilapia meat and pellets for shrimps at 29±2 °C. At the established times, a sample of 50 µL of haemolymph was withdraw to analyze the coagulation time between the groups and immediately a sample of 0.5 mL of hemolinfa was withdraw from the ventral sinus in a anticoagulant solution. Haemolymph was processed to determine haemocytes account, pro.Phenol-Oxidase content, Phenol-Oxidase activity, and protein concentration.

Results

Results showed uniforms conditions variables in groups 1, 2 and 4. Five days before harvest females group 3, showed a general alteration, pro-Phenol-Oxidase decrease in content, meanwhile the time in coagulation and Phenol-Oxidase activity increased.

Discussion

It was interesting way the increase in Phenol-Oxidase activity without apparent difference in contact with non-self material between groups, since they were reared in the same container. But this is not the cause of mortality. If females do not coagulate, they have to face blooding and the introduction of non-self material. After the addition of Transglutaminase in the 50 µL sample, coagulation time decrease suggesting a malfunctioning of this enzyme during reproduction.

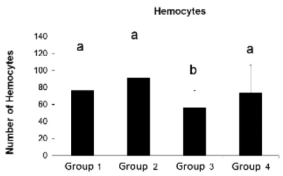

Fig 1. Number of Hemocytes In *M. americanum* females

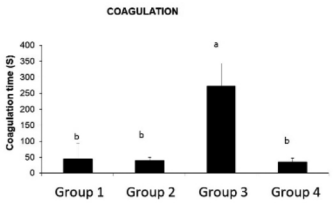

Fig 2. Time of coagulation In *M. americanum* females

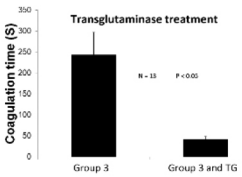

Fig 3. Effect of Transglutiminase In *M. americanum* females

IMPACT OF NOVEL OMEGA-3 LONG CHAIN POLYUNSATURATED FATTY ACIDS-RICH OILS ON ATLANTIC SALMON, *Salmo salar,* HEALTH

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Introduction

The use of terrestrial vegetables as a source of protein and lipid in aquaculture has led to a reduction of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) in salmon fillets. Genetic modification of oilseed crops such as Camelina or rapeseed as well as production of heterotrophic microalgae has allowed the production of land-based sources of LC-PUFA with the potential to take the pressure off fish oil supplies, which mainly come from wild-caught oily fish – a finite resource. Although several studies have investigated the impacts that these new lipid sources have on fish performance and product quality, limited information exists regarding their impact on fish health or adaptation to stress (Napier and Betancor, 2023., Ruyter et al., 2022; Santigosa et al., 2020). The aim of the present study was to assess the impact of different novel lipid sources on Atlantic salmon (*Salmo salar*) parr on their stress response during smoltification by investigating the production of lipid inflammatory mediators (LIM) as well as the head kidney lipid and fatty acid composition and transcriptomic response.

Materials and Methods

A conventional nutritional trial was carried out both in freshwater Atlantic salmon parr from 37.6 ± 6.7 g up to smoltification (109.3 ± 30.3 g). Fish were fed one of eight experimental feeds, in triplicate, containing different commercial and experimental lipid sources. Briefly, oils included two GM-camelina oils (high EPA and high EPA+DHA; ECO and EDCO, respectively), a canola GM oil (high DHA; Aqua), a microalgal oil (high EPA and DHA; Ver), a northern- and southern-hemisphere fish oil (reference feeds, NHFO and SHFO, respectively), krill oil (positive control; KO feed) and sunflower oil (SFO; negative control) (Fig. 1). Feeds were manufactured by BioMar (Brande, Denmark). All feeds used the same base pellet formulation with a fishmeal level reflecting current commercial practice for salmon and were formulated to satisfy all the known nutritional requirements of salmon, including a basal EPA+DHA content of >2.5% of total fatty acids.

Prior to sea water transfer, six fish per tank were humanely euthanized and samples from head kidney either quickly frozen for lipid extractions or stabilized into RNALater for RNASeq analysis (Illumina Sequencing). Plasma was also collected from the six sampled fish, quickly frozen in liquid nitrogen until LIM by LC-MS-MS. Additionally, five fish per tank were subjected to a sea water challenge, by transferring the fish for 24h at a salinity of 35 ppt, after which the fish were euthanized and plasma and head kidney collected.

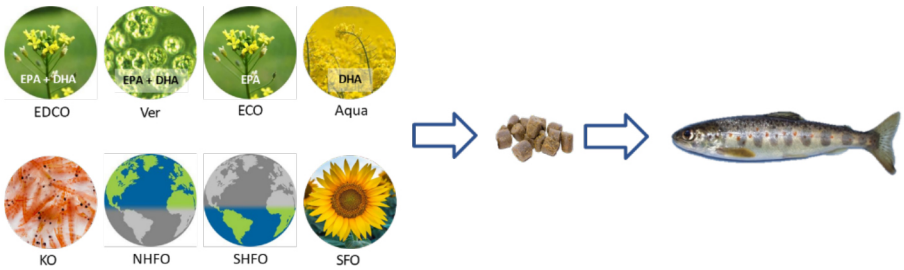

Fig. 1 – Diagrammatic representation of the experimental treatments used in the present trial, including GM-Camelina derived (EDCO and ECO), GM-canola derived (Aqua), microalgal (Ver), krill oil (KO), the positive controls south and north hemisphere fish oil (SHFO and NHFO, respectively) and the negative control, sunflower oil (SFO).

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Results and Discussion

Despite experimental feeds being isolipidic, the kidney lipid content varied among the different dietary treatments. In this sense, fish fed NHFO displayed the largest fat content, whereas those fed EDCO displayed the lowest content, with the remaining treatments displaying intermediate values. These different lipid contents also had an impact on the lipid class composition of head kidney, with fish fed NHFO showing the highest levels of neutral lipids, which are mainly used as energy source, specifically triacylglycerols. The opposite trend was observed in EDCO-fed fish, which in contrary displayed higher contents of structural or polar lipids than NHFO-fed fish. The head kidney fatty acid profile mainly reflected dietary input, not indicating any biosynthetic capacity, as expected. In this sense, the lowest n-3 LC-PUFA levels were detected in the group fed SFO and the highest in fish fed the microalgal oil.

The analysis of LIM demonstrated how the levels of those derived from shorter chain fatty acid (e.g. 18:3n-3 and 18:2n-6) were regulated by dietary input, rather than stress (salinity challenge). On the contrary, levels of those derived from n-6 (20:4n-6) and n-3 LC-PUFA (EPA and DHA) tended to increase/decrease mainly along with stress. Further RNASeq results will also be presented and related to LIM production in head kidney.

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Acknowledgements

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SINGLE NUCLEI RNA SEQUENCING UNCOVERS THE CELL TYPE-SPECIFIC RESPONSES TO SEA LICE WITHIN THE SKIN OF RESISTANT AND SUCEPTIBLE SALMONID SPECIES

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Introduction

Sea lice parasitism is one of the greatest economic, environmental, and animal welfare issues facing the Atlantic salmon (*Salmo salar*) aquaculture industry (Torrissen et al. 2013). A potential solution may exist in coho salmon (*Oncorhynchus kisutch*), a species that mounts a massive inflammatory response to sea lice, resulting in their swift detachment from the skin (Fast et al. 2002). However, it is not known which of the many cell types present in fish skin underlie this remarkable resistance. We therefore employed single-nuclei RNA sequencing, a technique facilitating transcriptomic profiling of thousands of individual cells, to investigate the cell types and gene expression patterns characterizing the response of coho and Atlantic salmon to sea lice.

Materials and Methods

Atlantic and coho salmon were reared in a RAS at the Centre for Aquaculture Technologies (PEI, Canada). Juvenile fish were exposed to copepodid stage sea lice (*Lepeophtheirus salmonis*) and pelvic fin and skin where lice had attached were sampled at 12h, 24h, 36h, 48h, and 60h after exposure. Fin and skin were also taken from unexposed control fish reared in identical conditions. Nuclei were isolated from one skin and one fin sample from the control and each of the five treatment time points for each species (N = 24 samples total) using a custom protocol (Ruiz Daniels et al. 2023). Samples were processed with Chromium (10X Genomics) and sequenced with Illumina technologies. Library mapping was performed with STAR (Kaminow et al. 2021). Resulting outputs were analysed with Seurat (Stuart et al. 2019). Samples were analysed by species and also integrated into a single dataset using 1:1 orthologs identified with OrthoFinder (Emms & Kelly 2019).

Results

The variety of cell types we detected (Fig.1) were largely consistent in identity and marker genes across species. Integration of both species data using 6494 1:1 orthologous genes detected most of the cell types observed in each of the species-specific analyses. These results suggest the same cell types are present in the skin and fin of both species.

We identified 4567 and 1799 unique genes in Atlantic and coho salmon, respectively, which were differentially expressed between control and any of the treatment time points (Fig.2). Both species upregulated immune genes in response to lice, intriguingly in both immune and non-immune cell types. However, coho salmon uniquely demonstrated a down-regulation of iron-sequestration related genes in red blood cells, potentially to starve lice. Coho salmon keratinocytes also dramatically upregulated genes associated with inflammation and immunity. Our results confirm the importance of previously identified candidate genes associated with lice-resistance but also identify new candidates that may have been missed by previous bulk RNAseq studies due to their expression in multiple cell types.

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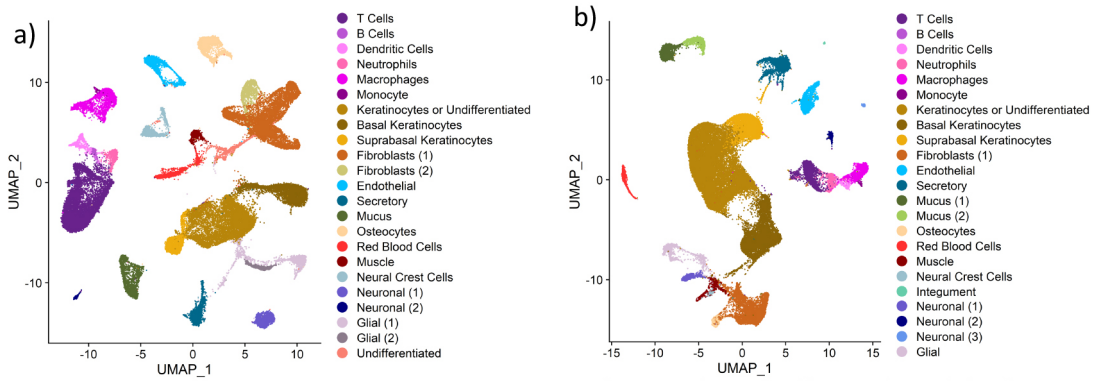

Fig.1 UMAPs of cell types in a) Atlantic salmon, b) coho salmon.

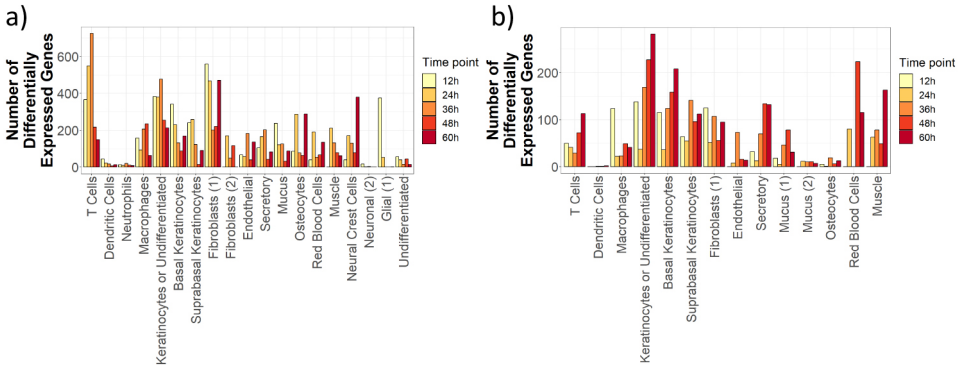

Fig.2 Number of differentially expressed genes between the control samples and each infection time point for each cell type in a) Atlantic salmon, b) coho salmon.

Discussion

Clearly, multiple cell types, common to both species, are involved in sea lice response in Atlantic and coho salmon. Our results also suggest that coho salmon use multiple strategies (with different cell types and genes) to repel lice. This may explain why a single locus for lice resistance has proven elusive. Yet, the candidate genes we found to underlie coho salmon's multiple resistance strategies to lice could be targeted in isolation or in combination via gene editing to confer this innate immunity to Atlantic salmon.

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DEVELOPMENT OF A MULTIPLEX SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ARRAY FOR EUROPEAN COMMERCIAL MOLLUSC SPECIES

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Introduction

Aquaculture is an age-old activity that has evolved slowly, often based on traditional knowledge and advances obtained through needs, experience or cooperation. It has expanded and developed over centuries, integrated with social, economic and cultural environment, hand by hand with scientific progress, and today supplies half of the world's fish and seafood (Yue & Shen, 2021).

The application of genetic and genomic tools has boosted aquaculture production to a higher level, which has enabled improving the main target traits for valuable commercial species. This has been accomplished both through deepening into their genomic architecture and improving genomic predictions in breeding programmes.

In this study, we present a high-density single nucleotide polymorphism (SNP) array for some of the most significant bivalve species in European aquaculture: grooved carpet-shell clam (*Ruditapes decussatus*), Manila clam (*R. phillippinarum*), Mediterranean mussel (*Mytilus galloprovincialis*) and common cockle (*Cerastoderma edule*) taking as reference their highly contiguous published or ongoing genome assemblies. The chip will help for the exploitation of genetic resources associated with traits of commercial interest along with the sustainable management of shellfish beds (Boudry et al. 2021).

One of the experiments that has been designed to validate and apply this chip has to do with a genome-wide association study (GWAS) to understand the genetic architecture of the resilience to *Perkinsus olseni* in Manila clam.

Material and methods

SNP discovery was carried out through whole-genome re-sequencing of pooled genomic DNA samples from 10 males and 10 females of each species taking as reference the published genomes of *C. edule* and *R. phillipinarum*, and new ongoing assemblies of the other *M. galloprovincialis* and *R. decussatus*. Raw data was filtered and aligned against the reference genome and the millions of SNPs called using SAMtools. The resulting list of SNPs for each species was filtered according to the following criteria: 1) coverage, 2) minor allele frequency (MAF), 3) lack of nearby genetic polymorphisms (± 100 bp).

In addition, previous candidate markers associated with productive traits, such as resilience to species-specific pathologies and sex, were included in the array together with geographical diagnostic variants, when available.

For the GWAS in Manila clam, 1000 individuals challenged with *Perkinsus* will be genotyped using the SNP chip, and 500 will be used as controls for comparison across the progression of the infection. The parasite load will be estimated in each individual using qPCR as the phenotype for parasite infection. Parasitic load will be estimated by qPCR in each individual and genotypes will be performed on the same individuals with the array to identify quantitative trait loci (QTL) associated with parasite resilience. This will provide key information to identify candidate genes for resilience and to design the best model for genomic or marker assisted selection to control this parasitosis.

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Results and Discussion

An Affymetrix Custom array (EUMOLCHIP) has been designed including 15.000 markers from the SNP list selected for each species and will be validated and applied in ongoing GWAS and population genomic screening studies across the natural distribution range of the species.

In particular EUMOLCHIP is being applied to understand the genetic architecture of resistance to perkinsosis in Manila clam through a GWAS experiment, providing key information to face the main issue for Manila clam producers.

This technology will be fundamental to promote the sustainability and competitiveness of our aquaculture in the European and global production framework, contributing to food security in the international framework of population growth.

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INFLUENCE OF TWO DIFFERENT STRESSORS (DENSITY AND RESTRICTED FEEDING) ON THE EPIGENETIC SYSTEM, THE CIRCADIAN CLOCK AND LIPID METABOLISM IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax*): THE IMPORTANCE OF THE TIME OF THE DAY

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Introduction

The circadian clock is based on genes that give positive and negative feedbacks to create a self-sustainable molecular clock that is entrained by different environmental stimuli or *zeitgebers*. The transcriptional factors that are part of the clock system can target many physiological variables like lipid metabolism, which in turn will present a 24-hour periodicity or variations during the day (Pando and Sassone-Corsi 2002, Paredes et al., 2015). This system has also mutual connections with the stress and epigenetic systems. In the first case, several stress parameters display differences during the 24 hours. At the same time, cortisol can affect the clock system by binding the glucocorticoid responsive element present on clock genes like *per2* (So et al. 2009*)*. In the second case, it has been recently proved that some epigenetic factors involved in methylation in fish display rhythms in the 24 hours (Paredes et al. 2018), but at the same time *dnmt3* is considered responsible for the methylation of the promotor of some clock genes and thus it can regulate the clock system activity. Moreover, the epigenetic system is tightly related to stress through the glucocorticoid receptors (gr) (Barlett et al. 2019). Finally, the connection between clock and methylation, suggest that feeding can exert an important role, since methylation relies on the availability of a methyl group given by diet. The aim of this study was to understand how different stressors like density and a low food ratio can affect the circadian clock, the epigenetic system and an output of the clock as the rhythms in lipid metabolism and fatty acid composition. In addition, we also aimed to understand how the time of the day can make a difference in terms of the response. Due to its importance in aquaculture and main concerns related to welfare, we selected the European sea bass (*Dicentrarchus labrax*) as the experimental model in this study.

Materials and methods

Fish were kept in an open system in a 13:11 Light/Dark cycle and were fed once during the light phase. Four experimental groups were designed based on two feeding ratios (F) and densities (D): high feeding-low density (HF - LD), high feeding-high density (HF – HD), low feeding-low density (LF – LD) and low feeding-high density (LF – HD). All groups were performed in triplicate (3 different tanks). Feeding differed between groups depending on the food ratio provided: 1.5 % for the high feeding (HF) and 0.5% for the low feeding (LF). The densities selected were 5 kg/m^3 for the low density (LD) and 50 kg/m^3 for the high density (HD). Fish were maintained under these conditions for 60 days. Samples were collected at day 0, 5, 10, 25 and 60. Each sampling day, samples were collected both at the middle of the light phase (ML) and at the middle of the dark phase (MD). At each day and time point, 3 fish from each tank (n=9 for each experimental group, 3 fish from 3 tanks per group) were collected and sampled. First, fish were anesthetized, and blood samples were collected by caudal puncture. Then, fish were euthanized, and we collected samples from brain, liver, perivisceral fat and muscle. In the liver samples we analyzed, by qPCR, the relative expression of clock genes (*clock*, *bmal*, *per* and *cry*), genes from the epigenetic mechanisms (*sirt1*, *dnmts*, *tet2*, *gadd45aa*, *mbd4*), and genes from the lipid metabolism (*cpt1a*, *ppars*, *fas*, *elvol5*, *lxr*, *srebp*) as the output signal of the clock. In addition, we evaluated the fatty acid profile of the liver, muscle, adipose tissue (perivisceral fat) and plasma by gas-liquid chromatography.

Results

We expect differences in the response of clock and epigenetic genes due to the stressor with significant differences based on how long these conditions were maintained. Moreover, due to the importance of the time of the day for the response, we expect to describe differences when comparing day and night sampling times. In the end, all these factors will impact the fatty acid composition of the body.

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FEEDING TIME EFFECTS ON EPIGENETIC MECHANISMS AND THE CIRCADIAN CLOCK IN LIVER AND BRAIN OF THE EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

Fish have evolved circadian system to synchronize to different environmental cyclic factors like light or food availability. These entraining factors can be referred as *zeitgebers* and they boosted the evolution of a self-sustainable molecular clock formed by positive and negative feedback loops. *Clock* and *bmal* represent the starting point and the positive loop; they work as transcriptional factors activating, among many genes, the negative loop formed primarily by *per* and *cry*, which in turn will work to repress *clock* and *bmal* transcription. This self-sustainable molecular system is the core of the vertebrate molecular clock, and its rhythmicity is transmitted to many physiological processes (Pando & Sassone-Corsi 2002). Even if this system is self-sustainable, several levels of regulation can be present. For instance, epigenetic mechanisms are involved not only as regulators of this system, but also as a target. Mechanisms of deacetylation and methylation seems to be crucial in this bidirectional system. In the deacetylation process, *sirt1* plays an important role (Nakahata et al. 2009) counteracting the intrinsic acetylation activity of *clock*, which is responsible for chromatin remodeling that is associated to circadian control of gene expression. DNA methylation is another process that could be involved in the regulation of the circadian system since it has been proposed that *dnmt3* might be responsible for the methylation of *bmal's* promoter (Satou et al. 2013). At the same time, the same genes involved in methylation and de-methylation have been described as rhythmic in zebrafish gonads (Paredes et al. 2018), suggesting again the double nature of the interaction between the two molecular patterns. Moreover, sirtuins and DNA methyltransferases are linked to nutrient availability (Su et al. 2016), indicating that feeding time can act on the clock mechanism not only as a *zeitgeber*, but also passing through the epigenetic way. Therefore, the aim of this study was to investigate how feeding time affects the rhythms of the circadian clock and genes of the epigenetic system in the liver of the European sea bass (*Dicentrarchus labrax*). We also focused on the hypothalamus to understand if feeding time has the potential to affect the central pacemaker.

Materials and method

Fish were kept in an open system in a 14:10 LD cycle and fed with 1% of the fish body weight, once a day. Fish activity was monitored by mean of photocells. Two groups were made based on the feeding time: mid-light (ML) and mid-night (MD) feeding. After 30 days under these conditions, fish were sacrificed in a 24-h cycle at seven sampling points after light onset: ZT 0.5, 4, 7.5, 12, 16, 20 and 24.5 h to collect liver and hypothalamus. In the liver, clock genes and genes of the epigenetic system were analyzed, while in the hypothalamus clock genes were considered. Liver was also used to investigate the presence of a rhythm in the SAM/SAH ratio, which is an index of methylation potential.

Results

In the liver, the epigenetic genes analyzed (*sirt1, dnmt1, dnmt3a, gadd45aa, mbd4* and *tet2*), presented rhythm in the ML group, but only *dnmt3a* conserved the rhythm also in the MD group. Additionally, all the acrophases were nocturnal. The analysis of the clock genes revealed that all of them (*clock, bmal1, per1, per2, cry1* and *cry2*) presented rhythms in the ML group, while only for *per2* the rhythm was maintained in the MD group. Finally, rhythms were found in the hypothalamus for the clock genes both for ML and MD groups, except *cry2*, where no rhythm was found. Concerning fish activity, both the groups displayed a diurnal activity.

Discussion and Conclusions

In the liver, the feeding time had a strong impact on the clock genes, since when the fish were fed in MD the rhythm disappeared with only one exception (*per2*). Moreover, when comparing ML with MD mean expression, in most of the cases they displayed a significant difference in most of the ZT points, demonstrating that also transcriptional levels are affected by feeding time. *Sirt1* was rhythmic in ML group, but not in MD group and the differences between the two groups were significant in most time points. The explanation could be related to the availability of its cofactor, NAD+, that could

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have been affected by feeding time as well. *Dnmt1* seems to be more affected by the feeding time than *dnmt3a*, since it presents rhythm only in ML group. This can suggest that the maintenance of the pattern of methylation in the cells could be affected as well by feeding. Also, the genes involved in de-methylation process present the same pattern observed for *dnmt1*, since the rhythm was only present in ML group. Moreover, comparing ML with MD group, is clear that feeding time has affected the transcriptional levels as well. In the hypothalamus, most of the clock genes analyzed displayed rhythm both in the ML and MD groups, and this is consistent with other data in fish that confirm that light is the main synchronizer in central tissues like the brain. However, when the two groups are compared, significant differences are present between the same ZT points. For this reason, it's not possible to exclude an important effect of feeding time in the central pacemaker. Since both groups were diurnal, and most of the genes displayed a nocturnal achrophase, we can speculate that during the resting phase fish prepare the factors that are involved in the connection between clock system and epigenetic machinery. To better understand the connection between clock system and epigenetic mechanism, our research is currently focusing on the analysis of the methylation potential, to understand if methionine metabolism (1-C cycle) can be connected as well with the clock system.

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NATURAL SPAWNING AND LARVICULTURE OF *Holothuria grisea*: A NEW CANDIDATE FOR MARICULTURE IN THE WESTERN ATLANTIC COAST

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Introduction

The control of hatchery protocols for seed production of Holothurian species is key for successful aquaculture, ocean ranching, and stock enhancement of these endangered species. Therefore, in this study we present the results of broodstock collection in the wild, transport to the hatchery, conditioning for natural spawning, and embryonic and larval development of *Holothuria grisea*.

Materials and Methods

Adult *H. grisea* (n=72) were collected from the wild, off the Brazilian Atlantic coast (27.8° S – 48.6° W) during the Spring (2022). They were placed in a container equipped with pure oxygen supply and transported in a pickup truck for 800 Km. On arrival at the hatchery in Rio Grande (Brazil), they were separated into three tanks in a RAS. During five months the sea cucumber were kept along with juvenile *Pogonias courbina* (Sciaenidae) and allowed to feed on fish biodeposits and waste feed.

After five months, the sea cucumbers and fish were transferred from these tanks to another RAS, with the same water quality. Two days after transfer, we observed broodstock releasing gametes and less than 1h after that, fertilized eggs were collected from the tank through a sieve (80μm). Eggs were incubated in cylindric-conical tanks using a semi-static system, larviculture was performed in the same tanks. Larvae were fed on microalgae (*Chaetoceros calcitrans*). Glass plates colonized with the same diatom were placed in the larviculture tanks once the late auricularia stages were observed. The juveniles were later transferred to flat bottom tanks. Embryonic, larval, and early juvenile development were followed using photography taken under a microscope.

Results

Adult individuals kept in a RAS with juvenile *P. courbina* (Sciaenidae) spawned within a couple of days after they were stimulated by disturbance and movement to new tanks.

The first cleavage was observed approximately 1h after the gametes were released in the broodstock tanks (Figure 1 B). Gastrula were first observed around 30h after fertilization (Figure 1 E). Two days after hatching the early Auricularia stage was first observed (Figure 1 F). The first hyaline spheres were observed 28 days after fertilization (Figure 1 I)

Abundance of larvae decreased with development towards the late auricularia stage. After reaching the doliolaria and pentactula stage, larvae assumed demersal behavior and were no longer available in the water column. Early juveniles were observed in the glass plates placed in the larviculture tanks (Figure L).

Five months after fertilization, juvenile length achieved between 1-2 cm. Their color is yellow, and they are successfully fed on organic debris siphoned out of juvenile *P. courbina* rearing tanks.

Discussion and conclusion

The results presented here show *H grisea* can spawn in captivity and the fertilized eggs can develop into juveniles using techniques common to other Holothuriidae species. Adult sea cucumbers kept in a RAS with juvenile *P. courbina* successfully spawned. Fertilized eggs were successfully collected and transferred to an incubator in a semi-static system. Auricularia larvae fed on microalgae (*C. calcitrans*) and developed to the juvenile stage.

The description of these breeding techniques is the first step to the introduction of a new species of sea cucumber to aquaculture, allowing diversification of mariculture operations in the Atlantic Ocean, with focus on low trophic species.

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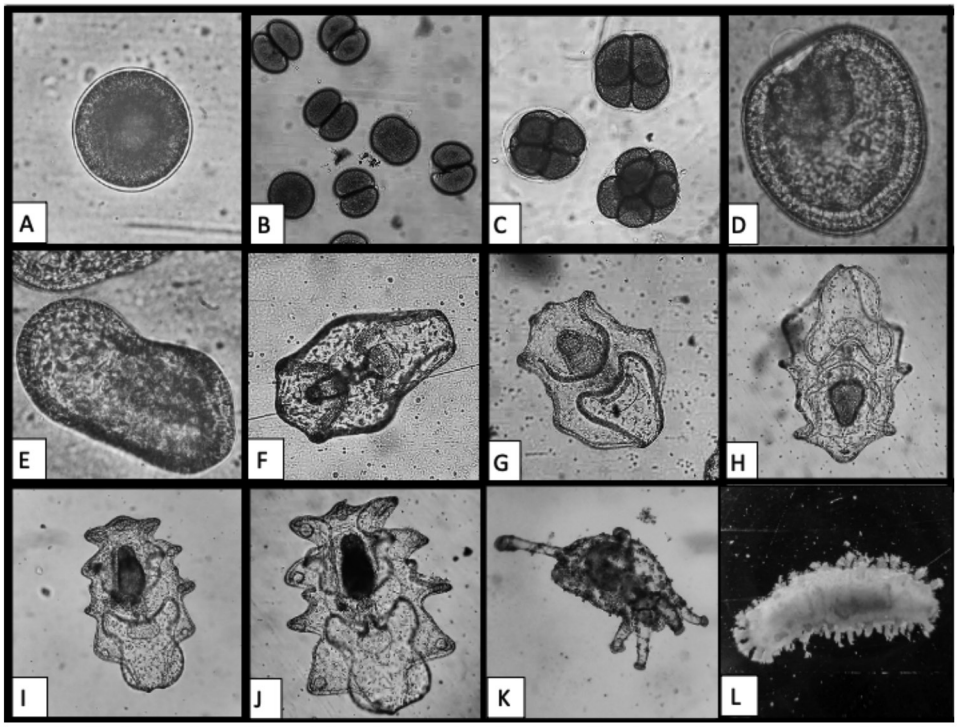

Figure 1 – Embryonic and larval development to the juvenile stage of sea cucumber *Holothuria grisea*. A: fertilized egg; B-C: cleavage stages; D: blastula; E: gastrula; F: early auricularia; G-H: mid auricularia; I-J: late auricularia; K: pentactula; L: juvenile.

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CAN STIMULATING MUCUS PRODUCTION REDUCE VIRAL INFECTION IN FARMED FISH? BACKGROUND: SAV AND ISAV CAUSE FISH DEATH AND ECONOMIC LOSSES

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Virus infection and disease outbreaks are a massive problem in aquaculture. Salmonid alphavirus (SAV) and infectious salmon anemia virus (ISAV) causes economic losses as well as welfare and sustainability issues in farmed fish. To enter their hosts, fish viruses must cross mucosal barriers. This includes skin, intestines, and gills, and the latter is identified as the primary entry site [1]. Mucus is known to provide protection against pathogens, both physically and chemically [2].

...Enter: Substance X! We hypothesise that Substance X has the potential to increase mucus production, and thus decrease viral infection levels in salmonid cells.

Methodology: In vitro experiments
We will perform basic research on substance X through in vitro experiments with two salmonid cell lines: RT-gillW1 (gill) and ASK (kidney). Cells will be infected with SAV (subtype 3) and ISAV. Effects on cellular responses and virus dynamics will be evaluated with and without the presence of Substance X.

Workflow: Our research design in three steps

1. Expose salmonid cells to four different treatments
   - Control
   - Virus
   - Substance X
   - Substance X and virus
2. Harvest cells at different timepoints
3. Analysis:
   Microscopy: Dye cells for mucins. Do the cells produce more mucus?
   RT-qPCR: Virus dynamics. How much virus is taken up in the cells?
   RT-qPCR: Cellular responses. RT-qPCR Do we see a higher expression of mucus-related genes, or other interesting changes?

In the future...
We hope that we are able to elucidate the mechanisms and potential usefulness of Substance X. Work package 2 in the project will perform in vivo trials with farmed fish.

(Disclaimer: Unfortunately, at this stage of the project, we cannot reveal the chemical name of Substance X. Stay tuned for results.)

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Can stimulating mucus production reduce viral infection in farmed fish?

BACKGROUND

SAV and ISAV cause fish death and economic losses

Virus infection and disease outbreaks are a massive problem in aquaculture. Salmonid alphavirus (SAV) and infectious salmon anemia virus (ISAV) causes economic losses as well as welfare and sustainability issues in farmed fish.

To enter their hosts, fish viruses must cross mucosal barriers. This includes skin, intestines, and gills, and the latter is identified as the primary entry site [1]. Mucus is known to provide protection against pathogens, both physically and chemically [2].

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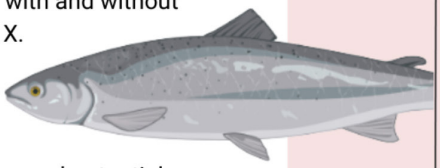
We hypothesise that Substance X has the potential to increase mucus production, and thus decrease viral infection levels in salmonid cells.

METHODOLOGY

In vitro experiments

We will perform basic research on substance X through *in vitro* experiments with two salmonid cell lines: RT-gillW1 (gill) and ASK (kidney).

Cells will be infected with SAV (subtype 3) and ISAV. Effects on cellular responses and virus dynamics will be evaluated with and without the presence of Substance X.



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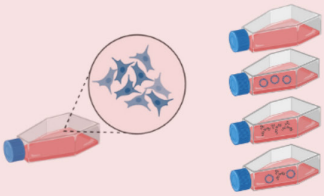
WORKFLOW

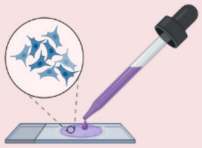
Our research design in three steps

1 Expose salmonid cells to four different treatments



2 Harvest cells at different timepoints

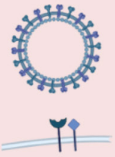
3 ANALYSIS



Dye cells for mucins

MICROSCOPY

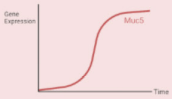
Do the cells produce more mucus?



Viral dynamics

RT-qPCR

How much virus is taken up in the cells?



Cellular responses

RT-qPCR

Do we see a higher expression of mucus-related genes, or other interesting changes?

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DIETARY *Debaryomyces hansenii* PROMOTES FISH PERFORMANCE, INTESTINAL CONDITION AND SKIN DEFENSE IN A MARINE FISH MODEL

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Introduction

The development of a sustainable business model with social and consumer acceptance makes it necessary to develop new strategies and tools to guarantee the growth, health, and well-being of farmed animals. In this context, the introduction of new ingredients and feed additives has managed to improve not only the productive performance but also improve the sustainability in the production of feed. In the recent years, the use of yeasts has shown to be a good health modulator and growth enhancer. However, these probiotic effects depend on several factors like the type of species, sources, and feed level inclusion. Among the most used species, *Debaryomyces hansenii* (CBS 8339) is an efficient probiotic in marine fish aquaculture. Among its reported properties are: i) immunostimulatory effects, ii) gut microbiota modulation, iii) enhanced cell proliferation and differentiation, and iv) enhancement of the digestive function. However, less is known about its effects on mucosal health, and specifically on the gut and skin mucosa, the main defensive tissues of fish.

Materials and methods

- Diet composition

To evaluate the effects of *D. hansenii* as a probiotic in aquafeeds, two diets were formulated with similar levels of protein content (48.4% crude protein), lipid content (17.2% crude fat), and energy levels (21.7 MJ/Kg gross energy). The diets were formulated with low levels of fishmeal (7%), which only differed in the inclusion of *D. hansenii* (CBS 8339) at an inclusion rate of 1.1% (17.2 x 10^5 cfu). *D. hansenii* was provided by CIBNOR (La Paz, Mexico) and grown as described in Tovar et al. 2021.

- Experimental Model and Design

A total of 500 *S. aurata* juveniles (body weight, BW= 14.5 ± 1.2 g; mean ± SD) were obtained from a commercial farm (PISCIMAR S.L.; AVRAMAR, Burriana, Spain), and acclimated in in two tanks of 2,000 L connected to a IRTAmar® recirculating system under natural conditions. The trial lasted 70 days, 200 fish (25 fish per tank; 4 tank replicates per diet), during which fish were fed at 3.5% of the stocked biomass by means of automatic feeders, monitoring the temperature, salinity, and uneaten pellets daily, and the nitrogen-derived compounds weekly.

- Transcriptional Analysis

Total RNA from the anterior-mid intestine and skin of nine randomly selected fish per dietary treatment (n = 3 fish per tank) was extracted and analysed as described in Reyes-Lopez et al., 2021. Microarray Hybridization and Analysis from both experimental groups was carried out using the Aquagenomics Sparus aurata Oligonucleotide Microarray v2.0 (4 × 44 K) (SAQ) platform, and detailed information and transcriptomic raw data are available at the Gene Expression Omnibus (GEO) public repository at the US National Center for Biotechnology Information (NCBI), accession numbers GPL13442, and GSE162504, respectively.

- Statistical analysis

Data on growth and feed performance indicators are expressed as mean ± standard deviation. Following confirmation of normality and homogeneity of variance, an ANOVA was performed followed by the Tukey's multiple range test when statistically significant differences were detected among experimental groups ($p < 0.05$).

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Results and Discussion

After 70 days of a nutritional trial in which a diet with low levels of fishmeal (7%) was supplemented with 1.1% of D. hansenii (17.2×10^5 CFU), an increase of ca. 12% in somatic growth was observed together with an improvement in feed conversion in fish fed a yeast-supplemented diet. Considering that feeding costs represent up to 50%–70% of total production costs in intensive fish farms (Rana et al., 2009), and fishmeal being amongst the most expensive raw materials in aquafeeds (Tacon et al., 2008), the nutritional strategy tested in our study with the supplementation of a live probiotic seemed very promising when KPIs related to growth and feed utilization were considered. In addition to those findings, the microarrays-based transcriptomic analysis found 232 differential expressed genes (DEGs) in the anterior-mid intestine of *S. aurata* (mostly related to metabolic, antioxidant, immune, and symbiotic processes), and 712 DEGs in the skin (mostly related to metabolism and immunity, mainly with a specific barrier function). Angulo et al. 2020 reported that *D. hansenii* supplementation promote gut protection in several aquatic and terrestrial species at immunological and gene expression levels, which agree with our results in terms of gut and skin gene expression. In that sense, it has been postulated that *D. hansenii* might modulate host's immunity through its wall-related β-glucan, mannan and polyamine content, increasing functional and decreasing deleterious immune responses in fish (Reyes-Becerril et al., 2011; Fernández-Montero et al., 2019).

Thus, these results support the dietary administration of *D. hansenii* as an excellent tool to promote the gut and skin mucosa defensive capacity, the first line of fish defense against environmental and biotic challenges, when included in low fish meal based diets for *S. aurata.*

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BIOCHAR: A PROMISSING ADDITIVE TO IMPROVE FEED EFFICIENCY AND GROWTH MODIFYING INTERMEDIATE METABOLISM IN PRE-FATTENING OF SENEGALESE SOLE

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Introduction

Nowadays aquaculture provides animal protein of quality for human consumption, necessary to maintain the high world population, thus supporting fisheries production constrained by overexploitation of fishing grounds (FAO, 2022). However, it is important to produce it in a cost-effective and sustainable way. Since 2012, biochar (i.e., activated carbon) is starting to be included in animal feed (Gerlach & Schmidt, 2012). Biochar is obtained by pyrolysis from organic waste in a low-to-no oxygen thermal process, reusing by-products from other industries (European Biochar Foundation - EBC, 2012). This product has been shown to improve nutrient intake efficiency, absorb toxins and generally improve animal health besides improving the quality of the effluent water (O'Toole et al., 2016; Toth and Du, 2016; Schmidt et al., 2019), making it a good candidate for its inclusion in aquafeeds. This study aims to test the putative improvement produced by the dietary inclusion of biochar in Senegalese sole, a species of great interest in Spain.

Material and Methods

A total of 135 Senegalese sole juveniles (initial body mass, ~41.13 g) were distributed in an open system circuit with 9 tanks of 500 L, which constituted the 3 experimental groups (in triplicate): i) Control group (CT) fed with a control diet mimicking the commercial formulation for this species; ii) group fed 0.5% Biochar (B0.5), introducing 5 g biochar/kg control feed and iii) group fed 2% Biochar (B2), introducing 20 g biochar/kg control feed. After 180 days of the feeding trial, a biometric sampling was done, and samples from plasma, liver and muscle were taken. Somatic and zootechnical indices were calculated, and samples from each tissue were analysed.

Results and Discussion

No significant differences were observed in the final body mass among experimental groups, although B2 group showed a clear a positive trend towards related to the significantly higher feed efficiency obtained. Therefore, it can be suggested that in a longer feeding trial, greater growth could be obtained in the group with the highest inclusion of biochar (B2), as obtained in previous studies in other species, indicating a growth performance improvement due to increased feed efficiency (Lan et al., 2018). In terms of organosomatic indices, only the intestine length index (ILI) showed different, with a dose-dependent decrease, being significant in the B2 group respect to the control fish. These results are in controversy with other studies with low inclusion of nutraceuticals derived from microalgae, which obtained an increase in the intestine length by increasing the dose, possibly due to lower digestibility of the compounds (Perera et al., 2020; Molina-Roque et al., 2022). However, biochar seems to facilitate the digestive processes, producing an intestine shortening, and agreeing with other authors who suggested that biochar could facilitate the formation of biofilms as habitat for gut microbiota which could be the reason for the improved growth rates (Lan et al., 2018; Schmidt et al., 2019). Regarding intermediary metabolism, the results showed a reduction of all plasmatic metabolites with the biochar inclusion (even at the lowest inclusion), suggesting a decrease in plasma metabolite mobilization or even an increased metabolite consumption. Respect to energy reserves, fish fed biochar-supplemented diets maintained homeostatic levels in the liver, but not in the muscle, which may be associated with the higher (but physiological) levels of cortisol detected, also in a dose-dependent manner, causing energy mobilization from this tissue and not allowing its accumulation in the muscle. This hypothesis also explains the dose-dependent increase in muscle lactate by glycolysis, which could be transported to the liver for acting as a lactate sink (Milligan and Girard, 1993).

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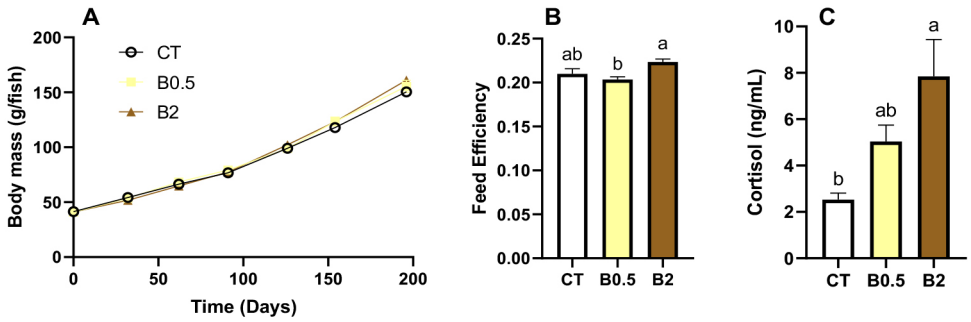

Fig. 1. Evolution of body mass (A), feed efficiency (B) and plasma cortisol (C) in *Solea senegalensis* after 180 days of feeding experimental diets. Results are the mean ± SEM (n = 12 fish). Different letters indicate significant differences among treatments based on one-way ANOVA and Tukey's test ($p < 0.05$).

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DIFFERENT WATER SALINITIES IMPACT MICROBIOTA DYNAMICS OF RAS SYSTEM AND BARRAMUNDI (*Lates calcarifer)* DIGESTA UNDER TEST DIETS SUPPLEMENTED WITH THE MARINE MICROALGAE *Microchloropsis gaditana*

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Introduction

Barramundi (*Lates calcarifer*) is a euryhaline fish species considered as an ideal model for optimizing aquaculture production based on resource availability [1]. Its adaptability to a wide range of water salinity is attributed to various evolutionary and physiological adaptations [2], that are expected to be accompanied also by changes in the host-environment microbiota [3]. In this study, our objective was to assess the extent to which different water salinities can influence both environmental and fish microbial communities, focusing on potential changes induced by the supplementation of *Microchloropsis gaditana* in the test diets.

Materials and Methods

The trial was carried out in two independent recirculating aquaculture systems (RAS) of 6X500L tanks each at AquaBioTech Group. Asian seabass, *Lates calcarifer* (50 fish/tank; initial weight of 64.9 ± 0.1g) were reared at two different salinities, 38+/-2ppm (S1) and 14 +/- 2ppm (S2). Fish in both systems were fed four times daily to satiation with either a control diet (CTRL) or the CTRL diet supplemented with *M. gaditana* liquid extract top coated at 3% (W/V) (ALG). Samples of fish digesta, sludge, surrounding and inlet water, and the biofilter were collected at the beginning of the trial, after 5 weeks and at the end of the growth phase (10 weeks) and characterised for bacterial populations. Bacterial diversity was assessed through 16S rRNA sequencing. Raw data were processed with QIIME2 and taxonomy analyses performed with R software.

Results

The characterisation of the samples showed that water salinity significantly impacted the environmental and fish microbiota composition, independently of the diet applied (Fig. 1). Environmental bacterial communities were observed to exhibit an increase of different potential probiotics genera depend on the salinity, being the genus *Ruegeria* increased under the S2 and the genus *Pseudoalteromonas* under the S1. Member of these genera were characterized by their antagonistic effect against *Vibro* pathogenic strains and their involvement in protein utilization. Albeit the bacterial communities within the digesta exhibited differences from those in the environment throughout the experiment, an increase in the *Ruegeria* genus was also observed in fish subjected to S2 conditions. Nevertheless, the *Mycobacterium* or *Legionella* genera were found to be significantly increased in the same fish stock under S2 conditions. Both of these genera contain high pathogenic strains for fish and humans (e.g. *Mycobacterium marinum, Mycobacterium tuberculosis, Legionella pneumophila*). In terms of growth, the variation in salinity, diets and microbiota composition did not show a significant impact.

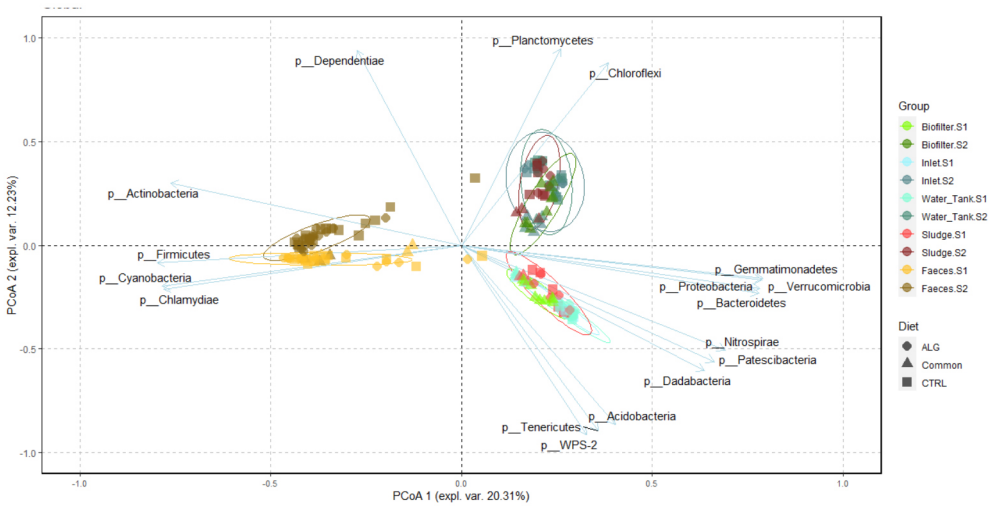

Fig 1. Beta diversity Principal Coordinate Analysis (PCoA) computed upon the Bray-Curtis index. Samples have been colored and grouped by the salinity condition; different shapes were used in terms of the diet utilization. Significant correlated phyla (p < 0.001) with bacterial communities changes were represented by arrows.

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Conclusion

This aquaculture microecology research provided has offered comprehensive insights into the independent dynamics of the barramundi digestive tract and the microbiota associated within the RAS system. Despite being strongly affected by the water salinity level, the microbiota, which did not experience significant changes under the *M. gaditana* supplemented diet, has no measurable effects on barramundi growth. Further studies on the dynamics between probiotics and potential pathogenic strains in waters with widely varying salinities should be conducted in order to ensure the safety of culturing euryhaline species.

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DISCRIMINATION OF LOWER FEED INTAKES UNDER HIGH STOKING DENSITIES: A CASE STUDY ON GROWTH PERFORMANCE, METABOLISM AND WELFARE

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Introduction

In aquaculture, as in others economic activities, the main objective is to obtain the maximum possible economic benefit. For this reason, several studies have focussed on the influence of an increase in density, considered as a stressful situation, on the growth and physiology of farming specimens (*e.g.* Montero et al., 1999 in *Sparus aurata*; Laiz-Carrion et al., 2012 in *Pagrus pagrus*). It is known that stress situations cause the activation of the HPI axis with the release of cortisol as the end product (Schreck and Tort, 2016). If this situation is maintained, it will lead to chronic stress that will decrease welfare, and for instance also appetite, feed efficiency and feed conversion ratios (Arends et al., 1999; Montero et al., 1999; de las Heras et al., 2015; Skrzynska et al., 2018). In this sense, it is known that crowding situations cause these responses in terms of performance, but it is unknown how much stress is related to this decrease in feed intake and how much to crowding *per se*. Our study proposes to determine the effect of stocking density in terms of growth, metabolism and welfare, discriminating the metabolic orchestration derived from the reduction in feed intake.

Material and Methods

A total of 371 gilthead sea bream juveniles (initial body mass, ~ 31.3 g) were distributed in an open system circuit with 9 tanks of 400 L, constituting 3 experimental groups (in triplicate): i) Low Stocking Density (LSD) fed *ad libitium* with an initial density of 3.6 Kg/m^3; ii) High Stocking Density (HSD) fed *ad libitium* with an initial density of 38 Kg/m^3 and iii) Low Stocking Density Pair Feed (LSD-PF) with the feed intake of HSD fish set at LSD. After 90 days of the feeding trial, a biometric sampling was done, and samples from plasma, liver and muscle were taken. Somatic and zootechnical indices were calculated, and samples from each tissue were analysed in terms of metabolism and welfare.

Results and Discussion

Significant differences were observed in the final body mass of fish between treatments, being statistically lower in the HSD group from the third week of the experiment. The condition factor (K), feed efficiency (FE), weight gain (WG) and specific growth rate (SGR) were also lower in this group. However, LSD-PF group maintained the same growth potential as LSD with less feed conversion rate (FCR) and feed intake, thanks to an improvement in feed efficiency, as observed in previous studies, indicating a growth performance improvement by optimizing digestive processes (de las Heras et al., 2015). In terms of somatic indices, only the hepatosomatic index (HSI) presented significant differences, with a decrease in HSD group respect to LSD, related to the lower hepatic glycogen reserve (see below). A metabolic orchestration, due to the lower feed intake situation, were observed in the HSD and LSF-PF groups, which could be related with i) a decrease in plasmatic mobilization of metabolites, ii) the major consumption of them, or even iii) after reaching a homeostatic load during the medium/long-term feeding trial (TAG, cholesterol and proteins). Regarding to energy reserves, fish under HSD condition maintain homeostatic levels of hepatic and muscle TAG, but not in hepatic glycogen, which may be associated with the increased levels of cortisol detected (although not significant) that could cause energy mobilization and stimulating mainly carbohydrate metabolism to cope with stress. This trend also explains hepatic lactate decrease in HSD group, as it has been demonstrated to be used to produce energy by gluconeogenesis in the liver through the Cori cycle (Perera et al., 2020). Therefore, with the present experimental approach we can suggest that the metabolic modifications (and growth performance) that occur under high stocking densities previously demonstrated (*e.g.* Skrzynska et al., 2018), are mainly produced by crowding chronic stress, but with a certain contribution of decreased feed intake.

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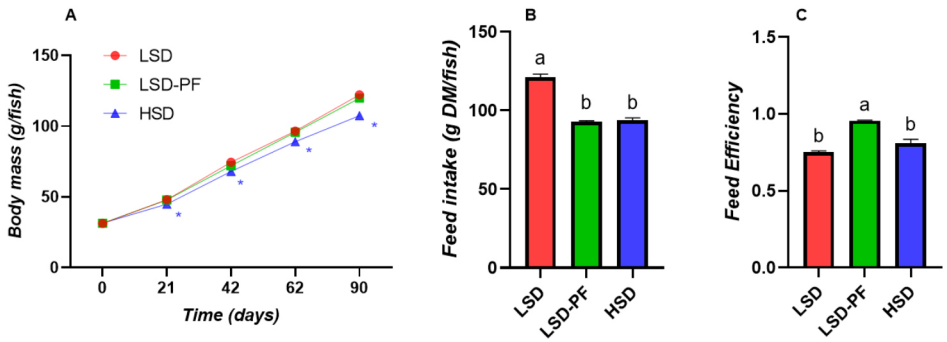

Fig. 1. Evolution of the wet weight (A), feed efficiency (B) and feed intake (C) of gilthead sea bream juveniles for 90 days. The results are expressed as the mean ± SEM (n = 12 fish). Asterisk and different letters indicate significant differences among treatments based on one-way ANOVA and Tukey's test ($p < 0.05$).

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SALMON HYDROLYSATE AS A PROTEIN SOURCE FOR ATLANTIC SALMON (*Salmo salar*)

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Introduction

Finding novel sustainable feed ingredients for the aquaculture industry is crucial to reduce the climate footprint and increase the production volumes in this industry. Marine by-products such as viscera, head, skin, backbone and cut-offs from fish are local and sustainable raw materials for feed production. Enzymatic hydrolysis of by-products facilitates use of hydrolyzed proteins as feed ingredients for the same species as it came from (Council Regulation (EC) No 142/2011, 2011). The aim of this study was to partially replace fish meal with salmon hydrolysate in feed for post smolt Atlantic salmon, and evaluate the effects on growth, digestibility and gut health. Salmon hydrolysate was also characterized and analyzed for prion content by proteomics.

Materials and methods

Salmon protein hydrolysate was produced by Nutrimar AS by enzymatical hydrolysis of salmon by-products followed by separation, concentration and spray drying of the liquid hydrolysate. The high molecular weight peptides in the hydrolysate was concentrated by dialysis (10 kDa cut-off) and analyzed for salmon prion protein content by targeted and shotgun proteomics analysis with LC-MS/MS. A feeding trial with Atlantic salmon (*Salmo salar*, Rauma strain, mean weight 141.5 g) post smolts was conducted to test isonitrogenous diets with different levels of salmon hydrolysate (FPH) replacing fish meal (FM) protein. The control diet with 30 % FM was compared with test diets containing 20 % FM with 9 % FPH (FPH-09), and 10 % FM with 18 % FPH (FPH-18). Atlantic salmon were fed until doubling of weight before collecting intestinal samples for histological evaluation of gut health as described in (Nordvi et al., 2023). Digestibility was examined by stripping feces and analyzing for protein, fat and ash content compared to inert yttrium oxide added to the diets.

Results and discussion

Proteomics analysis of the salmon hydrolysate used in this feeding trial did not detect any salmon prions. Salmon protein hydrolysate (FPH) increased the initial specific growth rate (SGR) significantly compared to the FM control diet (Figure 1). This is probably due to attractant effect of free amino acids and peptides that stimulate feed intake (Kousoulaki et al., 2018). The FM fed salmon showed compensatory growth by having the highest SGR in the second growth period (day 25-58). The weight difference was not significant after 58 feeding days but the stimulatory effect on feed intake is still industrially relevant for the sea-transfer phase and other changes in feed during fish farming.

The salmon hydrolysate amino acid profile is excellent for salmon in addition to being highly digestible as peptides. The apparent digestibility of protein was significantly higher in the FPH-18 diet compared to the other diets (Table 1), likely due to the free amino acids and peptides in the salmon hydrolysate (Hevrøy et al., 2005). Interestingly, the apparent ash digestibility increased linearly ($p < 0.001$) with increasing FPH content (Table 1). Histological analysis of midgut and hindgut from salmon fed the FM and FPH-18 diets did not reveal any significant differences between the diets, and the intestines looked normal and healthy.

Conclusion

Salmon hydrolysate is a promising novel feed ingredient for Atlantic salmon, due to its high protein digestibility and attractant effect that stimulates feed intake and growth. Atlantic salmon fed 9 % and 18 % salmon hydrolysate showed higher initial growth rate compared to a 30 % fish meal control diet in this feeding trial. Including 18 % hydrolysate in the diet significantly increased the protein digestibility, and the ash digestibility increased linearly with the hydrolysate inclusion level. Morphological analysis of gut samples revealed no significant differences among the diets. No salmon prions were found in the hydrolysate used in this feeding trial.

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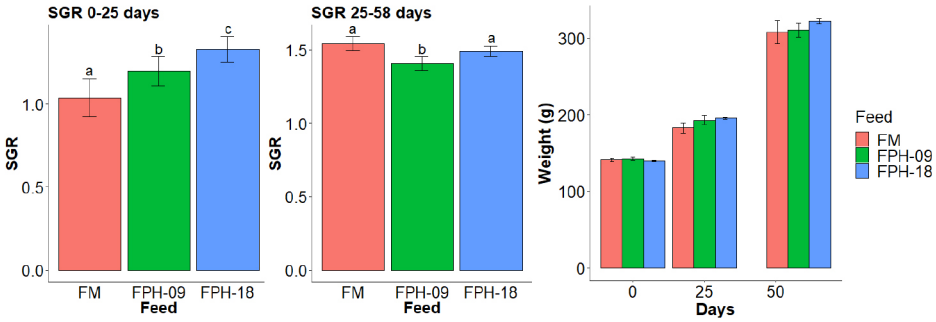

Figure 1: Mean (n = 3) specific growth rate (SGR) in the first 25 feeding days (left) and last feeding days (middle). Mean weight (g) as a function of feeding days (right) for the different feeds: Fishmeal (FM) control diets, 9 and 18 % salmon protein hydrolysate (FPH-09 and FPH-18, respectively). The SGR calculations are based on individual fish (that were PIT tagged). The error bars represent standard error (SEM) and the significance is presented as different letters based on p-value 0.05 from a nested One-way ANOVA analysis.

Table 1: Apparent digestibility coefficients (ADC, %) of the dietary protein, fat and ash using yttrium oxide as inert marker.

Diet	**FM**	**FPH-09**	**FPH-18**	**ANOVA**
ADC_Protein	87.3 ± 0.4 [a]	88.0 ± 0.4 [a]	90.0 ± 0.4 [b]	**
ADC_Fat	93.4 ± 0.4	93.5 ± 0.3	94.1 ± 0.6	
ADC_Ash	8.2 ± 1.3 [a]	16.4 ± 1.3 [b]	24.9 ± 2.0 [c]	***

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CORONARY LESIONS IN SALMONID FISH - IMPACT ON ENVIRONMENTAL AND AQUACULTURE STRESS TOLERANCE

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Background

Some fishes including salmonids have a coronary circulation that supplies the outer compact myocardium of the heart with fully oxygenated blood. This arterial blood supply complements the luminal venous O_2 supply that delivers relatively poorly oxygenated blood to the inner spongy myocardium. The role of the coronary circulation may be of particular relevance in farmed salmonid fishes, as our group (and others) have shown that they more frequently develop lesions and arteriosclerosis in the main coronary artery; a condition that narrows the vessel lumen and may decrease blood flow to the myocardium (Brijs *et al,* 2020; Farrell, 2002). This condition can, in turn, reduce overall cardiac and metabolic performance, and possibly the tolerance to environmental stressors that incur an elevated metabolic challenge. There is also a growing body of evidence demonstrating that coronary lesion development is positively correlated with factors promoting rapid growth in the smolt production phase (*e.g.*, supra-optimal rearing temperatures; Brijs *et al,* 2020), and may be a key factor underlying adult fish mortality in aquaculture (Poppe *et al,* 2021). Yet, it is still not fully understood how coronary blood flow affects overall cardiorespiratory performance. Moreover, its functional role in setting tolerance limits to environmental extremes such as heat waves and hypoxia, and how it affects resilience to stressful metabolic challenges in aquaculture, has not been extensively studied. Thus, to analyze the functional significance of coronary oxygen delivery in salmonids, we measured *in vivo* coronary blood flow under various thermal regimes in male and female rainbow trout (Ekström *et al*, 2017). In an additional series of experiments, we surgically ligated the coronary artery to mimic extreme coronary arteriosclerosis and explored its impact on cardiovascular performance and metabolic scope (Ekström *et al*, 2017; Morgenroth *et al*, 2021), as well as hypoxia and warming tolerance limits (Ekström *et al*, 2019; Morgenroth *et al*, 2021).

Material and methods

Fish were anesthetized in aerated freshwater (10°C) containing buffered MS-222. In one experiment, the coronary artery was dissected free and a Transonic transit time blood flow probe was placed around the vessel. In other experiments, the vessel was exposed and either ligated with a silk suture or left intact resulting in surgically ligated and sham operated experimental groups, respectively. In some experiments, sham and ligated fish were additionally instrumented with a flow probe around the ventral aorta to measure cardiac output or custom-made subcutaneous electrodes to record the electrocardiogram (ECG) and heart rate. Fish were typically left to recover from surgery for 24-48 h in respirometers to record oxygen consumption rate and then exposed to various metabolic and environmental challenges including exhaustive exercise, acute warming and aquatic hypoxia.

Results and Discussion

Routine coronary blood flow at 10°C was markedly higher in females than males (0.56±0.08 vs. 0.30±0.08 ml min^{-1} g^{-1} ventricle). Warming increased coronary blood flow in both sexes until 14°C, at which it peaked and plateaued. This meant that the scope for increasing coronary blood flow during warming was 101% in males, but only 39% in females. While coronary ligation reduced routine stroke volume relative to sham operated trout with intact coronaries, cardiac output and standard metabolic rate was maintained via an increase in heart rate. However, coronary ligated trout had reduced maximum oxygen consumption rate and aerobic scope when subjected to a 5 min exhaustive exercise protocol, and they had a markedly reduced ability to increase stroke volume to maintain cardiac output when bradycardia developed during acute hypoxia exposure. This was associated with a slightly reduced hypoxia tolerance in coronary ligated trout. During acute warming, coronary ligation caused cardiac function to collapse at lower temperatures and significantly reduced the overall acute warming tolerance relative to sham operated trout with intact coronaries.

Conclusion

Collectively, our findings show that coronary perfusion improves cardiac O_2 supply and overall cardiorespiratory function at environmental extremes, which benefits tolerance to environmental and anthropogenic metabolic challenges. The aquaculture industry should, therefore, consider the potentially detrimental effects of rapid smolt growth rate on coronary health and functionality, as this may result in raising fish that are more susceptible to environmental and metabolic stressors in challenging farm environments. In light of these findings, we are currently conducting several new experiments to shed light on how juvenile rearing conditions subsequently impact cardiac and coronary health of the adult fish.

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EFFECT OF AN HERBAL AND MINERAL PREPARATION ON ZEBRAFISH SWIMMING BEHAVIOR AS AN INDICATOR OF SEDATION STATUS

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Introduction

Fish sedation may help to secure fish health, welfare, preventing damages and negative side effects, when carrying out some farming procedures such as fish selection, transportation, vaccination and fish handling. To supply a slight sedation, or synthetic and natural (plant-based) sedative can be used. For aquaculture industry, the use of plant-based sedative has become a suitable alternative to the synthetic product, being more appreciated by the consumers and potentially claiming a reduced environmental impact (Aydın & Barbas, 2020). Lemon balm (*Melissa officinalis*) is well-known for its pharmacological effects. It contains different classes of phytochemicals, such as polyphenolic compounds which have many biological properties, including antioxidants, anti-anxiety, antidepressant and neuroprotective properties. Moreover, this plant affects the nervous system decreasing the symptoms of neurological disorders such as sleeping disorders, stress, anxiety, and irritability (Petrisor et al., 2022). Among mineral feed additives, $MgCl_2$ is use as relaxant for research and commercial applications because it acts as a calcium channel blocker, preventing calcium ions (Ca^{2+}) from entering the cells and blocking the release of acetylcholine (Azizan et al., 2021). Also, zebrafish (*Danio rerio*) has emerged as a model in neurobehavioral research; there are several studies that examined zebrafish behavior and their response to different drugs and sedatives products. The aim of the present study was investigating the potential toxicity and sedative effects of a commercial blend of *Melissa Officinalis* and soluble magnesium (AFI calm prototype®, Artic Feed Ingredient AS, Norway), on zebrafish larvae and adults.

Material and methods

The experimental procedures were approved by the Animal Welfare Board of the University of Pisa and the Italian Ministry of Health (B290E.N.0F7.). The study consisted of two trials, the first carried out on zebrafish larvae and using 5 different water concentration of AFI calm prototype® (0-, 0.5-, 1.0-, 2.0-, and 4.0- ml L^{-1}). Since the 2nd day post-fertilization (dpf) onwards, the larvae were exposed to the solution and on 5th dpf tested for locomotor behavior (distance moved, velocity, movement cumulative time) using DanioVision® (Noldus©, The Netherlands). Based on the observed results, the second trial was carried out on 260 adult zebrafish. Four treatments (CTRL, CTRL-R, T1 and T2) and three different AFI calm prototype® water concentration (0-, 1.0- and 2.0- ml L^{-1}) were used. On the 3rd, 6th, 9th day of treatment, fish behavior was tested using the Novel Tank Test (NTT). Two video cameras were used for recording fish behavior from the side and the upper view. This latter camera was connected to a pc and video elaborated by Ethovision XT 16® software (Noldus©, The Netherland). Moreover, after 3 days of suspension of the treatment, all the fish were tested once again for evaluating the recovery time. Finally, for groups CTRL, T1 and T2 the same individuals were tested at each time point, while in order to highlight possible inurement test effect, for CTRL-R group naïve fish were used. Each NTT lasted 4 minutes and each minute was analyzed both individually and as a whole period.

Figure 1a, 1b and 1c: Zebrafish larvae behavior performances of the different treatments

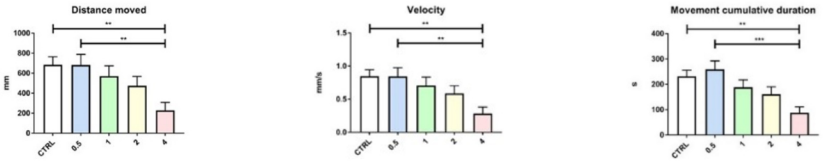
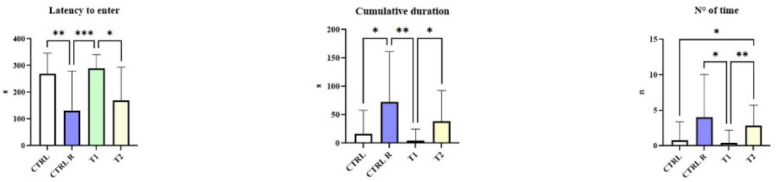


Figure 2a, 2b, 2c: Zebrafish adult behavior performances of the different treatments



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Results

The test carried out on zebrafish larvae showed toxicity effect and reduced larvae survival rate only when 4.0 ml L^{-1} water concentration was used (44±32 %). Locomotor performances showed sedative effect already at 1.0 ml L^{-1}, with a dose-dependent effect (Fig. 1a, 1b, 1c). Based on locomotor performances, no sedative effects were observed on adults on 3rd and 6th days of treatment. Effects were detected on 9th day of treatment at 2.0 ml L^{-1} concentration and on 4th bin (4th minute of test) fish of the treatment T2 showed higher exploration attitude than T1 in terms of distance moved (645.80±303.48 and 332.40±359.59 cm, respectively), movement cumulative duration (45.61±18.66 and 30.37±25.16 s, respectively) and velocity (11.29±5.27 and 5.79±6.25 cm s^{-1} respectively). Moreover, at 9th day of treatment, T2 showed shorter latency time to reach the top section of the water column and spent more time exploring this section of the tank than T1 (Fig. 2a, 2b). Also, T2 entered more time the upper section of the water column than T1 and CTRL (Fig. 2c). Furthermore, at 9th day of treatment, CTRL-R showed shorter latency time to enter, and spent more time exploring the upper section of the water column than CTRL and T1 (Fig. 2a, 2b); CTRL-R, also showed more entries to the upper section of the water column than T1 (Fig. 2c). Finally, at recovery CTRL-R showed a shorter latency time to enter the upper section of the water column than CTRL and T1 (37.74±64.90, 16.86±35.10 and 14.50±18.21 s, respectively).

Conclusion

The water exposure of zebrafish larvae to 1.0 ml L^{-1} and 2.0 ml L^{-1} of the tested herbal and Mg blend, showed no negative effects on larvae survival rate and induced lower locomotor performances. Similarly, adult zebrafish exposed for 9 days to 2.0 ml L^{-1} of the tested herbal and Mg blend, showed more capacity to cope with the stress supplied through the NTT test, demonstrated also by an increased environment exploration attitude. Repeatedly supplying the NTT stressor, induced in fish a certain grade of habituation. Hence, the observed results (CTRL-R group) support the hypothesis that the exposure to the herbal and Mg blend may reduce the stressor sensitiveness of fish through the sedation effect. These results open to interesting applications in the aquaculture sector. Despite that, it might be of great interest investigating about the possible administration of the sedative compounds through the inclusion in aquafeeds.

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EFFECT OF 1,3-1,6 β-GLUCANS ON MACROPHAGE ACTIVATION STATUS FOR THEIR USE IN AQUACULTURE

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Introduction

β-glucans are polysaccharides extracted from bacteria, fungi, yeast, algae, and plants. They have several biological activities and function, including immunomodulation, antioxidation and metabolic regulation (Ma et al., 2021; Liu et al., 2022). To promote fish health and reduce the use of antibiotics and/or other drugs, immunostimulant dietary supplementation has been proved a powerful tool (Liu et al., 2022). In mammals, β-glucans immunomodulatory effects are largely studied, and more recently particular attention has been given to the use of β-glucan in fish diet (Hadiuzzaman et al., 2022). Zebrafish has been widely used as a model organism for aquaculture nutrition research (Ulloa et al., 2014), such as fishmeal replacement (Barca et al., 2023), dietary nutraceutical effects, interactions between gut microbiota and dietary additives (Imperatore et al., 2023) . The zebrafish model is a valuable model to directly observe the immune cell function (Sanderson et al., 2015; Kaveh et al., 2020). Zebrafish larvae are optically transparent, this allowing *in vivo* observation of specific cell populations (López Nadal et al., 2020). The fish gut is a physical barrier to pathogen entry and host the gut-associated lymphoid tissue (GALT) (Salinas & Parra, 2015). The gut has the largest aggregation of immune cells of the vertebrate body and zebrafish GALT include macrophages and neutrophils (Hadiuzzaman et al., 2022). These immune cells play a role in the inflammatory immune response of most fish species against different gut parasites (Salinas & Parra, 2015). Macrophages are essential effector cells in the immune response to injury, infection, and disease. They take on a different phenotype based on their activation state, which can be proinflammatory (M1) and anti-inflammatory (prohealing, M2) (McWhorter et al., 2013). M1 and M2 phenotypes can be distinguished by a different cell elongated shape, indeed M2 phenotype is characterized by a more elongated cell shape than M1 phenotype (McWhorter et al., 2013). *Tg(coro1a:eGFP;lyz:DsRed)* is a double transgenic zebrafish line, in which macrophages and neutrophils can be distinguished by green and red fluorescence, respectively. Microglia/macrophages express eGFP only and neutrophils express both eGFP and DsRed (Li et al., 2012). For this reason, *Tg(coro1a:eGFP;lyz:DsRed)* are a powerful tools for studying the effects of dietary interventions on macrophage activation status with the aim of improving fish health in aquaculture (Sanderson et al., 2015).

Material and methods

Five treatments were carried out on 24 hpf (hours post-fertilization) zebrafish larvae until they reached 120 hpf. The *Tg(coro1a:eGFP;lyz:DsRed)* double transgenic zebrafish line was used. Forty-five embryos per treatment (3 replicates each) were exposed to 5 different water concentrations (0-, 200-, 20-, 2-, and 0.2- ml L^{-1}; CTRL, T1, T2, T3, T4, respectively, of 1,3-1,6 β-glucans (Glucan+, Arctic Feed Ingredients, Norway). On 48 hpf, egg hatching rate was determined and larva survival rate on 120 hpf. Moreover, at 120 hpf imaging of live anaesthetized larvae was performed using confocal microscopy (Axio observer, Zeiss©) of the mid- and hind- gut portion. Microphages number and activation status was then assessed on confocal images using Zeiss Zen Lite 3.7© software. Moreover, M2 phenotype percentage on the total number of macrophages was calculated and statistically analyzed (Chi^2 for hatching rate; One-way ANOVA and HSD Tukey Test for % survival rate, total number of macrophages and % of M2 on total macrophages).

Results

No differences were observed for larvae hatching and survival rate (Figure 1 and Figure 2), this meaning that at the tested β-glucans concentrations no toxicity effects were detected. Also, no differences were observed for total number of macrophages (Figure 5). The images analysis showed that the two different macrophage phenotypes (M1 and M2) were present (Figure 3 and Figure 4) in all the treatments. Significant difference (* $p<0.05$) between CTRL an T2 was observed for M2 activated macrophages (7.96±10.43 and 44.15±25.43 %, respectively) (Figure 6). Moreover, all treatments exposed to β-glucans showed higher incidence of macrophages in the M2 state of activation in comparison to the CTRL group (Figure 6).

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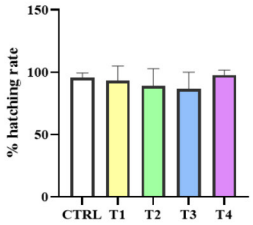

Fig. 1) Larvae hatching rate at 120 hpf

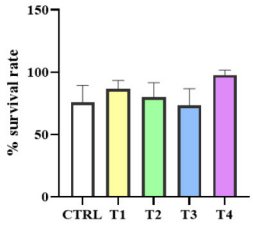

Fig. 2) Larvae survival rate at 120 hpf

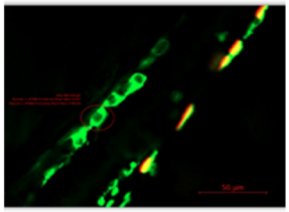

Fig. 3) Live imaging (20x) of CTRL (0 mg L^{-1}) larva, where M1 phenotype are mainly visible

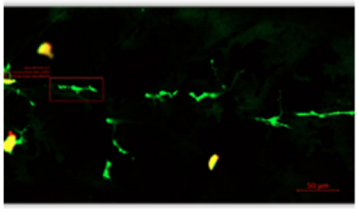

Fig. 4) Live imaging (20x) of T2 (20 mg L^{-1}) larva where M2 activated macrophages can be observed

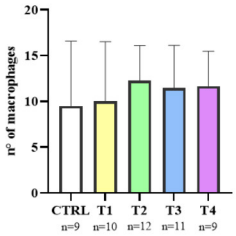

Fig. 5) Macrophages total number per treatment

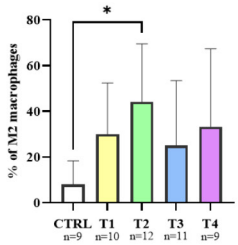

Fig. 6) Percentage of M2 macrophages on total macrophages

Conclusion

The 1,3-1,6 β-glucans water exposure of zebrafish larvae showed no negative effects on larvae hatching and survival rate at the tested concentrations. Already at the lowest β-glucans concentration used, the total number of macrophages was higher than in the control group. Moreover, the number of macrophages at the M2 activation state were mostly present in larvae exposed to β-glucans being significantly higher when larvae were exposed to 20.0 ml L^{-1}. Thanks to the use of *Tg(coro1a:eGFP;lyz:DsRed)* zebrafish line, it was possible to assess the macrophages activation status induced by dietary β-glucans in fish. These findings suggest that the exposure to 1,3-1,6 β-glucans enhance the intestinal immunity already at early developmental stage of fish (120 hpf larvae) with that promoting fish immunity, welfare, disease resistance and, ultimately, aquaculture production efficiency. Hence, it is of great interest to carry out further investigation on the mechanism of macrophage phenotype (M1 and M2) polarization induced by 1,3-1,6 β-glucans, in addition immune performances of the treated fish.

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BRIDGING THE FEED PROTEIN GAP IN AQUACULTURE WITH SINGLE CELL PROTEINS

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Because aquafeed accounts for up to 70% production costs and up to 80% of a producer's environmental footprint, the opportunity for more sustainable aquaculture is closely linked to availability of sustainable raw materials. Recent aquafeed price increases of up to 30% reflect in part the limited raw material basket, and the fact that our drive to extend this basket with novel, more sustainable proteins is only partly successful.

Microbial or single cell proteins (SCP) are the frontrunner when it comes to emerging proteins. Fermentation technology have a massive potential for scalability and other advantages include factors such as shorter generation times, the use of different feedstocks or substrates, and minimum requirements for land. In addition, single cell proteins generally have a high protein content and contain all or most essential amino acids required by fish for growth and development.

Early prototypes of a single cell protein produced by researchers at the DSM Bioscience Centre in Delft, The Netherlands showed excellent performance comparable to feeds containing fish meal and soy protein concentrate. Rainbow trout were grown for 12 weeks on different inclusions of SCP ranging from 0%, 5%, 10% to 20% on extruded feeds, with single cell protein replacing a combination of fish meal and soy protein concentrate. The results showed that single cell protein inclusion leads to equal fish performance measured by final body weight.

Availability of novel raw materials rich in protein would bring with it stability of supply and economics as the aquaculture industry grows. Collaborating across the value chain is key to drive the continued sustainable production of aquaculture, every member of the value chain has a role to make this happen and enable production at scale of single cell protein.

EFFECTS OF WATER TEMPERATURE ON GROWTH AND DIGESTIVE PROCESSES OF THE THICKLIP GREY MULLET *Chelon labrosus*

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Introduction

Water temperature is one of the most significant abiotic factors influencing fish energy partitioning and digestive capacity, which determines the amount of energy available for processes such as growth (Papoutsoglou & Lyndon, 1995; Nytrø et al., 2014). Knowing the optimal water temperature for the rearing of a given fish species is highly important from the productivity, nutrition and animal welfare points of view. The present work aimed to identify the optimal water temperature for the culture of the thicklip grey mullet *Chelon labrosus*, as well as to assess the effects of said temperatures on the activity and gene expression of its digestive enzymes.

Materials and methods

The fish (*C. labrosus*, N = 168, initial length 13.93 ± 0.96 cm, initial weight 32.09 ± 7.11 g) were distributed into 12 recirculating tanks (100 L), separated in four water temperature treatments (18, 22, 26 and 30 °C) with three replicates each. Fish were fed twice a day on a commercial feed (Gemma Diamond 1 mm, Skretting) containing 57 % protein and 15 % lipids for 90 days. Biometrical measurements (fork length and total weight) were recorded at days 0 (T0), 30 (T1), 60 (T2) and 90 (T3). At T1, 4 fish of each group were sacrificed and liver, muscle and gastrointestinal tract samples collected for biochemical composition, digestive enzyme activity and gene expression analyses. The same was done with the rest of the fish at T3.

Table 1: biometrical parameters and plasma, liver and muscle biochemical parameters of *C. labrosus* reared at different water temperatures at day 30 (T1) and 90 (T3). SGR = Specific Growth Rate; Gl = glycogen; Tg = triglyceride. Data are presented as mean ± standard deviation. Values highlighted in bold show significant differences ($p < 0.05$) between sampling times inside a given temperature treatment. Different superscripts represent significant differences ($p < 0.05$) between temperature treatments inside a given sampling time. Low letter superscripts: comparisons inside T1 sampling; capital letter superscripts: comparisons inside T3 sampling.

		18 °C		22 °C		26 °C		30 °C	
		T1	T3	T1	T3	T1	T3	T1	T3
Weight (g)		**40.3 ± 7.9^{a}**	**52.6 ± 9.6^{AB}**	**40.3 ± 8.8^{a}**	**57.7 ± 11.9^{A}**	**38.4 ± 7.1^{a}**	**55.7 ± 10.3^{AB}**	**34.9 ± 7.4^{b}**	**49.1 ± 8.7^{B}**
SGR (% / day)		0.92 ± 0.03^{ab}	0.80 ± 0.09^{BC}	1.02 ± 0.09^{a}	1.01 ± 0.02^{A}	1.01 ± 0.15^{a}	0.95 ± 0.09^{AB}	**0.51 ± 0.14^{b}**	**0.79 ± 0.06^{C}**
Plasma (mg/dl)	Tg	**178. 8 ± 20.9^{a}**	**243.3 ± 38.5^{A}**	**119.3 ± 12.8^{b}**	**175.0 ± 17.6^{B}**	**35.0 ± 28.7^{c}**	**97.5 ± 41.3^{C}**	**38.1 ± 23.3^{c}**	**96.7 ± 7.6^{C}**
Liver (mg/g)	Gl	13.2 ± 3.4	15.4 ± 2.0^{A}	7.5 ± 3.3	6.7 ± 4.0^{B}	10.7 ± 2.1	12.7 ± 3.4^{AB}	8.2 ± 2.9	10.8 ± 2.2^{B}
	Tg	86.0 ± 9.1^{a}	101.4 ± 25.6^{A}	55.5 ± 14.2^{b}	61.6 ± 7.2^{B}	63.9 ± 26.2^{ab}	49.4 ± 10.2^{B}	**47.5 ± 22.3^{b}**	**86.4 ± 15.6^{A}**
Muscle (mg/g)	Gl	**2.4 ± 0.3^{a}**	**1.5 ± 0.2^{A}**	1.3 ± 0.2^{b}	1.3 ± 0.1^{A}	1.5 ± 0.5^{b}	0.9 ± 0.2^{B}	**1.5 ± 0.2^{b}**	**1.1 ± 0.1^{B}**
	Tg	5.5 ± 2.0^{ab}	8.6 ± 5.9^{AB}	11.8 ± 5.9^{a}	10.6 ± 3.9^{AB}	9.3 ± 6.3^{ab}	5.3 ± 2.6^{B}	**3.5 ± 0.5^{b}**	**9.9 ± 1.3^{A}**

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Results and discussion

The best growth results were achieved at 22 °C, with a downwards trend at either side of the temperature spectrum (Table 1), although the only significant weight differences were observed at T3, between the 22 and 30 °C groups. This accurately reflects the dome-shaped growth curves typically caused by water temperature (Nytrø et al., 2014), due to the interaction between the acceleration of biochemical reactions, increases in feed intake (Davis & Hardy, 2022) and digestibility (Papoutsoglou & Lyndon, 1995), and the raise of basal metabolic demands (Kaushik & Schrama, 2022). In general, exposure to different temperatures did not produce changes in performance at the different samplings except the 30 °C group, as its SGR significantly improved at T3 when compared to T1. Even though this shows the ability of the fish to somewhat acclimate to this extreme temperature, it is still outperformed by the other groups. Our results suggest that the temperatures selected for the trial are at the upper part of the theoretical curve (except 30 °C), with the peak being close to 22 °C.

The group with the most energy reserves at both samplings, in term of carbohydrate and lipid contents (Table 1), was the 18 °C group. The fact that these fish did not grow as much as others despite their high energy availability indicates that at this temperature the metabolism of the fish is slower than at the other temperatures used, which causes a low energy expenditure and, concomitantly, growth. As already mentioned, the 22 °C group performed the best in terms of growth, which is reflected in containing lower energy reserves than the 18 °C group, suggesting that this group is expending much more energy, and directing it towards growth. This shows that this expenditure is not a sign of stress (Wendelaar Bonga, 1997). Energy reserves in the 26 °C group are similar or lower than in the 22 °C group, but with a slightly lower growth performance. It has to be taken into account that, in general, feed intake and digestibility increase alongside water temperature until a certain point (Papoutsoglou & Lyndon, 1995; Davis & Hardy, 2022), so groups 26 and 30 °C could have a higher energy and nutrient intake than the others. The fact that the 26 °C group did not reach the growth values of the 22 °C group, coupled with the theoretical higher availability of energy and nutrients, could indicate that 26 °C are stressful for the fish (Wendelaar Bonga, 1997), although not critically. In the 30 °C group, the lowest energy reserves and growth values were found, especially at T1, and even though they slightly improved at T3, it can be said that this temperature is out of the optimal range, and it is stressful for the fish (Wendelaar Bonga, 1997). Anyway, it is clear that raising the temperature above 26 °C could be dangerous for the well-being of the fish. The measurement of digestive enzyme gene expression and activity will help to clarify our observations. Nevertheless, those analyses have not been fully performed, and they will be key to shed some more light to the results obtained and draw conclusions that are more robust.

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WELFARE OF FARMED FISH UNDER CLIMATE CHANGE

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The welfare of cultured fish is of increasing concern for all stakeholders in the aquaculture sector. This rising interest is responsible for growing regulation at international level, as well as for the appearance of welfare standards in certification schemes. Concern for the welfare of cultured fish arises in part from ethical considerations based, for example, on the moral importance of caring for captive animals. Over and above this, good welfare potentially benefits all parties in fish culture. From an economic perspective, up to a point and in many cases, good production and good welfare go hand in hand, with poor welfare often impacting production-related traits. In addition, in some markets, there are premiums to be charged for fish cultured under welfare assurance schemes. Additionally, ensuring good welfare in farmed fish is an important aspect of job satisfaction for fish-farm workers. Finally, poor welfare often increases the adverse impacts of aquaculture on the environment, such as when appetite is suppressed in stressed fish which increases feed wastage into and pollution of the water surrounding farm cages.

Climate change is underway and is already having an impact in aquaculture, being set to alter profoundly the activity of fish farming, especially in open systems exposed to weather and climate such as pond and cage farming. Increasing our knowledge on cultured finfish biology and their diversity, as well as on welfare science will help us cope with the inevitable consequences of climate change.

The term 'finfish' ('fish' in what follows) refers to a great variety of animals that have little in common, except they are vertebrates that live in water, have fins and mostly respire using gills. More than 34,000 species of fish are recognized; less than 1000 are cartilaginous fish (elasmobranchs), the remainder being bony fish. The great diversity among teleosts in physiology, behaviour and ecology influences how they cope with environmental challenges. Additionally, there are also striking differences *within* a given species, associated with life history stage and among populations adapted to different habitats. In terms of coping style (or personality traits), for example, proactive and reactive animals flourish in different environments in the wild, proactive individuals being favoured when resources are abundant and predictable, population densities are high and predation is low, while reactive animals do best in the opposite conditions. This variability in personality traits is well documented to occur in farming systems and has clear implications for how well farmed fish adapt to aquaculture conditions, present and future.

To protect the well-being of farmed fish, much effort has gone into developing systems for improving fish health, directed at pathogen-induced diseases and other threats to health, such as malnutrition and injury. This is entirely appropriate because ill health is usually a powerful cause and indicator of poor welfare. This is not just because disease generates poor health and mortality, but also because survivors may experience welfare problems after the disease has passed. However, there are some interesting layers of complexity because 1) poor general welfare often makes fish more susceptible to disease, 2) disease treatments may also compromise welfare, 3) good health does not necessarily equate to good welfare and 4) poor health does not always equate to poor welfare.

Protection of fish welfare makes it imperative that climate changes are anticipated, and that coordinated action is taken before it becomes impossible to ensure that pond or cage-farmed fish have the environmental conditions they need for good welfare. It is more than likely that technological advances will allow not only a better understanding of farmed fish biology and their responses to farming contexts, but also the development of more adequate and effective welfare measures and their monitoring. This should include disease prevention (involving new diseases, emerging from climate change), precision fish farming, analysis of big data and more knowledge on positive welfare, for example.

Climate change is also likely to influence the range of species and strains of fish farmed in cages, in favour of those that are more tolerant of high temperatures and of environmental fluctuations. The welfare imperative here is to ensure that enough is known about the biology of the species and strains concerned to allow their welfare needs to be met, before intensive farming of such fish gets under way and lessons have to be learned by trial and error.

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Table I. Some interrelated effects of increased temperature on key physiological systems in fish.

Neuroendocrinology	Exposure to higher temperatures initiates neuroendocrine responses in the central nervous system, triggering release of corticosteroids and catecholamines. Chronically elevated plasma cortisol levels are costly, diverting energy from growth and reproduction and may result in morbidity and mortality.
Circulation	Both acute and chronic high temperature reduce cardiac function due to altered ionic balance and decrease blood flow to brain, liver, kidney and intestine, compromising biological functioning generally.
Respiratory metabolism	Standard metabolic rate (SMR), the minimum oxygen uptake needed to sustain life, increases rapidly with temperature, while maximum metabolic rate (MMR), the maximum rate of oxygen uptake (e.g. during intense exercise), increases to a plateau and then falls. Therefore, aerobic scope (MMR – SMR), the energetic potential of an organism to accomplish all of its tasks, eventually falls, constraining various functions in fish even at non-lethal temperatures.
Digestion	Increased metabolic demands may increase food requirements. High temperature may also influence feed conversion efficiency and the digestibility of specific nutritional categories, potentially leading to malnutrition.
Iono-regulation	Temperature change results in net ion loss in freshwater fish; the opposite occurs in seawater fish. The resulting ionic imbalances impair synaptic transmission in the central nervous system, with negative effects for general physiological functioning, amplified by rising salinities during droughts. Thus, maintaining the hydromineral balance becomes an increasing metabolic drain.
Immunology	Temperatures above the species' optimum have numerous adverse effects on immune function due to stress and increased maintenance costs are limited as energy is diverted to homeostasis. Impaired immune function at higher temperatures reduces the ability of fish to fight pathogens.
Reproduction	Deviations from optimal temperatures have numerous effects on sex ratios and the timing of and investment in reproduction in fishes, with implications for reproductive success in breeding fish. Early maturation in fish farmed for food has negative effects on welfare.

Besides the expected steady increases of temperature, fish in exposed farming systems will also experience an increasing number of acute events such as storms, algal blooms and heatwaves, which can only have adverse effects on their welfare (Table I). Little can be done to protect fish against such events, except making good use of available computational tools to predict these accurately and to have systems ready and in place to protect them.

In conclusion, measures to mitigate the effects of climate change could include, for example, upgrades in cage design, making the stronger and submersible; preference for resistant fish strains and species; relocation of fish farms; and improvement of fish monitoring systems and weather forecasting. All these responses will be challenging, but the fact that production and economic goals will be pulling in the same direction as the demands of protecting welfare is one reason for optimism.

ASSESSING RAINBOW TROUT WELFARE AT SLAUGHTER: AN INTEGRATIVE APPROACH USING BEHAVIOURAL, PHYSIOLOGICAL, PROTEOMIC AND QUALITY INDICATORS WITH A NOVEL TEMPERATURE STUNNING METHOD

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Fish are now widely recognised to be sentient animals. Good welfare practices are therefore not only ethical, but they also are demonstrated to improve fish quality and may even drive consumer choice. A critical point in the life of a captive fish is the final stages of their production. Not only this moment has the risk of dramatically affecting the welfare of the individuals, but it can also cause serious economic impacts to the farm, since the slaughter process can affect meat quality and carcass appearance. The most common method to slaughter fish (including trout) is by asphyxia either in ice-water or in the open air. However, this method induces prolonged and intense suffering, which is a poor practice in ethical, commercial, and legal terms. To achieve a humane slaughter practice, a stunning method needs to be implemented before slaughter, and it must render the fish immediately unconscious until death. In this regard, electrical stunning prior to slaughter has been proposed as a humane stunning method for trout, although there are anecdotal reports of trout carcass and fillet damage, with consequent decrease in value. In this experiment we tested a newly devised temperature stunner, in which the fish were immersed in water that achieves water temperatures of -8ºC still in liquid state. The objective was to evaluate and compare the effectiveness and welfare effects of four types of stunning methods in rainbow trout *O. mykiss*: cold shock by fast-chilling (FC) as a novel method, asphyxia (ASP) as the current method, electrical stunning (ES) as a humane method, and anaesthesia with MS-222 (AN) as a positive control. We used a multi-level approach to address the welfare of 176 juvenile trout, combining behaviour (individual swimming activity, equilibrium, opercular movement and eye-roll), physiology (heart rate and amplitude of electrocardiogram signal) and stress biomarkers (plasma cortisol and osmolality), and proteomics. We then proceeded to analyse the effects on shelf-life fillet and quality of fish subjected to each of these methods, using a wide range of indicators (namely *rigor mortis*, water content, fillet colour, pH and ATP degradation). This is, to our knowledge, the most detailed assessment of rainbow trout welfare at slaughter.

Behavioural indicators showed that ES presented quick and effective induction of unconsciousness (*i.e.*, absence of all consciousness indicators in 20s), while AN was slower yet 100% effective. Fish subjected to FC showed signs of extremely poor welfare (e.g., gill haemorrhage, brain damage, eye freezing and thawing, heavy mucus release) and this method was not only ineffective (only 13% lost consciousness) but also inconsistent (they recovered consciousness quickly). ASP also induced extremely poor welfare, with fish dying slowly (up to 20 min or more) and maintaining consciousness until death in 44% of cases.

Physiology indicators showed that ES had higher osmolality than AN, while ASP higher osmolality than AN and FC. Fish subjected to ASP had higher cortisol levels than AN, while ES and FC showed large variation in this hormone level. Regarding electrocardiogram signal, ES presented stronger heartbeat than ASP, while AN showed stronger heartbeat than ASP and FC. No differences were found in heart rate.

Regarding proteomics, FC and ASP had the highest impact on the brain proteome with bimodal effects. On one hand, FC registered a significant number of reactions, biological and metabolic processes. On the other hand, ASP results suggest suppression of the coping mechanisms and therefore an allostatic cost that may infer higher suffering. Interestingly, AN also induced a significant impact on the brain proteome as indicated by significant involvement of programmed cell death processes, which is probably linked to the opioid nature of MS-222.

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Regarding quality indicators, ASP and FC showed faster *rigor* onset than AN and ES until 6h *post-mortem*. Energy depletion was lower in AN and ES than in ASP and FC, while AN showed higher pH than all the other treatments, and ASP presented the lowest pH. Fish subjected to ASP showed the highest fillet weight loss after *rigor*, ES and AN the lowest.

Our results demonstrate that, other than anaesthesia (which is generally not permitted in fish farmed for food), electrical stunning consistently showed good results as a humane stunning method. This was presented at all levels of welfare analysis, as well as in fillet quality terms. The fast-chilling method, on the other hand, presented very poor results both in welfare and in quality and did not seem to be a viable humane alternative to asphyxia.

Additionally, the proteome analysis opens new windows into the selection of fine-scale biomarkers of welfare and, more specifically, provides very interesting insights into the brain mechanisms of rainbow trout at slaughter.

ANTIMICROBIAL ACTIVITIES OF *Nannochloropsis oceanica* FRACTIONS AGAINST BACTERIAL PATHOGENS

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Introduction

Algae inhabit a diverse range of ecosystems, contributing to their ability to synthesise diverse classes of highly active metabolites. These metabolites exhibit considerable antibacterial activity against a broad range of bacteria, thus making them promising sources of antibacterial compounds. Fish, due to their ecological environment, are in constant contact with pathogens, thus fish bacterial diseases are an important and recurrent problem in aquaculture. Natural antibacterial and/or immunostimulant compounds are greatly needed for a more sustainable industry. This study aimed to assess the antimicrobial properties of several *Nannochloropsis oceanica* fractions obtained through marine biorefinery concepts.

Material and Methods

Four different concentrations (0.5-, 0.25-, 0.1-, and 0.01 $mg.mL^{-1}$) of three *Nannochloropsis oceanica* fractions (lysed microalgae, crude polysaccharides, and sulphated polysaccharides-rich fractions) were tested *in vitro* for their antibacterial activity against *Vibrio anguillarum*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, *Aeromonas samonicida*, *Photobacterium damselae piscicida*, *Edwardsiella piscicida*, and *Tenacibaculum maritimum* species. Different fraction exposure times (1-, 2.5-, and 4-h) were also tested.

Results

The lysed microalgae fraction presented a higher bacteria inhibition spectrum. The adjusted means from Tukey's *posthoc* test indicated that aqueous lysed microalgae of *Nannochloropsis oceanica* inhibited all bacteria tested, with *Aeromonas salmonicida* being significantly affected ($p<0.05$; survival:12–55%). However, a range of bacteria growth inhibition was obtained (10–90%), with *Vibrio* sp. being less affected. Except for *V. parahaemolyticus* and independently of the fraction and concentration used, all bacterial growth was mostly inhibited at 1h post-fraction exposure, whereas a higher cell viability tendency was observed in longer fraction-exposure times. This phenomenon can be explained by the bacteria adaptation to growth conditions.

Discussion and Conclusion

Besides their appreciable levels of primary metabolites, such as polysaccharides, protein, carbohydrates, and vitamins, microalgae produce high-value secondary metabolites. Collectively these metabolites present antioxidant, antimicrobial, antitumor, anti-inflammatory, and anticancer capacities. The polysaccharides' antibacterial activity underlying mechanism has been hypothesised to be based on glycoprotein receptors in the polysaccharides, which bind to molecules on the bacterial cell wall, membrane, and nucleic acids.

The obtained results clarified that the crude lysed fraction maintains a potent antimicrobial activity, making it a promising source of new antimicrobial drugs.

However, antimicrobial susceptibility testing for aquaculture-relevant strains is highly recommended, as resistance to antibacterial agents may be strain dependent.

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INFLUENCE OF CARRAGEENIN-INDUCED INFLAMMATION ON SERUM AND SKIN MUCUS OF GILTHEAD SEABREAM (*Sparus aurata*) DURING A *Photobacterium damselae* sube sp. *Piscicida* INFECTION

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Introduction

Disease outbreaks are one of the major challenges to the sustainable development of marine aquaculture. Many species from the Vibrionaceae family are serious opportunistic pathogens responsible for relevant monetary losses to aquaculture producers (Ina-Salwany *et al*, 2019). However, to date, little attention has been paid to the susceptibility of fish to pathogen infections when they undergo an inflammatory process as a consequence of the intensive production conditions typically applied in the aquaculture sector. For this purpose, we used carrageenin, a sulphated mucopolysaccharide derived from the cell walls of red algae (Rhodophyceae family), as a model of acute inflammation in the skin of gilthead seabream (*Sparus aurata*), due to its local effects and high reproducibility (Campos-Sánchez *et al*, 2021). On the other hand, *Photobacterium damseale* subesp. *piscicida* (*Phdp*) is known to cause pasteurellosis, a septicemic disease with high mortality rate in farmed fish, and whose infectious process depends on the fish species, temperature, salinity, and other unknown factors (Santos *et al*, 2022). In this regard, the present study aims to evaluate the susceptibility of the immune status of gilthead seabream during the course of a bacterial infection by *Phdp* after an inflammation provoked by carrageenin.

Material and methods

In this study, 24 specimens (75.25 ± 3.77 g, 15.98 ± 0.22 cm) of gilthead seabream (*S. aurata*) from broodstock at the aquaculture Research Station of Olhão (Portuguese Institute for the Ocean and Atmosphere) and maintained in the same facilities, were injected intramuscularly with 50 μL of phosphate buffered saline (PBS, as control), or carrageenin solution (1%, Sigma) in PBS. At 3 h post-injection, half of the fish in each predefined group were injected intraperitoneally with 100 µL of PBS (control group), while the other half of the fish were injected with *Phdp* (10^6 UFC/mL, *Phdp* group). At 24 h after carrageenin injection, blood samples and skin mucus were collected by following the methods described by Guardiola *et al* (2014). Esterase activity, peroxidase activity, lysozyme activity, bactericidal activity against *Phdp* and *Vibrio anguillarum,* as well as total immunoglobulin levels were analysed in both serum and skin mucus (Guardiola *et al*, 2016). The results were expressed as mean ± standard error of the mean (SEM) and data were analysed by one-way ANOVA (followed by Tukey tests) to determine differences between experimental groups. The level of significance used was $p < 0.05$ for all statistical tests.

Results and discussion

The results obtained in serum showed an increase in esterase activity in the *Phdp* group compared to the control group. However, bactericidal activity against *Phdp* decreased in the carrageenin-injected and *Phdp*-infected fish group compared to the control and *Phdp* groups. Peroxidase, lysozyme and bactericidal activity against *V. anguillarum*, as well as total immunoglobulin levels remained equal among groups. Similar to serum, bactericidal activity against *Phdp* studied in the skin mucus decreased in the carrageenin + *Phdp* injected fish group compared to the control group and the carrageenin group. In contrast, bactericidal activity against *V. anguillarum* increased in the *Phdp* group compared to the other groups, while the total immunoglobulin level increased in both the *Phdp* group and the carrageenin + *Phdp* group compared to the control. According to the literature, *Phdp* is able to enhanced immune activity of gilthead seabream by activation and differentiation of phagocytes (Pellizzari *et al*, 2013). In addition, *Phdp* has a capsule of polysaccharide composition that confers protection against bactericidal activity (Magariños *et al*, 1996), which could explain why in our study there were

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no significant differences when studying the bactericidal activity against *Phdp* between the group of fish infected with the bacterium and the control group. However, since this activity decreased in the skin mucus of fish injected with carrageenin + *Phdp*, they might be more susceptible to infection than the control fish. In addition, due to the possible enhancing of the immunity caused by the bacteria, this group of fish could be on alert against other pathogens, explaining the increased bactericidal activity against *V. anguillarum* in the skin mucus of *Phdp*-infected specimens. Otherwise, no differences were found in esterase, peroxidase and lysozyme activities in the skin mucus between groups.

Conclusion

Our results provide an overview of the effect of *Phdp* on the immune status of gilthead seabream. Furthermore, our data suggest that fish subjected to an inflammatory process, such as the one tested in this trial (carrageenin), might be more susceptible to a bacterial infection. These results could be used in other future studies and contribute to a better understanding of bacterial infections in aquaculture and, therefore, to their resolution.

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IDENTIFICATION AND ENZYMATIC CHARACTERIZATION OF MARINE MICROBES ASSOCIATED WITH SEAWEEDS AND THEIR POTENTIAL FOR CAUSING ICE-ICE DISEASE AND PRODUCING BIOETHANOL

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Introduction:
Before initiating seaweed cultivation in integrated multi-trophic aquaculture (IMTA) or large-scale production in open ocean environments, it is imperative to address potential issues that could impede biomass production and result in significant economic losses for producers. While limited research has been conducted on the role of microorganisms and their interactions in the seaweed life cycle, their impact on seaweed cultivation is well recognized. To address this knowledge gap, the present study aimed to isolate marine bacteria and fungi associated with seaweeds and their role in causing Ice-Ice disease and to characterize their agar and carrageenan degrading abilities to assess their potential application in bioethanol production. While previous studies have reported on degrading agar and carrageenan microorganisms (Chi et al., 2012; Kang & Kim, 2015), identifying microorganisms with the ability to degrade both agar and carrageenan remains limited.

Materials and methods:
Our study focused on three species of red seaweed (*Kappahucus alvarezii*, *Gracilaria edulis*, and *Eucheuma cottonii*) collected from a seaweed cultivation site in Ramanathapuram, India. The seaweeds were transported to the lab in sterile plastic bags. The epiphytic and endophytic microbes were isolated for microbiological and biochemical analysis. Subsequently, the seaweed species were placed in glass tanks for growth assessment to evaluate whether the microbes could cause ice-ice disease in stressful environments. After five days, all seaweed species started losing pigments and turned pale white indicating symptoms of ice-ice disease, leading to complete degradation, as confirmed by visual observations Fig.1 and Fig.2. This confirmed that associated epiphytes and endophytes could degrade agar and carrageenan.

We isolated 18 marine microbes from the three seaweed species using spread plate techniques and pure culture methods. Qualitative assays were conducted to assess the ability of isolated microbes to degrade agar and carrageenan using lugol's iodine. DNA sequencing was performed for all the positive microbes in the agar and carrageenan qualitative assay, and their enzymatic activity was characterized using the 3,5-dinitro salicylic acid (DNSA) assay.

Results:
Out of the 18 isolated marine microbes' species, our investigation identified six with positive agar and carrageenan enzymatic activity, three with only agar, and three with only carrageenan. DNA sequencing revealed that ten species were bacteria, while the other two were yeast. Further, the results showed that *Bacillus kokeshiiformis* strain SM24, *Nitratireductor kimnyeongensis* strain E14, *Brevibacillus agri* strain 13, *Caldibacillus kokeshiiformis* strain FJAT-47861, *Lentibacillus salarius* strain BH139, *Bacillus thermolactis* strain SM10, *Micrococcus endophyticus* strain HCDB6, *Caldibacillus thermolactis* strain NWFY-11-4, *Oceanobacillus caeni* strain LNPC16, and *Bacillus thuringiensis* strain SH26 are the isolated bacterial species. The isolated yeast species are *Pichia fermentans* strain PM79 and *Geotrichum candidum*. The 3,5-Dinitrosalicylic acid (DNSA) assay results showed that *Caldibacillus kokeshiiformis* strain FJAT-47861 had the maximum carrageenase enzymatic activity of about 0.76 units/ml and *Pichia fermentans* strain PM79 had the maximum agarase enzymatic activity of about 0.52 units/ml. The highest agarase and carrageenase enzymatic activity was found in the *Pichia fermentans* strain PM79, with an average of 0.63 units/ml of agarase and carrageenase activity, the highest among all six species that tested positive for agar and carrageenan. Moreover, the average carrageenase enzymatic activity of all six microbes was found to be 1.5 times higher than their agarase enzymatic activity.

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Fig. 1. Pale white appearance of *K.alvarezii* thallus

Fig. 2. Pigment discoloration of *E.cottonii* thallus

Conclusion:

This investigation represents the first report of isolating four microbial strains: *Caldibacillus kokeshiiformis* strain FJAT-47861, *Lentibacillus salarius* strain BH139, *Micrococcus endophyticus* strain HCDB6, and *Caldibacillus thermolactis* strain NWFY-11-4, from red seaweeds. In addition, identifying species with bioremediation potential, such as *Pichia*, *Geotrichum*, *Caldibacillus*, *Lentibacillus*, *Micrococcus*, and *Bacillus*, is also significant as these species have been reported to remove organic pollutants, hydrocarbons, and polycyclic aromatic hydrocarbons (PAHs) from the environment. Using halophytic and thermophilic bacterial species, such as *Bacillus thermolactis* strain SM10, *Caldibacillus thermolactis* strain NWFY-11-4, and *Oceanobacillus caeni* strain LNPC16, for bioethanol production from macroalgae, offers a promising alternative to traditional biofuel production methods. Overall, our findings indicate that all 12 microbes can be used in bioethanol production from macroalgae. Their agarase and carrageenase activity indicates their ability to break down the complex carbohydrates in seaweed cell walls. The fact that the average carrageenase enzymatic activity was found to be 1.5 times higher than their agarase enzymatic activity is particularly noteworthy. Additionally, the higher carrageenase enzymatic activity of the *Caldibacillus kokeshiiformis* strain FJAT-47861 suggests that this microbe may be highly beneficial for producing bioethanol from seaweed. In conclusion, this study has contributed to the scientific understanding of the microbial diversity associated with seaweeds. It highlights the potential applications of the isolated strains in various industries, especially bioethanol production. Further research, such as the enzyme's ability to efficiently extract and hydrolyze the polysaccharides from red seaweed, the yield of fermentable sugars, the efficiency of the fermentation process, and the overall ethanol yield are needed to fully explore the potential of these strains and develop new biotechnological applications.

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NUTRIENT COMPOSITION AND POTENTIAL OF SEAWEED PROTEIN CONCENTRATE AS A SUSTAINABLE FEED INGREDIENT IN THE DIETS OF *Penaeus monodon*

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To address the shortage of cheap and sustainable feed-protein ingredient for aquaculture, this study developed *Gracilariopsis heteroclada* protein concentrate (GHPC) and evaluated its nutritional value as a replacement to soybean meal protein in the diet of black tiger shrimp, *Penaeus monodon*. Five isonitrogenous and isolipidic diets were formulated replacing soybean meal protein at 0% (control), 12.5%, 25%, 50% and 75%.

Results showed that the developed GHPC has a protein, lipid, fiber and carbohydrate content of 31.11±0.12%, 1.57±0.61%, 1.87±0.31% and 24.99±0.33%, respectively. The nutritional value of GHPC was found high, exhibiting an ingredient digestibility index of 91.84 ± 0.06% and Essential Amino Acid Index (EAAI) of 0.996. Feeding trial results revealed that the overall growth performance, feed assimilation efficiency and biochemical composition of the shrimp fed with the 50% GHPC diet were similar to the control group fed with full soybean meal based-diet (Table 1). The high acceptability of this feed ingredient to *P. monodon* is attributed to its high digestibility and adequate content of essential amino acids. However, shrimps fed with replacement levels beyond 50% exhibited significant growth depression that might be due to the presence of antinutritional compounds as observed with the reduction of digestive enzyme activities (Figure 1) and histomorphological changes (B-cell enlargement) (Figure 2).

Collectively, the findings suggest that GHPC has high nutritional value and could be used as a plant protein ingredient in the diet of *P. monodon*. Utilization of GHPC is a sustainable approach to meet the growing requirements of feed-proteins in the expansion of *P. monodon* aquaculture.

Table 1. Growth performance and survival of *P. monodon* fed with varying levels of *G. heteroclada* protein concentrate

Treatments	Parameters					
	%WTGN	SGR	PER	FCE (%)	Total feed given/intake (g) per individual and % body weight	Survival (%)
Control	5916.22±306.34^{b}	6.82±0.08^{b}	1.38±0.19^{b}	54.19±2.66^{b}	0.67±0.01^{ab}	94.86±2.57^{a}
12.5 % GHPC	6221.89±216.99^{b}	6.91±0.06^{b}	1.09±0.07^{ab}	52.69±2.23^{ab}	0.72±0.00^{b}	96.15±3.85^{a}
25 % GHPC	5920.05±460.02^{b}	6.82±0.13^{b}	1.25±0.13^{ab}	55.19±1.10^{b}	0.66±0.08^{ab}	97.92±2.08^{a}
50 % GHPC	5359.01±317.08^{ab}	6.66±0.10^{b}	0.97±0.18^{ab}	48.77±1.70^{ab}	0.61±0.07^{ab}	98.08±1.92^{a}
75 % GHPC	3752.40±261.95^{a}	6.08±0.12^{a}	0.74±0.08^{a}	43.70±1.03^{a}	0.53±0.04^{a}	93.93±3.34^{a}

Values are mean ± standard error of the mean
Within parameters, means with different superscript are significantly different ($p<0.05$)

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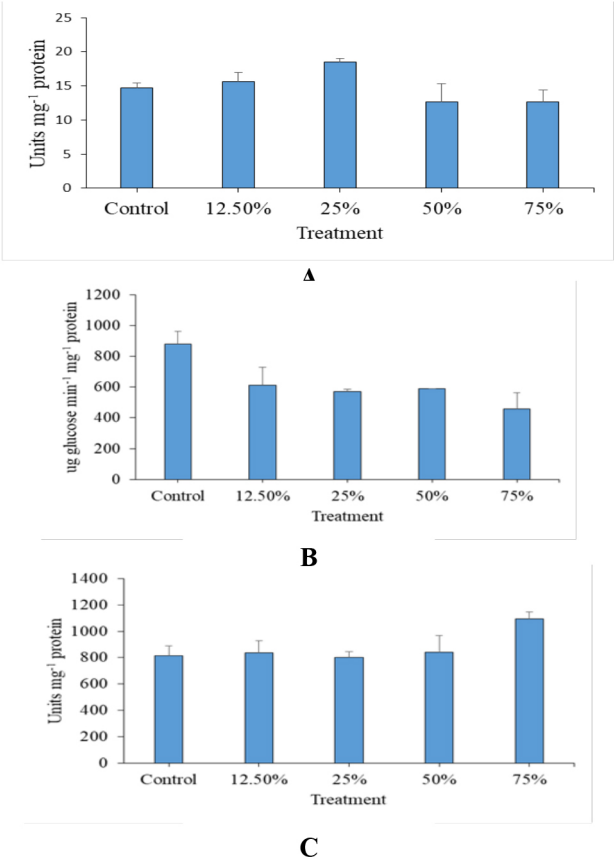

Figure 2. Digestive enzyme ctivity of *P. monodon* fed with varying levels of GHPC as soybean meal replacement (mean ± SEM). A- Protease, B-Amylase, C-Lipase.

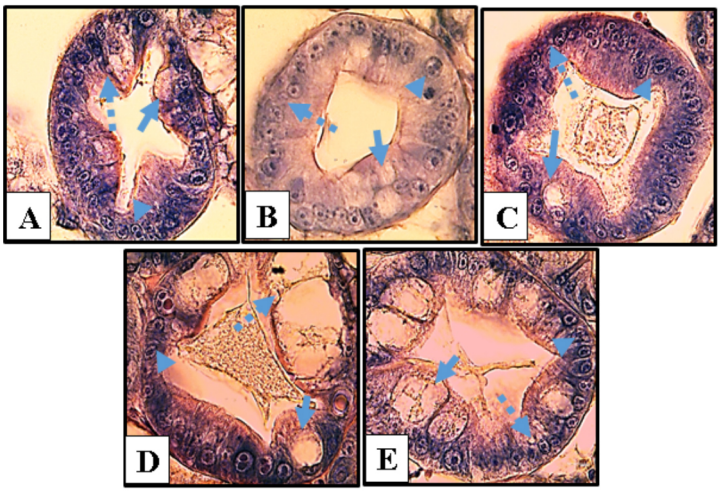

Figure 3. Transverse sections of the hepatopancreas of shrimp after 60 days feeding trial. A-Control, B-12.5% GHPC, C-25% GHPC, D- 50% GHPC, E-75% GHPC. Arrow- Blastozellen cell (B cell), arrow head-Fibrillar Cell (F cell), broken arrow-Restzellen cell (R cell). Longitudinal 5 µm paraffin section, H&E stain, Davidsons fixative, 100 x magnification.

USE OF STEREOVISION TO ASSESS VERTICAL SIZE-STRATIFICATION OF ATLANTIC SALMON (*Salmo salar*) IN A COMMERCIAL SEA-CAGE: A CASE STUDY

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Introduction

Norway is the largest exporter of Atlantic salmon (*Salmo salar*) in the world, with a production of 1,5 million tons in 2020 (FAO., 2022). Most of the grow out period happens in sea-cages which can host up to 200 000 individuals in a volume comprised between 18 500 and 80 000 m^3. Sampling fish for size and biomass estimation at such scale is challenging and time-consuming for farmers. They usually sample around 20 fish per week for lice counting and use this sampling periodically to measure and weight fish, which may be not representative for the whole cage. This can have a strong impact on feeding strategies and slaughtering prevision, followed by effects on production cost and yields. New technologies, like automated measurements from images of stereo-paired cameras to estimate salmon size and biomass in sea-cages, are developing fast for more representative and non-invasive samplings. Nevertheless, previous studies suggested that salmon distribution might not be as homogenously thought. Folkedal et al. (2012) showed that the vertical distribution of salmon in 2000 m^3 sea-cages is size-dependent, suggesting repercussions on biomass estimation depending on the sampling method. This needed to be confirmed at commercial scale. Similarly, Johannesen et al. (2022) suggested that horizontal distribution of fish in relation to depth might be very different in a commercial sea-cage. Both findings are important to consider for the deployment of automatic cameras to get representative results.

Materials and methods

Low-cost action cameras (GoPro Hero 8) were time-synchronized and deployed to cover 16 positions in a commercial sea-cage (18 850 m^3) of the salmon sea-farm in Gudmunset-Norway (62.45366, 6.60326). Four depths (1 m, 5 m, 9 m, 14 m) were covered at four horizontal positions (North, East, South, West) in the sea-cage. Cameras were set to record for at least 1h, four times in one day (4 deployments, during: 1st feeding and 2nd feeding periods, between feedings, and after 2nd feeding). On the east side of the cage, homemade stereovision rigs with paired GoPros were deployed. 3776 fish through the different depths and deployments were manually fork-length measured with Eventmeasure software (SeaGis 2006-2023). Fish abundance was scored from 0 to 3 (absence of fish, low, medium, high) and growth-stunt fish counted at every position in the sea-cage on screenshots taken every minute for 60 minutes per deployment. Fish swimming speed was also estimated on a portion of the fish length measured using a vertical reference line and counting the time the fish took to pass the reference line from snout to fork-length (BL/sec). Average fish length was compared per depth and per deployment and the same procedure was applied for swimming speed. Cumulative means for fish size were created per depth to estimate when a sample was representative for its depth and for the whole cage.

Results

Salmon fork-length averages were significantly ($p < 0.05$) different between depths with longer salmon found deeper in the sea-cage (9 m and 14 m) compared to the shallowest depth (1 m) and intermediate one (5 m). This was the case for every deployment that day. A strong variation in size, from 24 cm up to 70 cm, was present and mostly showcased at 1 m than at deeper depths. Distribution of fish appeared quite homogeneous on the horizontal plan, with higher fish abundance at 9 m and 14 m, intermediate at 5 m and low at 1 m depth. No difference was found between feeding or none feeding periods and fish were more abundant at the deeper depths during the day. Fish mean swimming speed was around 1 BL/sec. Most growth-stunt fish were located at 1 m and some individuals could clearly be identified and observed several times at the same position. The cumulative means per depth indicated that average size for a particular depth could be reached with 50 individuals measured in some cases, but most of the time after 150 individuals measured.

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Discussion and conclusion

These results are in accordance with the findings from Folkedal et al. (2012) and Nilsson et al. (2013) which found a vertical size-stratification with depth in smaller sea-cages. The use of stereovision to measure salmon in this study allowed to reduce potential bias in the selection of the fish to be measured as fish were not afraid of the stereo rig and were randomly selected to be measured. It appeared that this display did not disturb salmon in their "regular" sea-cage behavior. Results on fish abundance registered at the different positions in the cage suggest that in the absence of strong current or weather, salmon seem to use the whole cage quite homogeneously on the horizontal plan. The fact that high fish abundances were found at 9 m and 14 m, even during feeding times, suggest that the whole school of fish does not gather close to the feeding area at the same time. A different pattern would probably be observed in the center of the cage, not covered by our cameras, where feeding is occurring. From the different findings on vertical size stratification, horizontal and vertical distributions, and average size representativity per depths, several potential implications for an optimal deployment of automatic biomass estimating cameras can be mentioned. On one hand, if the primary objective of the farmers is to get the overall size/biomass variation in the cage, positioning the camera higher up appears more strategic as smaller fish tend to stay higher in the water column and might not be caught at deeper depths. This might be relevant for sea cages in which a large size stratification is present. On the other hand, if the objective is to get a close estimation of the mean size or biomass, it would be more interesting to stand were the larger part of the school is, in our case study: between 9 m and 14m. Anyhow, it seems difficult to obtain the exact average size if the camera is deployed at a single depth. Depending on the weather conditions, fish might use preferentially a part of the cage (Johannesen et al. 2022), and this should be accounted for when deploying equipment. Such studies should be conducted in different localities, during different seasons and production stages to be able to generalize, and automatization of measurements is necessary to conduct more experiments at that scale. This case study aimed to enrich what was previously known on salmon distribution at commercial scale and showed potential considerations for the use of innovative methods to measure salmon size.

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EFFECTS OF THE DIFFERENT MICROALGAL DIETS ON AMINO ACID COMPOSITION OF WATER FLEA (*Daphnia magna*)

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Introduction

Live preys are most important feed in larval fish nutrition. Rotifer and Artemia are first live feeds in fish larval feeding. Those live preys need intensive labour and energy. However, water flea (*Daphnia magna*) is become alternative zooplankton due to containing high protein and lipid levels (Turcihan et al., 2022). In addition, Daphnia culture can be performed in outdoor wastewater systems that lead to higher biomass production. Microalgae are commonly used for live prey, alternative microdiet ingredients, green energy production and wastewater treatments (Soto-Sánchez et al. 2023). In our study, fresh *Euglena gracilis* and *Pavlova lutheri* were used for Daphnia feeding. In addition, several commercial diets were evaluated such as Algome® (dried *Schizochytrium* sp.), Naturiga® (dried *Spirulina platensis*), AlgomeGrow® (dried *Chlorella* sp.) and ProteinPlus®. The aim of this study, was to evaluate amino acid compositions of *Daphnia magna* fed two fresh and four commercial microalgae products.

Materials and Methods

Daphnia magna cultured under controlled laboratory conditions. Daphnia stock cultures were maintained continuously aerated tap water at 21°C. Broodstock of Daphnia were fed baker's yeast. Daphnids were counted by pasteur pipette daily and settled at the same number (n=20) with triplicate in polyethylene culture vessels. Freshwater microalgae *Euglena gracilis* was cultured in 3N-BBMV medium whereas marine microalgae *Pavlova lutheri* was incubated f/2 medium. Culture medium and vessels of microalgae previously sterilized at 121 °C for 15 min. (Guillard, 1975). The microalgae species was scaled up in erlenmeyer flasks from 50 mL test tubes to 250 mL erlenmeyer flasks, followed by 1-L, and 5-L. Microalgae were counted in each experimental flask. Commercial microalgae feeds were ProteinPlus® (Aquafauna Bio-Marine, USA), Naturiga® (Naturiga Natural Foods, Turkey), Algome®(MarineBio, Turkey) and AlgomeGrow® (MarineBio, Turkey). All feeds were prepared at 2 g/L ratio. Total amino acids analyses were conducted at the Scientific and Technological Research Council of Turkey (MAM, Turkey). From each sample, 5 mg was added to a glass ampoule together with 5-mL lithium citrate loading buffer. Ampoules were sealed and placed in an oven (115 °C) for 2 h to facilitate hydrolysis. The samples were then removed, allowed to cool, and filtered with a 0.45 PTFE syringe filter. Samples were diluted as necessary to fall within the detectable range for the assay. Amino acids were measured with a Biochrom 30-amino acid analyzer (Biochrom, Holliston, MA, USA) (Hawkyard et al. 2016).

Results and Discussion

Protein contents of live prey are important for larval and juvenile fish in aquaculture for their optimum growth and survival. Daphnia studies are mostly related to their growth, fatty acids and proximate composition. It is reported that Daphnia can be alternative live prey for sustainable aquaculture production with partly used instead of Artemia (Chakraborty and Mallick, 2023). Microalgae species can affect Daphnia culture performance. For instance, *Cryptomonas* sp. had positive affect on reproduction whereas feeding with *C. reinhartdii* had resulted resting eggs in Daphnia. Moreover, addition some of amino acids such as Arginine and Histidine in microalgal diets could result less resting egg occurance in *D.pulex* (Fink et al., 2011). Each microalgae has different amino acid composition (Brown, 1991). From this respect, amino acid composition of microalgae and alternative diets have crucial importance in Daphnia reproduction. Karakaş et al. (2023) isolated cynobacteria *Phormidium lucidum* and evaluated for culture performance. In order to develop and commercialize Daphnia culture, alternative instant use diets should be introduced in aquaculture sector. However, commercial diets have not been evaluated for Daphnia culture. In this study, we used two fresh microalgae and four commercial diets and investigated effects on amino acids. The AlgomeGrow diet (dried *Chlorella vulgaris*) showed best results in amino acids of Daphnia. The present study showed that the Naturiga diet contained higher amount of amino acids, Daphnia individuals fed Naturiga did not contain higher levels of amino acids (Table 1). In fact, AlgomeGrow group showed higher levels of essential amino acids even though this diet had second levels of total amino acids. This result could be related to Nitrogen limitation in Daphnia diet (Wagner et al., 2017). Future studies are needed for large scale commercial experiments in daphnia culture with those commercial diets.

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Table 1. Amino acid composition of *D.magna* fed with the experimental diets.

	Initial	*Euglena gracilis*	*Pavlova lutheri*	Algome	Naturiga	Algome Grow	Protein Plus
Aspartic acid	105±4^{a}	78.5±0.5^{c}	86±1^{b}	85±1^{b}	97±1^{a}	101.5±0.5^{a}	85±1^{b}
Glutamic acid	195.5±0.5^{b}	143.5±0.5^{d}	173.5±1.5^{c}	142.5±0.5^{d}	152±1^{cd}	247.5±2.5^{a}	165±1^{c}
Serine	65.5±1.5^{c}	41.5±0.5^{d}	48±1^{d}	67.5±0.5^{c}	76.5±3.5^{b}	127±0^{a}	54±7^{cd}
Glycine	256.5±6.5^{c}	152.5±0.5^{d}	167.5±1.5^{d}	274.5±1.5^{b}	250.5±1.5^{c}	382±3^{a}	255±1^{c}
Alanine	226±2^{cd}	203.5±0.5^{d}	237.5±1.5^{cd}	313.5±0.5^{a}	244.5±0.5^{c}	270±0^{b}	324±0^{a}
Tyrosine	115±0^{c}	89.5±1.5^{d}	87.5±0.5^{d}	154±0^{b}	158±1^{b}	195.5±0.5^{a}	147±1^{b}
Proline	310±1^{b}	214.5±0.5^{d}	245.5±0.5^{c}	247±3^{c}	289±3^{b}	354±1^{a}	293.5±2.5^{b}
Non-essential amino acid	1315.5±7.5^{b}	905±2^{d}	971.5±1.5^{d}	1264.5±1.5^{c}	1267.5±0.5^{c}	1742±3^{a}	1323.5±7.5^{b}
Threonine	125±5^{d}	102±1^{d}	91.5±3.5^{d}	135±1^{c}	167.5±0.5^{b}	138±1^{c}	176±1^{a}
Arginine	121±3^{c}	115±1^{c}	121±2^{c}	116.5±2.5^{c}	123.5±3.5^{c}	197.5±2.5^{a}	140.5±1.5^{b}
Histidine	20.5±1.5^{c}	11.5±0.5^{d}	23±1^{c}	20±0^{c}	11±0^{d}	48±2^{a}	27.5±1.5^{b}
Valine	247.5±1.5^{b}	173.5±1.5^{c}	140.5±0.5^{d}	274±0^{b}	294.5±0.5^{a}	286.5±1.5^{a}	265.5±0.5^{b}
Methionine	45.5±1.5^{c}	58±1^{c}	39±0^{d}	75±0^{b}	87±0^{a}	73.5±0.5^{b}	79.5±0.5^{b}
Isoleucine	142±0^{d}	105.5±0.5^{e}	100.5±0.5^{e}	189.5±0.5^{b}	175±1^{c}	243±1^{a}	186.5±0.5^{b}
Leucine	281±2^{c}	200.5±2.5^{d}	197.5±1.5^{d}	333±1^{b}	299±1^{c}	450.5±0.5^{a}	315±0^{bc}
Phenylalanine	108.50.5^{d}	103±1^{d}	105.5±0.5^{d}	184±1^{b}	144.5±0.5^{c}	266±0^{a}	176±0^{b}
Lysine	217.5±1.5^{e}	166.5±1.5^{f}	209.5±1.5^{e}	409.5±2.5^{b}	255.5±1.5^{d}	650.5±2.5^{a}	372±1^{c}
Essential amino acid	1308.5±6.5^{d}	1035.5±5.5^{e}	1028±9^{e}	1756±2^{b}	1557.5±2.5^{c}	2353.5±6.5^{a}	1738.5±0.5^{b}

Note: Different letters show significant differences among groups (*p < 0.05); Duncan's multiple range test.

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AQUAKULTURINFO – OVER 10 YEARS OF COMMUNICATING ABOUT AQUACULTURE IN GERMANY

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Introduction

While the demand for fish and other aquatic food as well as the market share of aquaculture are on the rise globally, consumption of these products and the aquaculture sector in Germany is relatively stable on a comparably low level. Average per capita consumption ranged from 14.1 to 14.8 kg in the years 2016 to 2020 amounting to 1.231 million tons of total consumption in 2020 (FIZ 2022). In parallel, aquaculture production in Germany provides only a share of less than 3% of this consumption. Besides species mainly originating from capture fisheries, predominantly farmed species, such as salmon, trout or various crustaceans, are favorites among the market in Germany (FIZ 2022).

While fish farming has a long tradition in many regions across Germany, where mainly trout and carp production in flow-through and pond systems as well as mussel production on the shores dominate the sector, German consumers do not necessarily know much about aquaculture (Feucht und Zander 2017; Risius et al. 2017). Given the moderate consumption and small domestic aquaculture sector, contact between consumers and the primary production is fairly limited. Consumers are mainly confronted with aquaculture through products in supermarkets or restaurants. The dynamic development of the sector and the global trade result in ecological and social challenges. These and other topics, e. g., related to product quality, often put aquaculture into the public and medial interest. While fish is generally perceived as healthy food, impressions from neighboring sectors, agriculture and capture fisheries, are being transferred and the public perception of aquaculture and its practices remains ambiguous. This current state of comparably low knowledge, perception and awareness was also considered within the German Strategy Plan for Aquaculture highlighting the need for trustworthy information transfer and communication (NASTAQ 2020).

The project AQUAKULTURINFO

To allow for responsible, fact-based decision making by consumers as well as representatives of trade, processing industry, associations, NGOs, administration or politics, the IGB has launched the third-party funded project AQUAKULTURINFO in 2012. Mainstay and visible core of the project is the website www.aquakulturinfo.de (Fig. 1). The website offers in-depth, science-based articles on aquaculture related topics separated into thematic fields, such as husbandry, animal health and welfare, product quality, environmental interactions or economy and marketing. A section of the website offers species articles on fish, mollusks, crustaceans and algae. The articles are authored by aquaculture scientists and undergo a review process prior to publication on the website to ensure the independent character of the information and objectivity. The website also features a news section and embedded videos. Overall, the original content of the website results in very good rankings among search engines and is highly visible.

In addition to the website, AQUAKULTURINFO provides a variety of services at the interface of science and society and actively engages in public outreach. The project serves as a sought-after information hub for media, politics and administration, trade, industry and associations as well as NGOs and other organizations with interest in the field. Within the framework of the project, consumer and professional events related to aquaculture, fisheries, food, agriculture or general natural science are supported. Furthermore, AQUAKULTURINFO participates in research within the field of consumer perception and communication and supports other projects by dissemination of results. To further strengthen an active role in knowledge transfer, AQUAKULTURINFO seeks innovative approaches in science communication, e. g., through scientific gaming (Fig. 2). Through these means of PR in combination with the website, active and passive information transfer into the society is realized. In conclusion, AQUAKULTURINFO provides a unique link between science and society and offers comprehensive experience in aquaculture related science communication.

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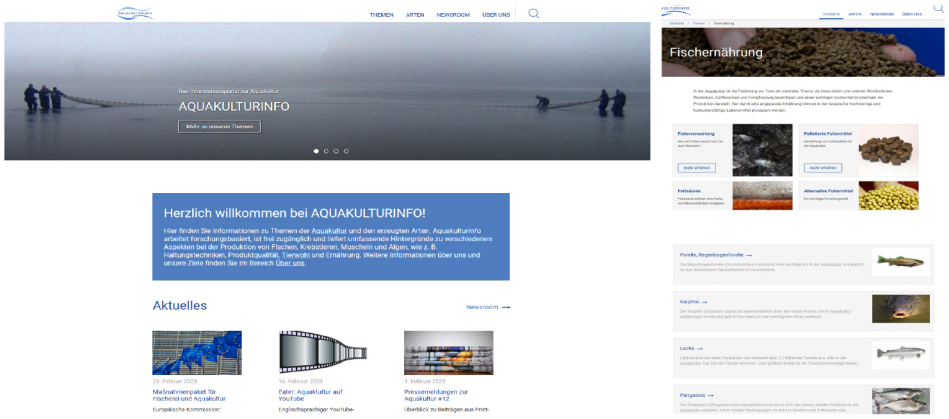

Fig. 1: Front page of the website www.aquakulturinfo.de (left), section fish nutrition (upper right) and fish species (bottom right) on 24th February 2023.

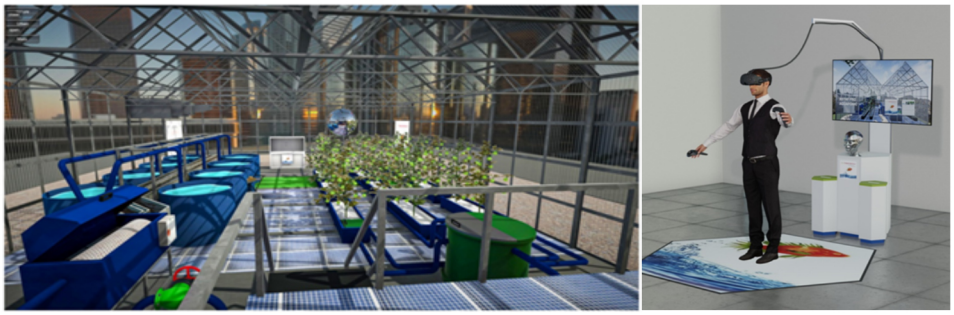

Fig. 2: Screenshot of the award-winning VR-Aquaponics game.

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METHOD TESTING TO UNRAVEL THE ECOLOGICAL IMPACT OF BACTERIA ASSOCIATED WITH CULTIVATED SUGAR KELP

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Introduction

Seaweed cultivation is rapidly developing in Europe (García-Poza et al., 2020). The cultivated brown sugar kelp (*Saccharina latissima*, Phaeophyta) has a diplo-haplontic life cycle (Visch et al., 2019). For cultivation, early life-stages are glued directly onto ropes and, while developing, attach firmly using own holdfasts until harvesting. A major cultivation challenge is the > 90 % loss of seeded seaweed. This study is part of the SEASEEDS project that aims to improve the sugar kelp attachment and as such limit such loss. One approach is to better understand the role of the associated microbiota (e. g., Proteobacteria, Bacteroidia or Bacillariophyta; Burgunter-Delamare et al., 2023; Liu et al., 2022), potentially involved in improving development and attachment. We focused on developing and improving a first method to specify bacteria associated with cultivated sugar kelp. As baseline, DNA extraction and HiSeq2000 sequencing methods were tested, also to get insight in the ratio between sugar kelp chloroplasts and bacteria. To do so, seeding material samples were provided by the Hortimare B. V. (NL) and harvest samples were provided by The Seaweed Company (NL).

Materials and methods

Sample collection
For method testing, the seeding material samples (i. e., gametophytes and early-stage sporophytes of the Dutch HNL strain) originally provided by Hortimare B. V were aliquoted in quadruplicates per three fresh weights tested (i. e., 12 samples) and stored at -20 °C. In addition, early-stage seeding sugar kelp samples (i.e., Dutch ZWG1 strain; cultivated in Autumn 2022) were aliquoted (i. e., at least 175 mg; 8 samples) and stored at -20 °C. In April 2023, late-stage sugar kelp samples from the seaweed cultivation site in Oosterschelde (NL), owned by The Seaweed Company, were harvested. For each of the four collected sugar kelp blades, 2 cm^2 discs in triplicates were cut out (i. e., 12 samples) and stored at -20 °C.

Method testing & application
Using the testing seeding material samples, the standardized DNeasy PowerSoil ProKit (ID: 47014) by QIAGEN followed by the established HiSeq2000 sequencing protocol (Indraningrat et al., 2022) were used to test the impact of 1) the sample fresh weights (i. e., 100 mg, 175 mg or 250 mg), 2) the sample dilution prior to DNA extraction (i. e., no dilution vs. diluted to 20 ng DNA/ μL nuclease-free water) and 3) the number of PCR cycles (i. e., 25, 27 or 30). Preliminary quantity and quality evaluation of this method testing (i. e., Qubit and gel electrophoresis) indicated that 175-250 mg initial sample fresh weight, no initial sample dilution and 27 or 30 PCR cycles are most promising for this study. To apply these findings, the early-stage seeding and late-stage harvest sugar kelp samples were investigated for their prokaryotic communities.

Data analyses
The Illumina 16S HiSeq 150bp amplicon sequencing data resulting of all samples was analyzed using the FastQC v0.12.0 (Andrews, 2010), the Galaxy NG-Tax2 Pipeline (Poncheewin et al., 2020), the SILVA 1.32 database (Quast et al., 2013) and R (RStudio Team, 2020).

Results

First, the higher the initial sample fresh weight (250 mg > 175 mg > 100 mg), the higher was the mean extracted DNA concentration ($p_{0.05;\ 250\ x\ 175\ mg} = 0.020$; $p_{0.05;\ 250\ x\ 100\ mg} = 0.004$; $p_{0.05;\ 175\ x\ 100\ mg} = 0.560$). Second, undiluted samples showed statistically significantly higher mean amplified DNA concentrations compared to diluted samples after running the 25-cyclic PCR ($p_{0.05;\ undiluted\ x\ diluted} = 3.16e\text{-}6$), independent on the initial sample fresh weight ($p_{0.05;\ sample\ dilution\ x\ amplified\ DNA\ x\ sample\ fresh\ weight} = 0.189$). And third, on average only 2-3 % of the total DNA reads of the undiluted testing seeding material samples were bacterial, independent on the initial sample fresh weight and the number of PCR cycles.

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Considering the number of PCR cycles, 27 cycles yielded the highest relative bacterial abundance (as compared to chloroplasts) for both, early- and late-stage sugar kelp. On average, 25 % of the total DNA reads of the early-stage seeding sugar kelp samples were bacterial and included Proteobacteria, Planctomycetota, Verrucomicrobiota and Bacteroidota. Only <1 % of the total DNA reads of the late-stage harvest sugar kelp samples were bacterial (i. e., Proteobacteria only), while 99% of the reads were sugar kelp chloroplasts. Proteobacteria only appeared throughout sugar kelp life-stages in cultivation.

Discussion

Method testing indicated that 1) using 175 mg wet weight for DNA extraction 2) no dilution of DNA before PCR, and 3) 27 PCR cycles are the most promising adaptations of the established laboratory procedures. It was successful for studying bacteria associated with early-stage sugar kelp, but highlighted limitations for late-stage-specific assessments. Specifically, for early-stage sugar kelp, the diverse taxa found are consistent with previous findings (e. g., Burgunter-Delamare et al., 2023; Liu et al., 2022; Tourneroche et al., 2020) confirming the method applicability and reliability. However, the low bacterial diversity and low relative abundance of bacteria found in the late-stage sugar kelp highlight the need of further method development. Also, to examine the interaction between cultivated sugar kelp and its associated bacteria (such as the found predominant Proteobacteria). Potentially, epiphytic microbial assessment by using sterile swabs instead of disc cutting may be more applicable (Liu et al., 2022). The high number of chloroplasts sequences could be reduced using primers amplifying the V5-V7 region (Tourneroche et al., 2020) as it prevents the amplification of microbiota-like 16S rRNA genes chloroplastic sequences (Beckers et al., 2016). Overall, fundamental understanding of proper bacteria assessment associated with cultivated sugar kelp could be fostered. This is valid for investigating their impact on sugar kelp to improve cultivation.

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MICROECONOMIC FRAME CONDITIONS AND RENTABILITY OF TYPICAL CARP FARMS IN GERMAN LUSATIA IN RELATION TO POLICY INSTRUMENTS

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Background

Lusatia, which extends partly over the German federal states of Saxony and Brandenburg, is the largest connected pond area of Middle Europe. The region has a history in carp production in man-made ponds of more than 750 years with management principles for the low-impact production having changed very little over time. Carp ponds are valuable habitats providing a variety of ecosystem services. However, changing framework conditions have caused severe economic pressure in the last three decades, and many businesses have been given up. Governance structures and policy instruments have been applied over the last decade in Saxony and Brandenburg trying to preserve carp pond farms including promotion of sustainable management practices. At the same time, only few in-depth economic analyses of production systems in carp pond farming exist. In the present study, the effects of existing regulations and policy measures on the economic efficiency of carp pond enterprises were evaluated by applying a microeconomic benchmark approach.

Methods

Typical carp production systems for the Lusatian region of Saxony and Brandenburg were defined according to the *agri benchmark* methodology (Lasner et al., 2020). Such typical farm datasets are based on real costs and prices and represent the production sector within the target region without including specific characteristics of individual enterprises. They can be used as tool to analyse short-, medium- and long-term profitability of the related sector in order to illustrate impacts of e.g. changing climatic, technical and political framework conditions. Within the present study, the datasets were used for microeconomic analyses of existing policy measures. Based on the results, economic improvement and adaptation potential of current support programs are explored in order to compensate for ecosystem services of ponds and to support long-term viability of the sector.

Results

Three typical carp farm systems were defined for the current study. In all models, fish are reared in traditional earthen ponds, producing table fish of around 2 kg/piece after a three-year production cycle.

DE-FCP-20 represents a small farm in Brandenburg, integrating nursery, grow-out and processing for a production of 20 mt carp, selling directly or to wholesalers. DE-FCP-80 and DE-FCP-200 represent two typical farm models for the region of Saxony producing 80 mt and 200 mt carp, respectively. Both DE-FCP-80 and DE-FCP-200 hatch their own fingerlings for on-growing. The carp is processed and sold directly, to the gastronomy market, to wholesalers and fish farms.

Short-, medium- and long-term profitability results with and without measures for all farm models on grow-out, grow-out with subsequent processing as well as on whole farm level revealed differences depending on farm size and specifics related to the federal states. DE-FCP-20 in Brandenburg showed the highest financial loss on long-term scale. For grow-out and grow-out including processing DE-FCP-20 was only profitable on short-term scale with subsidies, while on whole farm level no profitability could be reached independent of measures applied.

On whole farm level DE-FCP-80 and DE-FCP-200 were only profitable short-term but not on long-term scale. In the short- and medium-term DE-FCP-80 was profitable for grow-out and grow-out including processing. However, on long-term scale, grow-out without processing was only profitable with subsidies.

Grow-out including processing was profitable in DE-FCP-200, while grow-out without processing was only profitable on long-term when measures were included.

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Conclusion

The results revealed the high relevance of governance structures and policy instruments on the rentability of carp farms in eastern Germany. Measures are necessary, however still not sufficient, to secure long-term profitability on whole farm level in all three farm models. Moreover, farm size, location and fish processing seem to be of significance. As carp farming provides ecosystem services and cultural value, further development of governance and policy options could balance the mismatch between costs and returns to maintain this unique man-made landscape.

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MICROBIAL DIVERSITY ACROSS COMPARTMENTS IN AN AQUAPONIC SYSTEM

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Introduction

The aquaponic system, a combination of soilless hydroponic crop production with recirculating aquaculture, relies on diverse consortia of microorganisms (Schmautz et al. 2017; Bartelme et al. 2019), to convert nutrients from fish excreta and non-consumed fish feed into nutrients for plants (Goddek et al. 2016). In these systems, each of the components such as fish tank, biofilter, sump, hydroponic table, mechanical filters, and sludge reactors, feature unique environmental pressures, which may trigger the formation of different microbial communities (Bartelme et al. 2018). Most attempts to characterize the microbial communities of aquaponics have been focused on the sampling of the water column and biofilter material (Blancheton et al. 2013; Hu et al. 2015; Schmautz et al. 2017; Eck et al. 2019). However, little consideration has been given to the microbial populations that may be developing in other compartments of such systems.

Methods

Three identical aquaponic systems, run in parallel over a 12-week period, were used to investigate the microbial community composition during three cycles of lettuce production in the greenhouse facilities of the Zurich University of Applied Sciences (Wädenswil, Switzerland). A community pattern based on amplicon sequencing of bacterial and archaeal 16S rRNA genes was generated through localized sampling of individual compartments at the beginning and the end of every lettuce growth cycle. Samples were taken directly from the fish tank (FTW), sump (HPS), and hydroponic table (HTS) surface using a cotton swab, as well as the moving-bed biofilter material (BFO) by collecting around 20 biocarriers. The roots (ROT) were sampled by cutting a few fine roots from three randomly selected lettuce plants from each replicate system. Fish feces (FEC) were obtained by stripping one anesthetized fish per system. To obtain samples of the microbial community present in fresh sludge (FRS), digested sludge (DIS), and the supernatant of digested sludge returned to the system (RSS), 1.5 mL of each was collected.

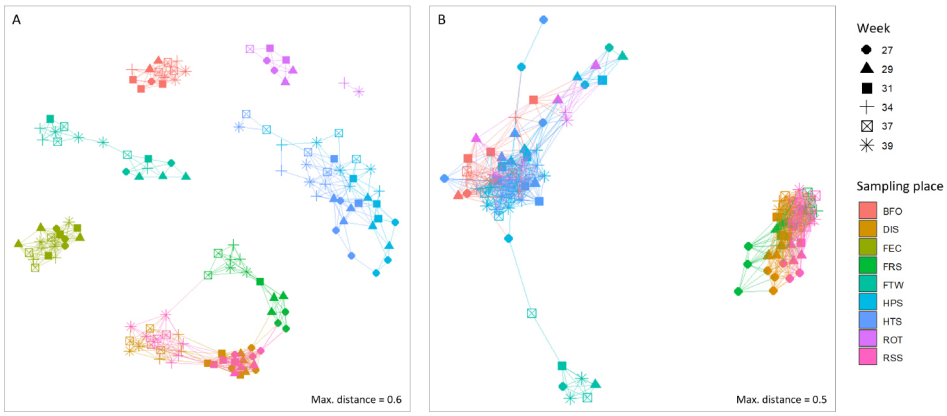

Figure 1. Network clustering using Bray-Curtis distance between different compartments of the aquaponic system, where bacterial primer set (A) could predict the origin of the sample with less than 5% error rate and archaeal primer set (B) with a more prominent 38% error rate.

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Results and discussion

Results indicated higher overall population diversity for bacteria than archaea, particularly in aerobic compartments, with lower diversity observed in fresh fish feces, disclosing a highly discrete gut flora composition (Figure 1). Furthermore, nitrifying bacteria were identified on the hydroponic tables, indicating that this compartment may play a more significant role than previously thought in the systems nitrogen cycle, transforming undesirable ammonium and nitrite to nitrate and improving water quality for the fish. Alongside the observed temporal changes to community compositions within the anaerobic compartments, higher archaeal taxa could be established in the anaerobic sludge samples. This was likely the result of the more extreme environmental pressures prevailing there. Finally, the most pronounced differences in microbial community compositions were observed between the aerobic and anaerobic loops of the system, with unique bacterial compositions established for each compartment.

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BROODSTOCK FISH SIZE AFFECTING SPAWNING SUCCESS OF BALTIC COD *Gadus morhua* IN CAPTIVITY

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Introduction

The cod (*Gadus morhua*) population in the Baltic Sea is drastically depleted due to decades of overfishing, unsustainable management and habitat degradation. Severe oxygen deficiency and decreased salinity in their spawning grounds further limited successful spawning to the Bornholm basin, which caused a decline in recruitment. To facilitate the populations' recovery, a range of measures are necessary, including the implementation of restocking programs. Baltic cod, which are genetically distinct from other cod populations, are however not successfully cultivated yet, creating the need for the development of breeding efforts. We therefore investigated the quality and quantity of the reproductive output from Baltic cod in relation to broodstock size. The results of this study will give insight into reproduction of Baltic cod, which is valuable for broodstock management to achieve high breeding success.

Material and methods

Broodstock fish of the eastern Baltic cod stock were caught between February 2021 and March 2023. At the research station Ar, Gotland, Sweden, they were separated into three different tanks according to capture time, in which they were kept in recirculating seawater (17 psu, 7 ˚C) and followed a natural light cycle. The study is carried out in 2023, from April to August, when the fish naturally spawn. Quantity of produced eggs is measured daily in each tank. Quality of produced eggs in each tank is assessed weekly, focusing on deformities, developmental stage, size and thiamin content. For each tank, 100 eggs are then transferred into a 400 mL beaker and incubated for a total of 14 days. Water change occurs daily, along with counting of dead eggs/larvae and hatched larvae. At the end of the incubation period, total survival, hatching success and quality of produced larva is assessed, including presence of deformities, length, and yolk-sac size.

Expected results

With this study, we expect to be able to describe the reproductive output of Baltic cod, a threatened population of small fish in bad condition (Ero et al., 2020), possible under controlled conditions in captivity. We will further compare the relation between Baltic cod female size and quality of reproductive output to known ratios of other cod populations (e.g., Chambers & Waiwood, 1996; Kjesbu et al., 1996). Additionally, we aim to find out whether even small sized broodstock can produce good quality reproductive output in a controlled environment with sufficient nutrient-rich feed supply.

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PROTOTYPE OF INNOVATIVE MINIMALLY PROCESSED FRESH OYSTERS (*Crassostrea gigas)* TREATED WITH COLD ATMOSPHERIC PRESSURE PLASMA

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Introduction

Oysters are abundantly harvested shellfish and are highly perishable due to their high water activity, neutral pH and chemical composition. Deterioration can be caused by enzymatic autolysis, oxidation and microbial growth of the natural spoilage microbiota. Refrigeration is used to limit the risk associated with potentially pathogenic microorganisms and to delay changes in freshness, undesirable odour, off-flavour and texture. However, maintaining low temperature throughout the supply chain can be difficult, making additional preservation measures desirable.

Post-harvest treatments based on coatings, electrolyzed or ozonated water, rapid chilling, irradiation, hydrostatic high-pressure processing, and vacuum or modified atmosphere packaging (MAP) have been investigated as additional measures to reduce the levels of pathogens and spoilage in oysters. Among the non-thermal intervention strategies, not extensively studied for fish products but considered promising for maintaining food quality and safety, cold atmospheric pressure plasma (CAP) has received increasing attention in recent years. Plasma, the "fourth state of matter," is a neutral gas containing various species such as electrons, ions, reactive atoms, free radicals, neutral molecules, and photons in a metastable state with a roughly zero net electrical charge. If enough energy is added to a gas or gas mixture, it generates a plasma that produces a wide range of unique species, including reactive oxygen and nitrogen species (ROS and RNS) and UV radiation. These reactive species play a crucial role in microbial inactivation by damaging microbial DNA and causing oxidative damage to cell envelopes and membranes. The CAP configuration and processing conditions must be optimized to maintain nutritional and quality characteristics, depending on the food matrix composition and chemical-physical properties. Plasma technology is a smart, green, non-thermal technology with relative advantages in extending the shelf-life of various foods, with limited side effects on quality parameters thanks to the low treatment temperature.

The aim of the present research was to develop a prototype of fresh oysters treated post-harvest with CAP. The setup of protocols and identification of the best performing conditions were based on a preliminary screening by modulating different processing variables such as the feeding gas (argon or atmospheric air), the main reactive species generated during the treatments (ozone or NO_x), and the processing time. The effects of the different conditions were checked by evaluating the reduction of the natural spoilage microbiota and retention of nutritional and quality features of the treated products. The selected conditions were then assessed through shelf-life tests by combining CAP treatments with two different MAP conditions.

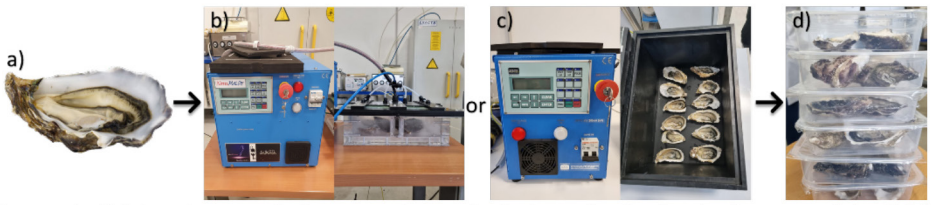

Figure 1. Main phases of the experimental design: fresh shucked oyster (**a**), CAP prototype used for atmospheric air or argon gas treatments (**b**), CAP prototype used for treatments generating ozone or NO_x reactive species (**c**), oysters packed in two MAPs (**d**).

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Materials and methods

Pacific oysters (*Crassostrea gigas*) (shell length 11±1 cm) were harvested from France and transported to the laboratory under refrigeration within 24 hours. The live oysters, after cleaning and draining excessive drip solution, were shucked and the meat left on one side of the valve used for experiments. Two CAP prototype were used (Figure 1):

- with argon gas or atmospheric air and treatment times of 20 and 45 minutes,
- with ozone or NO_x reactive species with treatment times of 20 and 45 minutes.

Untreated (control) and treated oyster samples were analysed for quality parameters (pH, dry matter, oxidation, texture, colour and sensory proprieties) and natural microbiota (e.g., *Pseudomonas* spp., aerobic mesophilic and psychrotrophic bacteria). After plasma treatment, oysters were packed in high-density polyethylene (HDPE) boxes and high barrier film under two MAP conditions (20% CO_2 + 80% N_2 or 80% CO_2 + 20% O_2) and stored at 4 °C as an additional intervention to prolong the product shelf-life. Microbial load and pH were analysed at storage times of 0, 3, 7, 10, 15, 21, and 28 days, while the other quality controls were carried out up to 7 days of storage.

Results

During the application of cold plasma at atmospheric pressure to fresh oysters, different processing parameters were selected to control the generation of reactive species that can significantly affect the quality of the final product. The pH, dry matter and texture of the innovative oysters showed no significant changes in most cases immediately after the CAP treatments. However, after 45 minutes of treatment, the oysters showed a darker colour, regardless of the feed gas, which affected their visual quality. Despite this effect, the CAP treated oysters scored very well overall in terms of sensory freshness. Nutritional quality, assessed by lipid oxidation, showed that the 20-minute treatment with argon did not affect this parameter, while the 45-minute treatment with argon, ozone and NO_x increased lipid oxidation, also if below the expected limit for seafood.

From a microbiological perspective, CAP treatment with argon gas resulted in an immediate reduction (1-2 Log CFU/g) of *Enterobacteriaceae*, aerobic mesophilic and psychrotrophic bacteria. Similar results were obtained after treatments with ozone as reactive species, while those generating NO_x mainly delayed and limited the extent of cell proliferation over storage of the microbiota that survived the treatments. Overall, the chosen CAP processing conditions in combination with MAPs helped to maintain the microbiological quality of the product within acceptable limits.

Conclusions

In summary, the results of the study showed promising effects of cold gas plasma treatment on some of the microbiological and physicochemical parameters investigated, with synergistic effects derived from the use of MAPs. However, given the inherent variability of the raw material and the multitude of parameters that need to be adjusted to optimize the quality and stability of the oysters during transport or storage, further studies are needed before considering actual industrial application of CAP. In addition, it is important to consider eventual potential toxicological concerns in future studies.

Acknowledgements

This research was conducted under the NewTechAqua project supported by the European Union's Horizon 2020 Programme (grant agreement No 862658). The project aims to expand and diversify European aquaculture production by developing and validating technologically advanced, resilient, and sustainable applications for finfish, molluscs, and microalgae.

WATER PURIFICATION THROUGH MICROALGAE AQUACULTURE - ANALYTICAL AND TOXICOLOGICAL EVIDENCE OF THE REDUCTION OF TOXIC POLLUTANTS IN WATER FROM SEALED SURFACES

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Introduction

On biogas plants, large areas are sealed (silo and driving surface) on which water accumulates primarily due to precipitation. This water from sealed surfaces (WSS) often contains pollutants as heavy metals or organic pollutants, as well as increased nutrient concentrations. Hence, it cannot be discharged directly into receiving waters. The collection, storage and ultimately regeneration of the polluted water is quite cost intense. However, the use of the nutrients and trace elements in WSS in cultivating algae biomass - sustainable value creation through production of economically usable microalgae biomass - could remedy the situation. Microalgae aquaculture can serve as bioremediation method to improve the water quality of WSS.

Materials and Methods

A microalgae-cyanobacteria mixture, resistant to germ pressure from WSS, was established and adapted to the WSS of the reference biogas plant. WSS was used as cultivation medium in laboratory trials to evaluate the cleaning performance of the microalgae mix in regards to several environmental pollutants. Further the mix was cultivated on WSS in a semi-continuous production with an algae reactor directly at the biogas plant. The water cleaning effect of the microalgae cultivation was analytically quantified looking at contaminants such as Pb, Cd, As, Hg, polychlorinated biphenyls (PCB) and mineral-oil-derived hydrocarbons (MOSH/POSH and MOAH). Which are the pollutants identified in preliminary tests to mainly occur in WSS from biogas plants.

Single water polluting substances can be analytically quantified and toxicologically evaluated regarding their effects on diverse organisms. However, the real toxicological effects of the exposure to the simultaneous presence of many different substances can still only be inferred to a very limited extent. So in addition to analytics, the water cleaning effect was assessed toxicologically. Therefore, the possible reduction of the mutagenic activity of the WSS after the algae cultivation was quantified with *Salmonella typhimurium* TA 100 in Ames Test.

Results

The results show that primarily heavy metals and PCB are cleaned from the WSS through the microalgae cultivation. While lead was cleaned from the water by up to 50% (fig.1), cadmium and individual polychlorinated biphenyls were cleaned by up to 60% after the algae cultivation.

Conclusion

Microalgae aquaculture in WSS can not only produce economically usable biomass but also significantly reduce individual pollutants in the water. Further, scientific assessment of mixed exposure effects on organisms is still very difficult and complex, but the use of contaminated natural matrices as WSS in toxicological tests might give an initial insight.

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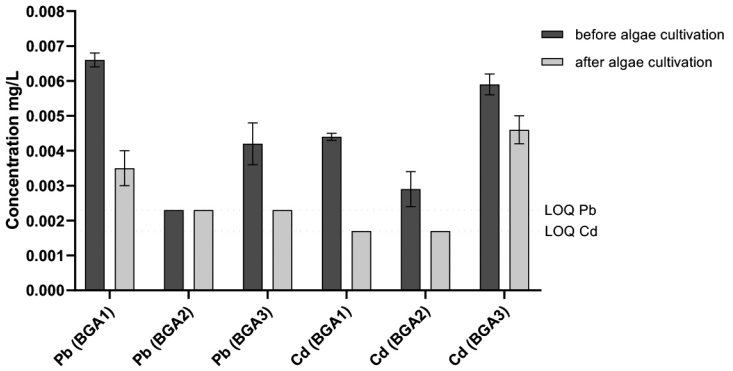

Fig. 1: Reduced concentration of Pb and Cd in the WSS from before to after the cultivation of the algae mixture for 14d in laboratory conditions.

As for the assessment of the mutagenic properties of the WSS the results show significant reduced revertants for the WSS in which the microalgae mixture was cultivated previously (fig. 2).

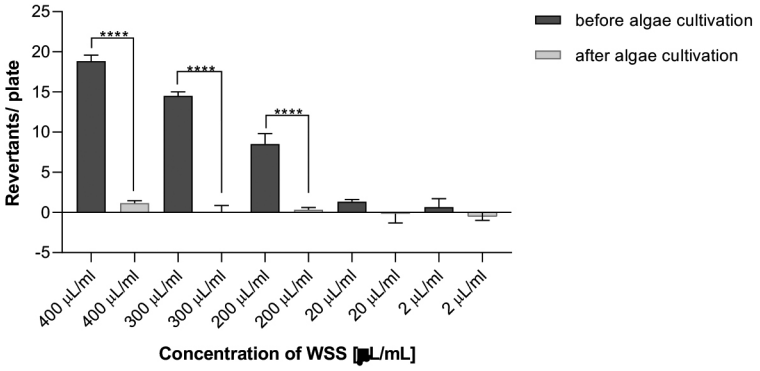

Fig. 2: Number of TA 100 revertants/ plate (corrected by negative control group (WSS 0 µl/mL)) when incubated in WSS concentrations of 400, 300, 200, 20 and 2 µL/mL with the addition of S9 cell lysate. Each comparison includes the same concentration of water from the WSS before and the after the microalgae cultivation for 14 d in the algae reactor (****=$p<0.0001$, n=3).

NEAR INFRARED SPECTROSCOPY AS A NOVEL NON-INVASIVE TOOL IN AQUACULTURE

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Introduction

In the last decades aquaculture production has expanded rapidly, due to the increasing demand for human nutritional purpose. Infectious diseases strongly impact on aquaculture sector. Lactococcosis, sustained by the warm-water pathogen *Lactococcus garvieae*, is a disease associated with high mortality and economic losses (1). Main symptoms are mono or bilateral exophthalmia, haemorrhages around the eye area, opercula, and mouth region, swollen abdomen, and anal prolapse. Since traditional laboratory investigation techniques are time consuming, expensive, and have limited sample flow, an effective system of surveillance activities on fish diseases is needed, that makes use of rapid, in situ and low-cost diagnostic tools. Near Infrared Spectroscopy (NIR) technique is an example of phenotypic approach for early diagnosis. Through this practice, it is possible to obtain information, directly by in-situ analysis, on the structural properties of matter, studying the interaction with electromagnetic energy, reducing considerably time and costs. This sector has found application in many fields (2), but not yet used for diagnostic purposes in aquaculture. Aim of this work was the evaluation of the potential of portable NIR, in particular SCiO miniaturized technology (3), in the aquaculture sector, to discriminate the health *status* of fish.

Materials and methods

Analyses were carried out in a natural outbreak of lactococcosis in rainbow trout, located in Nothern Italy. Two categories of samples were used: 20 symptomatic and 20 asymptomatic trout. SCiO acquisitions were performed directly on site. Prior to acquisition, excess of water and mucus were removed from each individual. Trout were submitted to measurement three times, scanning them on a flat bench top, with the device placed over the fish. Scans were initially acquired in one specific point on the dorsal and the abdominal area (caudal to the perianal area) to account for possible variations. Each sample, after morphometrical parameters evaluation, was subjected to necropsy to highlight the presence of pathological alterations and/or lesions of internal organs. Bacterial isolation was carried out from kidney and spleen, in parallel with specific PCR amplification and sequencing to determine presence of *Lactococcus garvieae*.

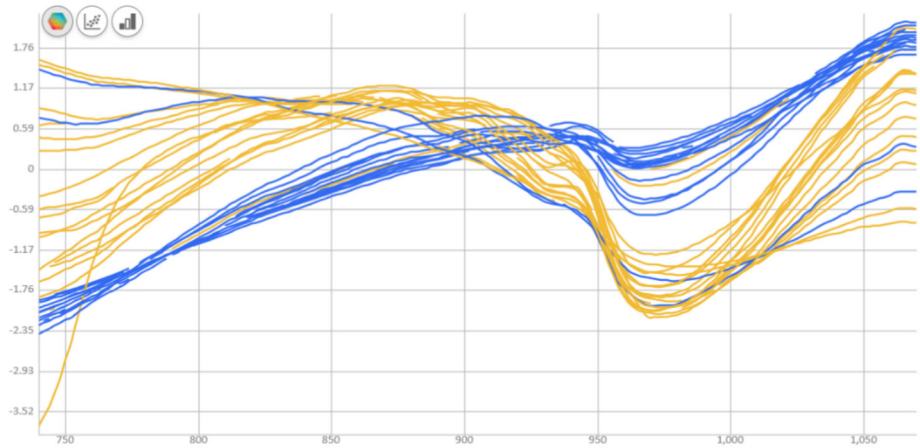

Figure 1. SCiO spectrum of the trend of healthy trout (yellow) and sick trout (blue).

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Results and Discussion

SCiO acquisitions provided more reliable results in the proximity of the abdominal area. SCiO obtained very good results, with sensitivity of 0.95, specificity of 0.87 and accuracy of 0.90. There are various hypotheses for the mechanism by which the device proposes differential spectra between sick and healthy individuals, but the most plausible one is the fatty acids profile of analysed tissues. Moreover, it is known that lactococcosis induces severe inflammatory and haemorrhagic lesions in the intestine and in the surrounding abdominal fat (4), which could be detected by the SCiO device as alterations of biochemical properties in affected tissues.

Conclusions

Results demonstrate high performance of SCiO to discriminate between healthy and sick animals. The rapidity of acquisition, low cost, and the possibility to use the device directly on site, preserving animal welfare, make the SCiO a prognostic tool useful to implement control on animals, in order to avoid economic losses.

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SEASONAL VARIATIONS OF GONADOSOMATIC AND MUSCLE INDEX OF *Pecten jacobaeus* L. FROM KRKA RIVER ESTUARY (CROATIA) – IMPLICATIONS FOR AQUACULTURE

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Introduction

Today, most of the shellfish products on the market comes from aquaculture, but this offer is often limited to a small number of species, so there is a need to introduce new species. The Mediterranean scallop *Pecten jacobaeus* (Linnaeus, 1758) is the largest bivalve of the Pectinidae family that lives in the Mediterranean and Adriatic Sea. Due to its size, fast growth, and high market demand, *P. jacobaeus* seems like a potential aquaculture candidate. Aim of this study was to provide information on some of the biological characteristics of *P. jacobaeus* from the Krka River estuary, through analysis of seasonal variations of some physiological indices, that can be of use for the potential introduction of this species into the commercial aquaculture in the Adriatic Sea.

Materials and methods

In the period from December 2021 to May 2023, approximately 20 specimens of *P. jacobaeus* were collected monthly from the Krka River estuary. Shell dimensions (length, height, and width (cm)), and shell and soft tissue mass (g) were measured, in wet and dry state. Based on the obtained measurements, water content (WC) and gonadosomatic index (GSI) and muscle index (MI) were calculated according to Lucas and Beninger, 1985:

GSI= gonad dry weight (g)/ total tissue dry weight (g) x 100,
MI= muscle dry weight (g)/ total tissue dry weight (g) x 100.

Results

Soft tissue WC was high (~82%) but varied seasonally and by soft tissue parts. Gonads' WC was lowest in early winter, and highest in summer period, while muscle's WC was highest in spring, and lowest in summer period.

Results of GSI suggest that there were three spawning periods in a year, the main one in early winter/spring season that lasted until summer, during which gonads seemed to be dormant. Second, shorter spawning period was in early autumn, and the possible third one was in early winter.

GSI showed significant inverse correlation to MI, which was highest during summer period, while lowest in early spring, when gonads were fully developed. GSI also showed significant inverse correlation to gonads' WC, while MI showed significant inverse correlation to muscle's WC.

Discussion

Gonadosomatic index is used for assessment of the reproductive cycle which can provide valuable information for collection of spat from nature or for conditioning of broodstock. Muscle index can indicate the state of nutrient reserves in the muscle, the most sought-after part of scallop meat, and can thus be used to estimate its quality for the market. Inverse correlation of GSI to MI, suggests that partitioning of energy is divided between storage of nutrient reserves in the muscle and gonads development. Water content in the gonads and muscle, can provide additional insights into gonads and muscle state, as an indicator of gonads ripeness or of muscle nutrient reserves.

Conclusions

For the possible aquaculture development, the most favourable period for setting up collectors for wild spat collection, as well as for collecting and conditioning of broodstock under laboratory conditions, would be during spring season.

For the market and consumer consideration, we suggest that harvest of natural populations would be best during summer season when the muscle is at its highest size and nutritional value.

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IS TAGGING OF BLUEFIN TUNA *Thunnus thynnus* FOR THE PURPOSE OF INDIVIDUAL GROWTH ESTIMATION IN CAGE FARMING FEASIBLE?

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Introduction

Tuna farming and fattening play an important role in Mediterranean aquaculture production and the tuna sushi sashimi industry. To preserve the tuna stock, the International Commission for the Conservation of Atlantic Tunas (ICCAT) establishes mandatory management measures to regulate fishing effort, catch, and farming capacity, and allocates annual state quotas (Fromentin, 2006). To better control Bluefin tuna farming/fattening practices, the Commission requested in 2018 an update on farmed fish growth rates, i.e., determining the growth of individual fish under farmed conditions. Therefore, the objective of this study is to present the novel tagging method, used for the first time in tuna farming, with the derived results of individual fish growth during the 18-month farming cycle.

Materials and methods

The tagging experiment targeted 2-3-year-old fish (> 8 kg) caught for commercial farming purposes and then kept under standard farming conditions for 18 months. In July 2019, a total of 206 tuna juveniles between 7.5 and 25 kg were PIT tagged in the head muscle. The fish were individually weighed, measured, and distributed in equal numbers in the two experimental cages for farming. During the farming cycle, fish growth was monitored seasonally for each cage using a stereo camera and image analysis system. All fish were harvested at the end of the season and subsequently measured individually.

Results and discussion

A total of 206 fish distributed between two cages were tagged with PIT tags, most of which were successfully captured at harvest, allowing 157 individual growth rates to be determined. PIT tagged fish were mostly evenly distributed across all size classes in the experimental cages. During the farming cycle, juvenile Bluefin tuna reached the overall harvested weight between 58 and 64 kg. The average 500% increase in body weight during the experimental period did not differ between two experimental cages or between tagged and untagged fish. Recorded mortality of tagged fish throughout the farming period was negligible (1%). The unrecorded proportion of tagged fish (22%) could be due to a combination of factors, such as detector and reader failure due to routine harvest procedures. The PIT tag appears to be biologically inert, as there is no evidence of tissue inflammation in the wound area. In summary, an experienced and trained team is capable of performing mass tagging of juvenile tuna for scientific research purposes with acceptable losses.

Acknowledgments

Financial support for this study has been provided by Atlantic-Wide Research Programme For Bluefin Tuna (ICCAT GBYP) (Phase 10).

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DRIVING global CHANGE IN HOW SHRIMP PRODUCERS MANAGE PATHOGENS AND RISK DURING CULTURE

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Introduction

The concept of pathogen presence resulting in reduced production performance of livestock and aquatic species has been embodied in text books for decades. Despite this, many shrimp producers often don't understand and/or have not been able to quantify the true impact of multifactorial pathogen presence, load and prevalence on production. This is understandable when you consider that only until recently were the tools available for accurate multiple pathogen detection at an affordable cost and that shrimp can have any number of 13 commercially relevant (impactful) pathogens, often harbouring 3-4 of these at any one time. Establishing data points that give farmers a quantitative pathogen profile of their crop over time empowers them with data to quantify the true impact of pathogens on culture and on which to make management decisions that can change the outcome and profitability of a crop.

This study will present case studies from around the world on how the application of the multiple pathogen detection platform Shrimp MultiPath has re-defined how farmers think about pathogens, assess pathogen loading and manage pathogens throughout the production cycle.

Materials and methods

Statistically significant sample plans are designed with farmers to allow sampling of appropriate tissue types in a timely manner for testing on the Shrimp MultiPath system. Tissue types that provide accurate detection of virus, bacteria and microsporidians are pooled together for testing. A typical grow out farm samples at postlarvae stage 10, 25 days of culture 50 days of culture and 70 days of culture for early pathogen detection to characterise pathogen presence and prevalence before having clinically sick animals. This provides up to four weeks early warning of pathogen presence and prevenance during which time a farmer can change management protocols to maximise crop outputs and minimise pathogen impact. Management decisions include actions such as increased biosecurity, reduced shrimp stress and feeding of alternative diets depending on the pathogen.

Results

Latin America, for example are using this knowledge and technology as part of their broodstock selection program resulting in a 10-15% improvement in production and a 10% improvement in fertility. Asia-Pacific farmers utilise the technology to monitor pathogen presence and prevalence during grow-out to provide an early warning that allows simple but smarter management practices to be applied on a case-by-case basis.

EVALUATION OF 20 YEARS OF MONITORING DATA TO CORRELATE ENVIRONMENTAL AND BIOLOGICAL FACTORS RELATIVE TO SHELLFISH PRODUCTION – A NORTHERN IRELAND CASE STUDY

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Introduction

Shellfish are potentially hazardous foods, particularly when consumed raw, as in the case of oysters, and as a result can be the origin of foodborne illnesses due to the proliferation of pathogenic microorganisms or the build-up of natural toxins. Live bivalve molluscs, as filter-feeders, can bioaccumulate various harmful substances or marine contaminants prevalent in coastal estuarine regions that have been significantly impacted by human activities (Ferreira et al., 2018; Zuykov et al., 2013). Consequently, the biomonitoring of shellfish is crucial not only for ensuring food safety but also as a bio-indicator to keep track of waterborne pollutants. Currently in Northern Ireland, environmental factors affecting shellfish production are monitored and tested for separately using a diverse range of methodologies. On top of that, data obtained from these analytical approaches is often reviewed in isolation, whereby any related synergistic or antagonistic effects in the natural environment may go unnoticed.

Methodology

A historical compilation analysis was carried out to examine past surveillance data collated by the Food Standards Agency related to the coastal and estuarine waters of Northern Ireland across two decades, from 2001 to 2022. A total of 26,182 samples over seven water bodies in Northern Ireland were evaluated. The factors studied were phytoplankton species *Alexandrium* spp., *Dinophysis* spp., *Pseudo-nitzschia* spp.; *Escherichia coli* counts in shellfish flesh as a sentinel species for seawater quality; heavy metals (cadmium, mercury, lead); polycyclic aromatic hydrocarbons (PAHs); and marine biotoxins causing Diarrhetic Shellfish Poisoning (DSP), Azaspiracid Shellfish Poisoning (AZP), Paralytic Shellfish Poisoning (PSP) and Amnesic Shellfish Poisoning (ASP). Figure 1 shows the percentage of samples evaluated per environmental contaminant category. Additionally, chemical contaminants in water, biota and sediment samples monitored under the Water Framework Directive (European Parliament and Council of the European Union, 2000, 2020) and the Clean Seas Environmental Monitoring Programme (CSEMP) by the Department of Agriculture, Environment and Rural Affairs (DAERA) were also evaluated.

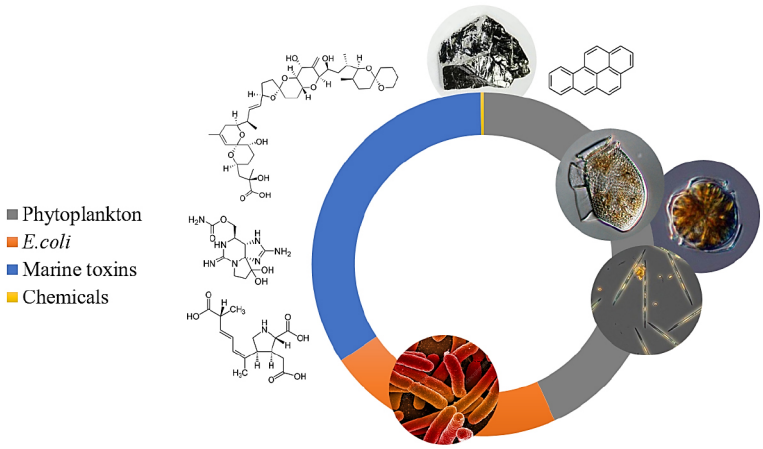

Fig. 1: Percentage of sample entries evaluated across datasets provided by the Food Standards Agency in Northern Ireland between 2001 and 2022 per environmental monitoring category.

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Results

Northern Irish Sea waters have historically presented and still hold good biological and chemical status for shellfish production. Phytoplankton and *E. coli* counts were generally below trigger levels. Positive incidents for marine biotoxins and chemical contaminants above regulatory limits in the region were extremely low, suggesting NI aquaculture production sites are an ideal safe location to sustainably develop the sector further.

Conclusion

This review provided a baseline reflecting the current status of information from a public health perspective. Our examination of historical data revealed gaps and inconsistencies, limiting its utility for tracking public and environmental health trends. Nonetheless, the data collected by environmental monitoring agencies still holds high value as it did allow for points in time analysis which may or may not be subject to change.

In order to create a coherent picture of marine contaminant trends for NI marine aquaculture moving forward, we need a structured, long-term monitoring strategy that incorporates more frequent and diverse data. Such an approach would bolster our Blue Growth Strategy by ensuring sustainable growth in the marine sector. As technology evolves, we must correlate analysis methods from different periods, helping future researchers and facilitating the development of predictive models, possibly through artificial intelligence. We suggest increased investment in rapid screening tests and citizen science programmes to facilitate frequent sampling. Simultaneously, sharing and centralising environmental data will empower us to address climate change and public health concerns more effectively, thereby contributing to a robust aquaculture strategy for Northern Ireland with potential for global application. By adopting a more holistic approach to monitoring, we can resolve current challenges and shape the future of sustainable aquaculture and food safety standards.

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EFFECTS OF FEEDING STIMULANT ON APPETITE, FEEDING BEHAVIOR, AND FEED INTAKE IN YELLOWTAIL (*Seriola quinqueradiata*)

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Introduction

Yellowtail (*Seriola quinqueradiata*) is a large oceanodromous fish and an important Japanese aquaculture fish species with strong carnivorous characteristics (Masumoto, 2002). Recent global developments in aquaculture have led to the depletion of resources such as sardines, the main feed source for aquaculture fish. Fish meal substitution with plant proteins has been recommended for sustainable aquaculture; however, feed intake has decreased because of the low preference for plant proteins in yellowtail. Mixtures of alanine (Ala), proline (Pro), and inosine 5'-monophosphate disodium salt hydrate (IMP) have been reported as feeding stimulants for yellowtail in non-fish meal diets (Senzui et al., 2020). Behavioral and physiological approaches are required to elucidate the mechanisms underlying this increase in feed intake. However, few studies have investigated yellowtail feeding behavior and appetite hormones. Therefore, the mechanism of increased feed intake is relatively unknown. The purpose of this study was to investigate feeding behavior, orexigenic hormone expression, and feed intake in response to feeding stimulants (Ala, Pro, and IMP) in yellowtail.

Materials and methods

Yellowtail was reared in fiber-reinforced 200 L tanks supplied with seawater maintained in a continuous flow-through system with a natural photoperiod. The stimulus solutions used consisted of 10 mmol each of Ala, Pro, and IMP. [Appetite] Hypothalamus was collected before the addition (0 min) and at 10 min and 30 min after the addition of the stimulus solutions ($n = 8$ at each point). Gene expression of the orexigenic hormone (neuropeptide Y [NPY]) was measured using RT-qPCR. [Feeding behavior] Two weeks before the feeding behavior test, fish were acclimatized to a commercial floating extruded pellet (EP) diet. The control was a water-soluble fraction of soy protein concentrate (SPC) that yellowtail showed a low preference. Cotton puffs soaked in the stimulus solutions were placed in baskets in the tanks, and feeding behavior was recorded for 10 min. Instances of "search" (moving to the upper zone of the tank) and "bite" (pecking at the water surface) were recorded using an action camera, and the frequency was counted. [Feed intake] After the feeding behavior test (15 min after adding the stimulus solution), a commercial floating EP diet was fed until satiation and feed intake was measured.

Results

[Appetite (Fig. 1)] Hypothalamic *npy* mRNA expression significantly decreased after Ala addition compared to that at 0 min (before addition). However, *npy* expression was significantly increased by IMP addition compared to that at 0 min. No change was observed in the *npy* mRNA expression for Pro addition. [Feeding behavior (Fig. 2)] The mean frequencies of "search" for control, Ala, Pro, and IMP were 169.0 ± 29.7, 458.01 ± 39.3, 420.7 ± 30.3, and 397.0 ± 5.3, respectively. The frequencies of "search" for Ala, Pro, and IMP were significantly higher than that for control ($P < 0.05$). The mean frequencies of "bite" for control, Ala, Pro, and IMP were 3.7 ± 2.7, 8.0 ± 1.2, 33.7 ± 5.7, and 6.0 ± 2.3, respectively. The frequency of "bite" for Pro was significantly higher than that for the other groups ($P < 0.05$). [Feed intake (Fig. 3)] The amounts of feed intake after addition for the pure water (vehicle), Ala, Pro, and IMP were 6.2 ± 0.38 g, 4.1 ± 0.41 g, 6.1 ± 0.48 g, and 7.6 ± 0.48 g, respectively. The feed intake of Ala was significantly lower than that of the other, and the feed intake of IMP was higher than that of the vehicle.

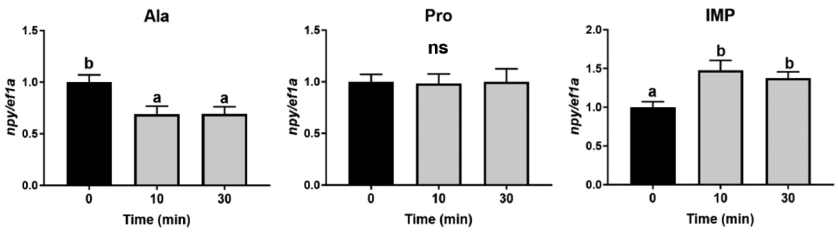

Fig. 1. Hypothalamic *npy* mRNA expression levels after Ala, Pro, and IMP addition to the rearing water ($n = 7$–8 at each point). The *npy* expression levels are shown as 0 min before stimuli solution and 10 min and 30 min after addition. All measurements were standardized using *ef1a* mRNA expression levels. Different letters indicate significant differences ($P < 0.05$).

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Discussion

The results of the feeding behavior and appetite tests suggested that Ala decreased feed intake by decreasing *npy* expression in the hypothalamus, the center of appetite. Pro increased "bite" but did not affect hypothalamic *npy* expression and feed intake. These results suggest that IMP increases feed intake by increasing hypothalamic *npy* expression. Ala, Pro, and IMP increased feeding behavior "search," and Pro increased "bite," but only IMP increased feed intake. This suggests that there may be a weak relationship between feeding behavior and feed intake. In contrast, Ala decreased feed intake along with hypothalamic *npy*, whereas IMP increased feed intake along with hypothalamic *npy*. These findings suggest that hypothalamic *npy* levels and feed intake are related.

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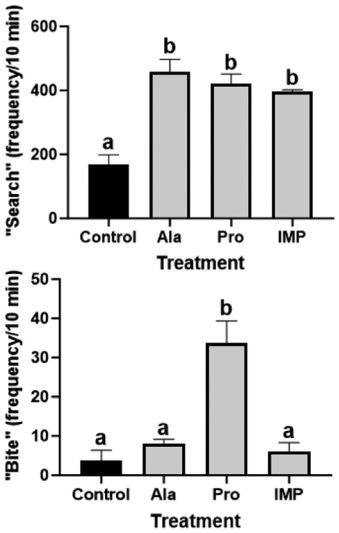

Fig. 2. Mean values of "search" and "bite" over 10 min in four test sections ($n = 3$). Frequency of feeding behaviors counted after added SPC soluble fraction (control), alanine (Ala), proline (Pro), and IMP solution (10 mmol/20 mL). Different letters indicate significant differences ($P < 0.05$).

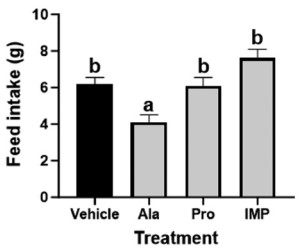

Fig. 3. Averages of feed intake 15 min after addition of cotton puffs soaked in pure water (vehicle), alanine (Ala), proline (Pro), and IMP solution (10 mmol/20 mL). Different letters indicate significant differences ($P < 0.05$).

EXPERIMENTAL VALIDATION OF A BIOPHYSICAL POND MODEL

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Introduction

Design and planning of pond aquaculture require a unified, quantitative pond model that can be linked also with the simplified quantitative models of neighboring areas of different land use. Considering the labor-consuming and expensive experimentation of individual production ponds, in this study we present the reusability check-based improvement of a formerly validated production pond model (Varga et al., 2020), applying the modeling framework of Programmable Process Structures (Varga and Csukas, 2022).

Materials and methods

The testing and improvements include both the reduced and the extended use of the reference model (i.e., appropriate reduction and extension of a selected reference model to describe the various pond managerial cases e.g., natural fishpond with no feeding and manuring, manured pond, manured and foraged pond with optional artificial fertilizer, etc.), and its application for the model-based scaling-up.

To check and improve the reference model, data generated during the vegetative seasons of 2021 and 2022, from carp-rearing experiments conducted in frequently monitored pilot ponds of MATE AKI HAKI, Szarvas, Hungary were used. This included data on stocking and harvesting of fish (mainly carp); on manuring, fertilizing, and feeding strategy; on manually measured and sensor-based water quality; on the concentration of food web elements (zooplankton, Chl-A represented phytoplankton); as well as about meteorology (Sharma et al., 2023). In order to pre-process this data, Matlab® Data Cleaner was utilized, and the moving median smoothing approach with a smoothing factor of 0.25 was also applied. The normalized root mean square error (NRMSE, %) was used to calculate the average deviation of simulated and calculated data in the absence of knowledge of the obvious sampling errors.

Results & Discussion

In the first phase of the suggested stepwise reusability check, each step ended with the necessary refinement and testing of the refined parameters or prototype models, while the already made improvements were fixed for the further steps. A simplified structure of the investigated model was created showing the contribution of the various nutrients (e.g., from feed, manure, and, inorganic fertilizer) to the food web of the produced carp. Finally, a hypothesis-based extension was suggested to distinguish phytoplankton's eukaryotes and cyanobacteria groups. After iterative testing and verifying computations in multiple steps, a unified model was created for a wider range of ponds, from natural lakes to heavily stocked and manured ones. The input files, describing the structure, the input data, and parameters, as well as the prototyped local programs of the applied Programmable Process Structures, can be found in the contributing Mendeley database (Sharma et al., 2023). The obtained large NRMSE values contain both sampling & measurement and model errors. The reusability checking procedure brought attention to the even more conscious planning of experiments. In order to develop the strategy for sampling and measurements, which must comprise a small but essential number of spatially distributed and simultaneous measurements, the experimental design must involve the model specialists and some preparatory modeling. The inclusion of the pond history and the comprehensive set of initial concentrations are quite crucial since food web models in particular are very sensitive to the initial conditions.

The resulting model was also tested for model-based scaling-up of production ponds in the knowledge of the very limited amount of case-specific input data. In addition to the estimated approximate fish production, the up-scaled simulation can give estimations also about the other characteristics and environmental impacts of the production pond (e.g., for the necessary water supply, nutrient emission, O2 production and consumption, CO2 sequestration, and emission, etc.).

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Acknowledgments

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COMPONENTS OF BLUE WHITING (*Micromesistius poutassou*) FISH WASTE AS GROWTH FACTORS FOR GUT-FRIENDLY BACTERIA

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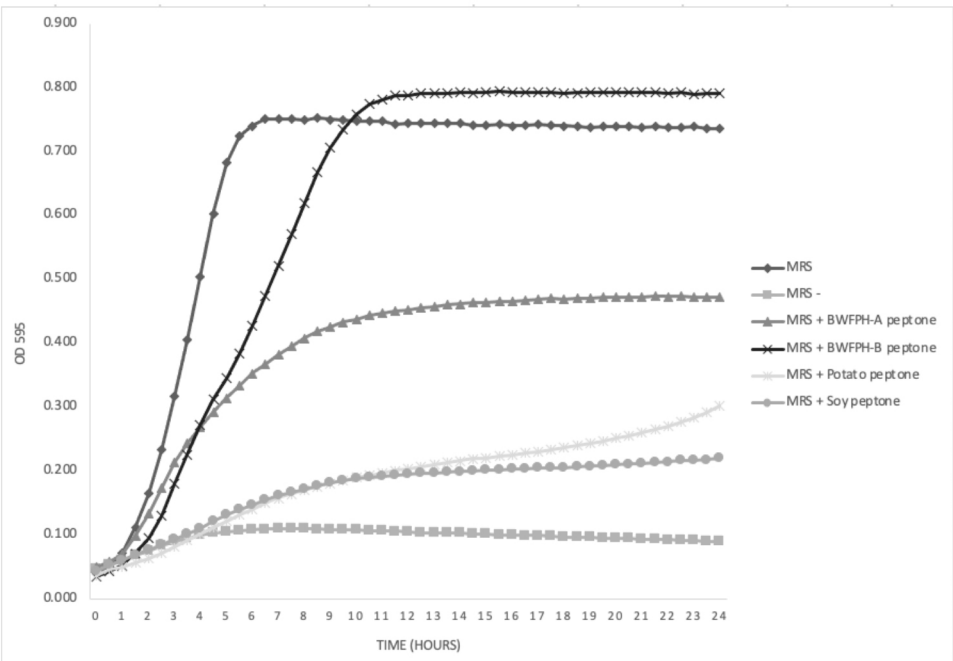
Introduction

Protein hydrolysates enzymatically derived from under-utilised fish waste can potentially serve as valuable sources of dietary protein. Although oligosaccharides are the most commonly utilized prebiotics, the prebiotic properties of enzymatically produced peptides in the digestive system are receiving attention for the development of functional foods [1]. Lactic acid bacteria (LABs) may use the free amino acids and peptides found in fish waste as nitrogen sources, allowing them to grow and multiply [2]. Additionally, the use of fish peptones as a suitable and economical substitute to currently available meat-derived peptones of pig or cow origin is being investigated. This chapter explores the development, advantages, and possible uses of prebiotics derived from fish waste as functional foods.

Materials and methods

Bio-marine Ingredients Ireland Ltd. (Lough Egish Food Park, Castleblaney, Co., Monaghan, Ireland) developed two products from frozen blue whiting (BWFPH-A & BWFPH-B). Following homogenization, the substrate was subjected to proteolysis by a microbial enzyme at a temperature of 50°C. Following hydrolysis, the soluble fraction (BWFPH-B) was separated from the insoluble fraction (BWFPH-A), and then spray dried and milled respectively to obtain powders. A microplate assay for seven lactic acid bacterial cultures was employed where their growth was measured after incubation at 37ºC overnight at 595 nm. The growth media deMan, Rogosa and Sharpe (MRS) was modified by substituting the nitrogen content with peptones derived from the hydrolysates. These results were compared to their growth in presence of commercial nitrogen sources like tryptone and peptones from soy and potato.

Figure 1: Growth of *Lactobacillus reuterii* in the presence of different peptone sources



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Results and discussion

Stimulated growth was observed in most bacteria in presence of the hydrolysates, but superiorly in BWFPH-B, when compared to the commercial nitrogen sources. An example is shown in Figure 1 where the growth of *Lactobacillus reuterii* is stimulated in the presence of peptones derived from BWFPH-B as compared to commercial MRS media or peptones from soy or potato sources. Our findings indicated that the hydrolysates produced from fish waste, which served as nitrogen sources, effectively promoted the growth of lactic acid bacteria. The hydrolysates thus have the potential to offer nutrients linked to prebiotics in addition to contributing to the economic and environmental sustainability.

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ANIMAL VERSUS PLANT PROTEIN SOURCES IN AQUAPONIC DIETS – A CASE STUDY ON NUTRIENT EXCRETION OF AFRICAN CATFISH (*Clarias gariepinus*) REARED IN RAS

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Introduction

Specialized aquaponic diets could help to reduce the need for artificial fertilizer use in on-demand coupled aquaponic systems. Following the logic of reducing the dependence on marine ingredients from capture fisheries and respectively using pertinent alternative ingredients, the present study aimed to compare the potential of a fixed combination of animal protein sources – poultry by-product meal (PM), catfish by-product meal (CM) and poultry blood meal (PBM) - with a fixed combination of plant protein sources – soybean meal, rapeseed meal, guar korma, corn gluten meal, wheat gluten and soy protein concentrate - for use in aquaponic diets that aim to provide an improved dissolved plant nutrient profile. While by-products of animal origin, particularly when high in bone content, could represent an ample source of phosphorus (P) in aquaponic diets [1], plant protein sources may provide higher levels of potassium (K) as one of the most abundant cations in plants [2]. In this sense, African catfish (*Clarias gariepinus*) were fed four different experimental diets ranging from entirely animal protein based to primarily plant protein based in a systematic RAS feeding trial in which growth performance was tracked and dissolved inorganic nutrient excretion of major plant macro- and micronutrients were compared, particularly N, P and K.

Material & methods

Four isolipidic and isonitrogenous (42 % crude protein - CP, 12 % crude fat) were designed to have a blend of animal by-products contribute 100% (A100), 75% (A75), 50% (A50) and 25% (A25) of the CP delivered through the protein ingredients in the diet with a concomitant increase of the CP contribution through a blend of plant protein ingredients. The ratio of the animal protein sources as well as the plant protein sources among each other was kept constant in all diets. All diets featured the same amount of monoammonium phosphate (0.5%) and phytase (1000 FTY/kg). Diets were fed at a daily ration of 3.8% to African catfish (initial weight 11 g) reared in recirculating aquaculture systems (RAS - 400 L system volume) in a 7-week trial (n=4). Feed rations were increased daily and individually per RAS according to determined

	Daily water exchange					N (mg/L)	P (mg/L)			K (mg/L)		
					Hoagland & Arnon (1938)	210	31.0			234.0		
					Tap water used in trial	1.29	0.03			5.58		
Diet	% of RAS volume		L/kg of feed/day									
A100	8.3	± 0.10	117.9	± 1.38		210	26.6	± 0.8	86%	10.7	± 0.4	5%
A75	8.1	± 0.16	115.6	± 2.25		210	20.7	± 1.0	67%	17.7	± 0.4	8%
A50	8.0	± 0.33	113.9	± 4.65		210	10.7	± 0.6	35%	27.5	± 0.4	12%
A25	8.2	± 0.25	117.7	± 3.61		210	4.5	± 0.1	14%	36.2	± 1.0	15%

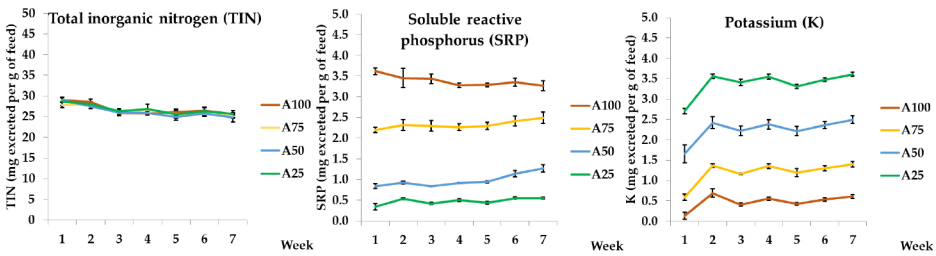

Figure 1. Excretion of major dissolved plant nutrients per unit of feed by African catfish reared in RAS.

Table 1. Modelled RAS water exchange rate required to achieve the nitrogen concentration of a standard hydroponic nutrient solution, i.e. 210 mg/L, and the consequently establishing concentrations of P and K; assumptions include a constant rearing density of 70 kg/m^3, a 2% daily feeding rate and the rearing volume making up 50% of the total RAS volume.

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feed conversion ratios (FCR). A daily water exchange of 10% was maintained and pH was kept above 6.5 by the help of daily NaOH dosing over 12-18 h. Fish growth performance was recorded and dissolved nutrients in the process water were analyzed weekly (continuous flow analysis (CFA), ICP-OES). Under certain assumption, the water exchange rate necessary to reach the N concentration of a hydroponic standard nutrient solution [3] was calculated on the basis of the determined nutrient excretion per unit of feed.

Results

Uniform growth performance between most diets was recorded with a biomass increase of 839-959% over the course of the trial and only at the maximum plant protein inclusion (A25) performance was slightly impacted; i.e. FCR and protein efficiency ratio was significantly lower for the A25 diet compared to the A75 and the A50 diet. Regarding the excretion of total dissolved nitrogen (TIN) per unit of feed, no significant differences were detected between the experimental diets, with diets occupying a range of 24.8-25.7 mg TIN excreted per g of feed (Figure 1). However, higher plant protein inclusion led to significantly elevated dissolved potassium excretion and higher animal protein inclusion to significantly elevated soluble reactive phosphorus excretion. Hence, the animal protein-based diets produced RAS process water with a better dissolved N:P ratio for aquaponics, whereas the plant protein-based diets resulted in RAS water with a better N:K ratio. At a similar RAS water exchange rate, it was modelled that the A100 diet could potentially reach 86% of the P concentration suggested by a standard nutrient solution and the A25 diet 15% of the respective K concentration (Table 1).

Discussion

The results support the notion that for the most part animal by-products as well as plant protein sources can support good growth performance in African catfish. However, they appear to have different advantages in terms of the plant macronutrients they supply to the hydroponic unit of aquaponic systems. While P-rich animal by-products seem superior over the used plant protein mix in terms providing plant available P, the plant protein mix provides higher K levels. Considering the illustrated potential to eliminate the need for P fertilization in aquaponic systems, P provisioning through strategic protein ingredient choice could be a focus point in aquaponic diet development in the future.

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EFFECT OF DIETARY LIPID QUALITY AND FISH SIZE ON CHOLINE REQUIREMENT AND FATTY ACID PROFILE IN DIGESTIVE ORGANS OF ATLANTIC SALMON (*Salmo salar* L)

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Introduction

The shift from a marine-based to plant-based diet in farmed Atlantic salmon has revealed new challenges concerning the dietary requirements necessary to ensure fish health. Among the nutrients discovered to be lacking in plant feeds, choline has been identified as essential to guarantee efficient lipid transport and metabolism[1]. The distinctive sign of choline deficiency is an excessive presence of fat within the enterocytes, a condition known as steatosis. A recent study estimated a choline requirement of 3.4 g/kg in Atlantic salmon weighing 200-400g raised in freshwater[2]. However, it is not unlikely that choline requirement is influenced by production conditions such as dietary lipid level, environmental temperature, life stage, growth rate, etc. In a previous experiment we investigated to which extent dietary lipid level and water temperature influence steatosis symptoms. The findings confirmed the influence of lipid level and water temperature. The results to be presented are part of a following screening study investigating the possible effects of dietary lipid quality and fish size on choline requirement in Atlantic salmon. To perform the statistical analysis, a Bayesian approach was used. This approach provides careful and robust reasoning as well as accurate uncertainty handling for resource demanding studies aiming to describe dose-response relationships.

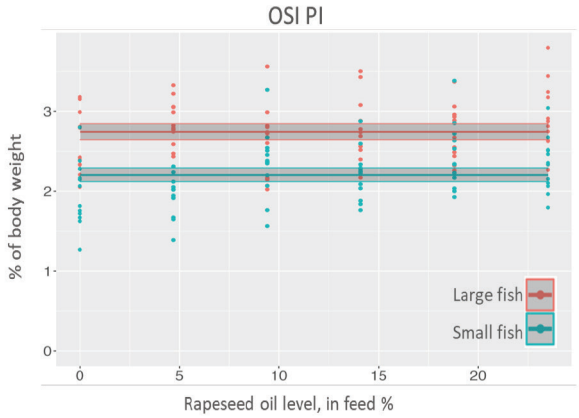

Figure 1: Pyloric intestine somatic index (OS PI) indicated as percentage of the total body weight ± 95% confidence range.

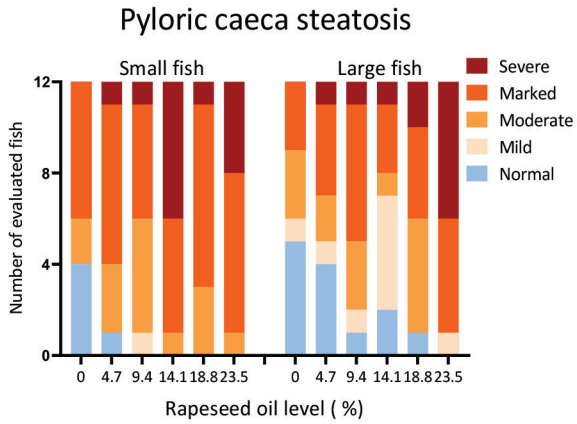

Figure 1: Scoring of steatosis level in the pyloric caeca tissue.

Materials and methods

Six experimental diets were formulated to contain 32 % fat, varying in ratios of rapeseed oil to fish oil, from 0/32 to 24/8. The diets were fed to two groups of Atlantic salmon raised in sea water: the smaller fish had an average initial weight of 1500g, while the larger fish weighted 4500g. At the end of the 8-weeks feeding trial, twelve fish from each tank were sacrificed and their body measures taken. The fish were then opened ventrally, and the intestinal package removed. The intestine was sectioned into pyloric, mid, and distal portion and weighed. The pyloric section was cleaned of mesenteric fat and weighed again. The liver was removed and weighed. Tissue samples from all the organs and sections were taken and processed for analyses of fat content and fatty acid profile, gene expression and histological characteristics. Enterocyte vacuolization was scored according to their morphological appearance and graded as normal, mild, moderate, marked and severe. Feed and feces were analyzed for dry matter, ash, crude protein, fatty acids and starch content. The digestibility of the macronutrients was assessed by using yttrium oxide as internal marker. Plasma was collected and stored for evaluation of biomarkers of nutritional status: glucose, free fatty acids, cholesterol and total triacylglycerides.

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Results

Growth performance, identified as Thermal Growth Coefficient (TGC), was higher in the large fish, whereas the increasing rapeseed oil level had no effect. A similar picture was observed for the organosomatic index of the pyloric intestine (OSI PI), which was higher in the large fish and not clearly influenced by lipid quality (Figure 1). Likewise, the histological assessment showed a higher vacuolization degree in the pyloric caeca of the larger fish. The vacuolization increased with the increasing rapeseed oil level, suggesting a dose-response effect (Figure 2), which was confirmed by analyses on fatty acids content. Neither fish size nor lipid quality affected the expression of the targeted biomarker genes.

Other data are under evaluation and further results will be presented at the conference.

Conclusions

The results obtained so far showed that steatosis symptoms and therefore choline requirement is mainly affected by fish size, whereas lipid quality showed less clear effects.

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FEEDING APULIAN NATURALIZED NILE TILAPIA WITH *Bacillus velezensis* MT9-BASED DIET

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Introduction

Aim of the INAQUA-2-O project is to improve the quantity and quality of the farmed freshwater fish through the definition of specific feeding plans and the control of their physiology. This to expand the offer of aquatic products of the Apulian aquaculture enterprises, support their productions, and increase their competitiveness. Studying the effects of regular and special feed with enhanced product characteristics falls into the objectives of the project. Emphasis is given to the research, development and use of probiotics, due to their now recognized beneficial properties on the health of the farmed fish, in terms of optimization of the processes of digestion and absorption of the food, immuno-modulation and enhancement of certain anti-inflammatory and protective effects, and control over pathogens and/or infectious agents (Zabidi et al., 2021). The analysis of essential physiological functions such as gastro-intestinal, endocrine and cardio-circulatory function, as well as the behavior of the animals in the tank, are also considered in the project in order to obtain high quality fish.

Materials and Methods

Fish. Apulian naturalized (Scordella et al., 2003) Nile tilapia (*Oreochromis niloticus*) were purchased from Azienda Ittica Agricola Residence San Nazario srls (Lesina, Foggia, Italy) and reared indoor in tanks in a recirculating aquaculture system set at the Laboratory of Urban Farming, Department of Innovation Engineering, University of Salento (Lecce, Italy).

Rearing system. The system consisted of tanks (360 L each; 300 L water) with UV-treated (Velda Clear Pond UV-C 7 W) urban tap water. Dissolved oxygen was maintained above 4 mg/L using an impeller aerator (aquael oxyboost 200 plus) per tank to provide continuous aeration. The aquaria room was at controlled temperature (air conditioning system: 21-25 °C). Fish were kept under natural photoperiod. Water temperature and dissolved oxygen were continuously monitored by probes (Hanna Instruments HI98193). Other water quality parameters, such as ammonia, nitrite and nitrate, were monitored three times a week. During the experimental period, water temperature was 22.9 °C, pH 8.2, oxygen dissolved 6,8 mg/L and total ammonia-nitrogen < 1 mg/L.

Probiotics. Among probiotics, *Bacillus* appears to be the second most studied/used genus (Arsène et al., 2021). Notably, *B. velezensis* is one of the most studied/used species for fish feeds (Yi et al., 2018; Khalid et al., 2021). *B. velezensis* MT9, as isolated in the Laboratory of Microbiology (DiSTeBA-University of Salento), was chosen to integrate the diet. Growth and tolerance tests were conducted in a range of temperatures (bacteria grown in liquid GYM medium for 6 hours) from 30 to 70 °C. Bacteria showed viability and good growth up to 40 °C, making it suitable for the purpose of this study (Figure 1A).

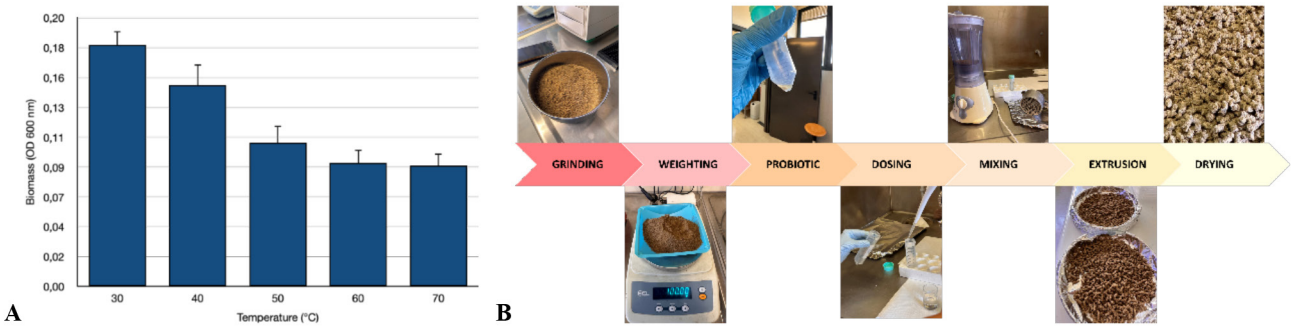

Figure 1. (A) Growth of *Bacillus velezensis* MT9 at various temperatures. (**B**) Preparation of the new feed.

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The serial dilution method was used to measure the bacterial load and standardize bacteria growth. When the culture reached OD = 0.7 (absorbance at 600 nm), 7 serial dilutions (1:10) were made in saline solution (NaCl 0.9%). 10 µL of each dilution were plated onto Petri dishes containing solid GYM. The plates were incubated at 28 °C for 24 hours and then the colonies were counted.

Diet. A diet was formulated, including the probiotic *Bacillus velezensis* (Khalid et al., 2021) as isolated in the Laboratory of Microbiology (DiSTeBA-University of Salento). This diet was tested *vs.* a control diet without probiotic. Fish feeds were prepared starting from a complete commercial feed (Veronesi CFW 4; extruded pellets, 4 mm diameter). The two diets were isoproteic (35% crude protein), isolipidic (10% crude fat) and isoenergetic (crude fiber 4,80%, ash 5,95%, calcium 0,60%, phosphorus 0,80%, sodium 0,12%). The commercial feed was first fine powdered and then mixed with water (50 ml dH_2O for 100 g feed powder) alone (control diet) or containing the probiotic *B. velenzensis* MT9 (Calcagnile et al., 2022) (7 x10^6 bacteria, OD = 0.7 resuspended in 50 ml dH_2O for 100 g feed powder). The ability of the probiotic to resist was routinely assayed at 40 °C for 6 hours. The mass obtained was roughly extruded with a potato smasher to get a new 1-2 mm diameter pellet. After desiccation overnight in a dryer (40 °C), the new feed was considered ready for use (Figure 1B).

Feeding trial. To start the experiment, 48 hours fasting fish were distributed into 2 tanks. In particular, out of 75 selected uniform-sized fish (average body weight: 41.5 ± 8.3 g) (means ± SD; n = 75), 2 groups of 25 fish each were randomly composed and distributed into the 2 tanks. After 1-week adaptation, an initial sampling (T0) was carried out followed by a second sampling (T1) after 21 days of feeding control or experimental diet (2% fish weight).

GI function. To monitor the health state of the fish GI tract, selected intestine and liver enzymatic activities were checked. Briefly, fish were euthanized by phenoxyethanol/chilled water. Intestines and livers were isolated and homogenized, and leucine aminopeptidase (LAP), lipase (LIP) and alkaline phosphatase (ALKP) activities measured by spectrophotometry using a suitable substrate (i.e., L-leucine p-nitroanilide for LAP, p-nitrophenyl myristate for LIP, p-nitrophenyl phosphate for ALKP; absorbance at 405 nm). The values of the initial sampling (T0) (means ± SD; n = 6) were as follows: (liver) ALKP: 142.1 ± 14.3 mU/mg protein; (intestine) ALKP: 680.6 ± 12.1 mU/mg protein; intestinal LIP: 31.6 ± 11.7 mU/mg protein; LAP: 91.4 ± 33.1. In addition, viscerosomatic (VSI) and hepatosomatic (HSI) indexes were calculated.

Results

We planned to calculate growth performance parameters and somatic indexes at the beginning of the experimental trial (0 day) (T0) and after 21 (T1), 42 (T2) and 63 days (T3). Here, we report the results referring to the T0 and T1 time points, while the experiment is still underway. The initial (T0) average weight (means ± SD; n = 6) was 47.3 ± 10.0 g, while after 21 days (T1) it was 60.4 ± 9.9 g for the group of fish fed the control diet and 69.6 ± 7.3 g for the group of fish fed the experimental diet containing the probiotic. The initial (T0) length (means ± SD; n = 6) was 129.7 ± 10.4 mm, while after 21 days (T1) it was 144 ± 12.4 mm for the group of fish fed the control diet and 157 ± 7.6 mm for the group of fish fed the experimental diet containing the probiotic. During the feeding trial, both VSI and HSI (means ± SD; n = 6) increased passing from T0 (VSI = 8.0 ± 0.3 and HSI = 1.2 ± 0.3) to T1 (VSI = 13.2 ± 2.5 and HSI = 1.8 ± 0.5 in the group of fish fed the control diet, and VSI = 11.5 ± 0.8 and HSI = 1.9 ± 0.4 in the group of fish fed the experimental diet containing the probiotic). Both diets were well accepted and the survival rate was 100% for both fish groups. During the experiment, current regulations on the protection of animals used for scientific purposes were respected, with particular emphasis on the health and welfare of the fish during the growth, testing and sacrifice phases.

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THE EFFECT OF GENETIC IMPROVEMENTS ON OPTIMAL FISH FEED FORMULATION: A TRAITS-TO-FEED FRAMEWORK

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Introduction

Fish nutritional requirements are generally estimated through analysis of dose-response studies involving essential nutrients, which condition the nutritional targets of fish feeds during formulation. Due to the high cost of undertaking such studies, they are often performed under simplifying assumptions (e.g., the effects of nutrients on growth are marginally independent) and the implicit notion that nutritional requirements are essentially static: once the requirements of a specific nutrient have been estimated for a specific species, at a specific size and under specific environmental conditions, its value is simply accepted as being universally valid and seldom re-evaluated.

On the other hand, genetic improvement programs of commercial fish species through selective breeding are known to have potential effects on fish traits which directly condition nutritional requirements (e.g., feeding and growth potential, retention efficiencies, body composition). Thus, and given the importance of the genetic-nutrition interaction in the process of fish growth, it seems relevant to be able to quantitatively predict the impact of such changes in fish traits on optimal feed formulation targets.

In this work, we develop analytical formulas that directly relate "relevant fish traits" to "optimal feed formulation" as a way of tackling this question without resorting to (possibly biased) observational data.

Materials and methods

In order to establish general formulas, we start by assuming energy and mass conservation to define a budget for energy, protein and phosphorus (i.e., gain = intake – losses). Then we consider a series of assumption sets with increasing levels of complexity (and realism).

Expanding the budgets under each of these sets of assumptions leads to different closed-form formulas that directly relate relevant fish traits to the optimal feed inclusion levels of energy, protein and phosphorus. The specific effect of each trait on optimal inclusion levels were then obtained and analysed both through an analytical sensitivity analysis and plots of the marginal effects of each trait (under varying conditions).

Results

The formulas obtained under a simplistic assumption of a linear relationship between intake and retention (of energy and nutrients) provide a useful first-order approximation to the effect of changes in fish traits due to genetic improvement on optimal feed formulation targets: the digestible amount of a certain nutrient (or energy) required in the diet is proportional to the relative amount of that nutrient (or energy) in the body of fish, and inversely proportional to FCR and to the retention efficiency of that nutrient (or energy). This implies that the effect of relevant fish traits on optimal feed formulation targets is, in this case, straightforward, since each trait multiplicatively and independently affects the optimal target in the same way.

On the other hand, the more realistic formulas, obtained under an assumption of a saturating (rational) relationship between intake and retention, display a slightly more complicated structure, where (unlike in the previous formulas) both the feeding rate and the growth/anabolic rate play a relevant role (see Figure 1 for an example), and where the effects of the different traits on the optimal inclusion rates is not necessarily multiplicatively separable. Despite these challenges, these formulas end up being more useful than the previous ones, not just due to the increased realism (e.g., effective retention efficiency should go down at high intake levels), but due to the fact that the nutritional and genetic effects are more clearly separated and identifiable, which is not the case for the first set of formulas (since FCR is not a feed-independent trait).

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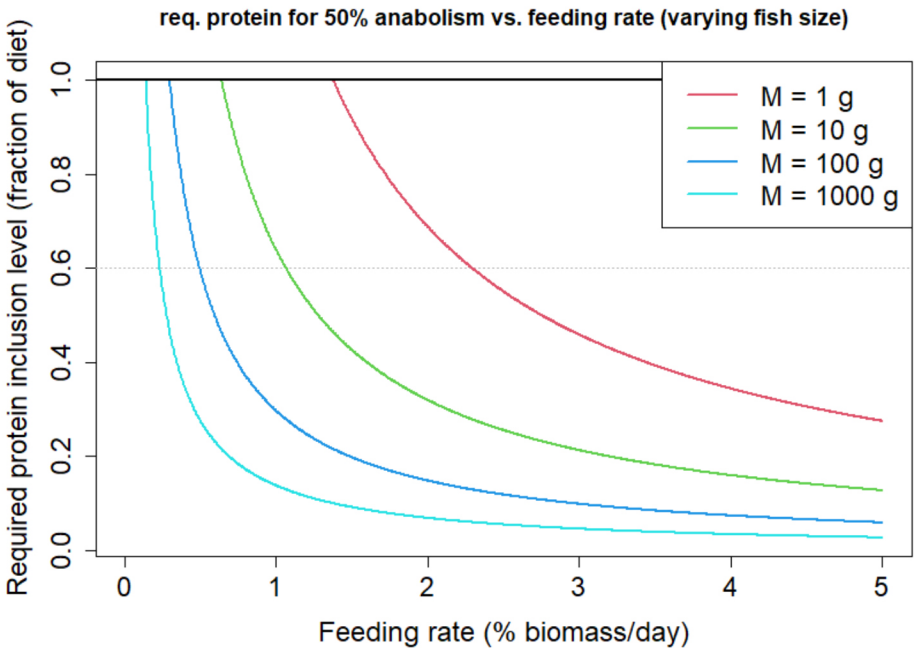

Figure 1 – Line plot showing an example of calculated required protein inclusion levels under the "advanced" estimation model, as a function of the relative feeding rate, for different fish sizes (all other parameters were set at hypothetical but realistic values). The grey dotted line indicates a generally high level of protein in the diet (60%).

Discussion and Conclusion

The formulas developed within the traits-to-feed framework seek to help aquaculture stakeholders make more informed decisions: for fish farmers, these formulas can be used to adjust feeding rates to achieve specific growth rates (as a function of fish size, diet composition and environmental conditions); for fish breeders, these formulas can inform them on the relative importance of different fish traits (and the relevance of estimating them within breeding programs); for fish nutritionists and aquafeed formulators, these formulas can guide the formulation of diets that are adapted to specific fish strains and under specific conditions and growth targets.

Acknowledgements

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MICROPLASTICS CONTAMINATION IN DIFFERENT SEAFOOD SPECIES: COMPARISON BETWEEN FRESH AND CANNED SAMPLES

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Introduction

Between 1950 and 2021, the annual production of plastics has continually increased from 1.5 to 390 million metric tonnes. Regarding microplastics (MPs), plastic particles smaller than 5 mm, there is a worldwide growing interest and awareness on its impacts in the marine environments and organisms. Seafood has a crucial role for human consumers because it is an important source of high-quality proteins, unsaturated fatty-acids, fat-soluble vitamins and minerals, with numerous nutritional and health benefits. Thus, understanding the potential contamination of seafood with MPs is critical for food security and human health. Seafood is also highly perishable, and several preservation methods (like salting, freezing, or canning) are traditionally used to ensure the quality of the products. Canned seafood corresponds to approximately 10% of the total 178 million tons of the world fishery production, with the existence of a wide variety of canned organisms (*e.g.,* fish, molluscs, crustaceans), immersed in different edible liquids (*e.g.*, sunflower oil, olive oil, tomato sauce). Since consumers eat these products without any additional cleaning process, from a health perspective it is crucial to have a better understanding on possible MPs contamination both in the food tissues, as well as in the respective immersive liquids. The occurrence of MPs has been widely studied for several seafood species but, regarding canned seafood, there is still limited information. The main objective is to better understand possible MPs contamination during the canning processing of the seafood. From our knowledge, this is the first study comparing the occurrence of MPs in fresh and canned samples from the same seafood species.

Materials and methods

Four seafood species with relevance for human diet were selected and studied, both in fresh and canned samples: sardine (*Sardina pilchardus*), chub mackerel (*Scomber colias*), octopus (*Octopus vulgaris*) and mussels (*Mytilus galloprovencialis*). Fresh seafood was obtained directly from fisheries harbor, and canned seafood were obtained from local markets. MPs were extracted using 30% H_2O_2 to digest organic content, followed by vacuum-filtration and subsequent observation under a stereomicroscope, according to a previously optimized protocol. For canned samples, the edible tissues and immersion liquids were separately analyzed; for the fresh samples, MPs contamination was investigated in subsamples from the dorsal muscle of the organisms.

Results

For all the fresh samples (n= 107), an average (± SD) of 0.086 ± 0.088 MPs/g of was obtained, while for the canned samples (n= 50), an average (± SD) of 0.040 ± 0.070 MPs/g of seafood tissue was observed. In general, some variability among MPs concentrations were observed in the two types of samples: sardine presented an average of 0.211 ± 0.074 MPs/g for fresh samples, and 0.020 ± .0.052 MPs/g for canned samples (in tomato sauce and sunflower oil); for octopus, an average of 0.006 ± 0.009 MPs/g in fresh samples, and 0.070 ± 0.106 MPs/g in canned samples (in tomato sauce) were obtained; concerning mussels, fresh samples presented an average of 0.066 ± 0.095 MPs/g, while canned samples (in escabeche sauce) presented 0.050 ± 0.071 MPs/g; for chub mackerel, an average of 0.060 ± 0.115 MPs/g for fresh samples, and

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0.040 ± 0.052 MPs/g for canned samples (in sunflower oil) were reported. Also, an average of 0.004 ± 0.012 MPs/mL was obtained for the immersion liquids from the cans, an important finding since some of the liquids are ingested by human consumers. Microplastics were observed in all the studied seafood species including fragments, films and fibers from eleven different colors, and with a size range from 250 to 3000 µm. Plastic polymers such as polypropylene, polyester, polyethylene, rayon, polyvinyl and nylon were identified through FTIR analysis.

Conclusion

The present study shows MPs contamination in four relevant and commercialized seafood species, in both fresh and canned samples. This is an important finding, indicating that MPs contamination in seafood may result from two paths: *i)* direct and continuous contact between marine organisms and the surrounding contaminated environment, or *ii)* human handling and industrial process as canning. Quantifying and regulating MPs in canned seafood is crucial to increase food safety and human health, and a better knowledge on the possible MPs contamination from the capture of seafood organisms until the final products sold to consumers (considering all the human handling and industrial canning steps) is of major importance for a better understanding of MPs occurrence in canned seafood.

Acknowledgements

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S2AQUAcoLAB' AND IPMA'S COLLECTION ON THE FRAMEWORK OF THE PORTUGUESE BLUE BIOBANK

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In face of global biodiversity losses, due to habitat degradation and pollution, the creation of biodiversity biobanks has been explored as a promising approach for the conservation of endangered species. In Portugal, the creation of a national biobank for marine resources is deemed a strategic priority, not only to promote the country's role in increasing the current knowledge of the planet's biodiversity, but also to contribute to the international visibility of national bioresources and its economic valorisation in international chains. In view of the above, the Portuguese Blue Biobank project was established in late 2022, focused on the implementation of a national network of marine resources Biobanks, with a dedicated infrastructure and increasingly digitalized tools to enable mapping the national resources, monitorization of their end-uses and distribution (both for commercial exploitation and scientific research), as well as the application of the international Nagoya Protocol on Access and Benefit-sharing.

In order to facilitate industrial research, the Portuguese Blue Biobank main goals are to 1) establish a demand-centered distribution system, by creating a single, regulated system for distribution of isolates and samples (with special focus on microorganisms, macrophytes, invertebrates and marine vertebrates), and expanding the available samples typologies (strains, DNA, extracts, and tissues), 2) develop a repositorium for the national marine biodiversity (mainly microorganisms that present high biotechnological value), and 3) develop a quality management system for the Biobank, by operating a standardized management protocol and introducing a certification process (ISO 20387-2018, for the competence, impartiality and consistent operation of biobanks).

Two partners of this Portuguese Blue Biobank are S2AQUAcoLAB, a collaborative laboratory for a Sustainable and Smart Aquaculture, and IPMA/EPPO, a state laboratory that has a pilot station to improve aquaculture research, product development and experimental procedures. As part of the Portuguese Blue Biobank Consortium S2AQUAcoLAB' collection will be focused on pathogenic bacteria isolated from different marine fish species and confirmed by biochemical and molecular tools, fish-derived cell lines with high potential for cellular *in vitro* research, and macroalgae collected from the Portuguese coast. IPMA's collection will consist in marine fish broodstock from different species with the main goal to increase the aquaculture biodiversity, external parasites isolated from fish cultured in IPMA's facilities, and microalgae. The project's idea is to collect and cryopreserve these marine bioresources to push forward advancements in aquaculture research.

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EFFECT OF DIETARY MICRO AND MACROALGAE ON THE PERFORMANCE, GENE EXPRESSION, AND OXIDATIVE STRESS OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Aquaculture industry progress depends on fish health and welfare. Nowadays, with the production intensification to meet the needs of a continuous growing human population, fish are exposed to challenges (e.g., diseases, stress) that limit their performance with consequences on the overall productivity. It is essential to implement sustainable strategies to prevent, mitigate and overcome these deleterious events. Hence, functional nutrition is key for creating precise solutions to improve fish performance and robustness. Algae, both micro- and macro- algae, are high value ingredients due to the diverse bioactive compounds present in their composition (Ampofo and Abbey, 2022). The inclusion of these bioactive biomasses and/or extracts in fish diets for commercial species will be a differentiating element, as it may improve overall fish resistance to stressful events (Reis et al., 2021). This trial aimed at evaluating the biological efficacy of different functional microdiets with different amount of micro- and macro-algae inclusion for gilthead seabream (*Sparus aurata*) post-larvae on survival, growth, robustness, and resistance against pathogenic stresses.

Materials and Methods

The trial was performed at EPPO/IPMA (Olhão, Portugal) and was divided in two phases: 1) 33DAH post-larvae started an experimental feeding that consisted in four different feeds: Winfast (control diet, SPAROS Lda.), BLEND1, BLEND2, and BLEND3 (three different diets with different amounts of micro- and macro-algae). Fish were fed respective diets until 42DAH, where they changed to a control feed for 1 week until 50DAH, and then back to the functional feeds for another week, until 58DAH. Fish were sampled at that point (S1) for growth assessment and effects of blends in gene expression and oxidative stress related enzymes activity. 2) The second stage of the trial initiated at 58DAH where all fish groups were fed a boost diet for 1 week (BLEND6). BLEND2 was eliminated to give place to a CTRL group that did not receive a boost diet. Fish were sampled previously to stress exposure (S2) to evaluate boost effects. After this period, larvae were subjected to a stress event (air exposure) and samples were collected 6h hours after exposure (S3) for gene expression analysis, oxidative stress enzymes' activity, and survival.

Results

At the end of trial 1 (S1), the seabream post-larvae fed with diets containing micro- and macro-algae showed no differences in growth. Although not significant, a higher fish survival was observed in the fish fed with the BLEND diets. Regarding gene expression, an increase in stress-related genes, such as GPX1 and GPX4, was observed in all BLENDS when compared to CTRL. In S2, the addition of a boost diet (BLEND 6) decreased the expression of antioxidant enzymes to control levels (S2 vs S1). After the stress event (S3), the addition of a boost diet (BLEND 6) increased the expression of antioxidant enzymes such as GPX1 and GPX4, in a dose dependent manner.

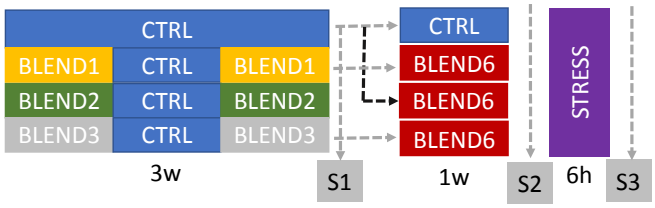

Figure 1 – Experimental scheme of the trial. CTRL, BLEND1 BLEND2 and BLEND3 diets were used in a first trial. BLEND6 was used as a boost diet before the stress in the second trial. S1, S2 and S3 represent sampling times. S1 was performed for 3 weeks (3w), boost period was 1 week (1w) and sampling after the stress was performed after 6h.

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	CTRL	BLEND1	BLEND2	BLEND3
Weight (mg)	2.7±1.9	2.6±1.8	2.8±1.6	3±2
S1 GPX1	1.0±0.4	1.8±0.9	2.0±0.3	3.1±3.5
S1 GPX4	1.2±0.9	2.9±2.3	5.2±1.9	5.8±8.4
	CTRL	CTRL-B6	B1-B6	B3-B6
S2 GPX1	1.7±1.8	1.1±0.5	1.1±0.6	1.1±0.3
S2 GPX4	1.3±0.9	1.1±0.6	1±0.7	1±0.2
S3 GPX1	1.2±0.9	3.8±1.8	3.8±1.8	6.1±2.4
S3 GPX4	1.3±1.1	3.4±0.9	4.1±1.1	3.1±1.9

Figure 2 – Summary of the results from the trial. Results are expressed as mean ± SD. Weight is expressed in mg, and gene expression data is expressed as relative expression to *EF1a/ bActin* housekeeping genes, using the Pfaffl method. S1, S2 (pre-stress) and S3 (post-stress) are related to the sampling times in the trial. *Gpx1* and *gpx4* gene expression was accessed.

Conclusions

These results suggest that in the early stages, the performance of seabream larvae might benefit by the incorporation of micro- and macro-algae in the diets. The results observed show that the inclusion of these algae in the fish diets increase the expression of antioxidant enzymes after a stressful event. Nevertheless, in order to validate these results further studies are needed and the identification of the mechanisms involved in this response, as well as the long-term effects of these substitutions, are of paramount importance.

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EFFECT OF DIETARY NON-PROCESSED ALGAE MEALS ON INFLAMMATORY RESPONSE OF EUROPEAN SEABASS (*Dicentrarchus labrax*)

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This study assessed the two unprocessed microalgae (*Nannochloropsis oceanica* and *Tetraselmis* sp.) and macroalgae (*Gracilaria gracilis* and *Ulva rigida*) meal in sea bass diets to enhance the utilization of micro- and macro-algae on immune response of European seabass. As a reference (control, CNTR) commercial-based diet was used and experimental diets were prepared by replacing 30% of the control diet with each algal meal used. Triplicate groups of fish (initial 23.9 g) were fed five experimental diets containing 30% *Nannochloropsis oceanica* (Nano-D), *Tetraselmis* sp. (Tetra-D), *Gracilaria gracilis* (Gra-D), *Ulva rigida* (Ulva-D) and CNTR for 60 days. IL-1β expressions in Gra-D were significantly higher than those in Ulv-D and were significantly lower than those in Tet-D, Nan-D and CNTR diet. The strongest response to diets containing macroalgae was seen in juvenile sea bass, induced the secration of anti-inflammatory IL-1beta and IL18 genes in pathways for pro-inflammatory response, modulating both innae and adaptive immune response. HSP70 expression levels in Tet-D were significantly higher than those in Nan-D, Ulv-D and Gra-D. However, there are still crucial knowledge needs to assess of these algae sources in the aquafeeds, enabling to prove comprehensively effects on immune response/ mechanisms in fish having high trophic levels or commercially cultured marine species.

EUROPEAN SEABASS HEAD-KIDNEY PRIMARY CELLS RESPONSE TO FRACTIONS OF *Nannochloropsis oceanica* WHEN STIMULATED WITH INACTIVATED *Photobacterium damselae piscicida*

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Introduction

Disease outbreaks in fish farming are expected to intensify in the next years, which could compromise global food production and security [1, 2]. Some nutritional strategies can be applied to ameliorate the detrimental effects of disease outbreaks, including dietary functional feed supplementation [3]. Owing to several bioactive compounds in its composition, microalgae have been suggested for this purpose. Particularly, *Nannochloropsis oceanica*, a microalgae cultured industrially by Allmicroalgae Natural Products and other companies, has received great attention as it is rich in bioactive compounds with antioxidant [4], anti-viral, and anti-bacterial properties [5]. Recently, a group of aqua-soluble sulphated polysaccharides (SPS) obtained from *N. oceanica* showed immune stimulant properties [6], revealing its great potential in innovative animal health therapeutic products. Although *N. oceanica* has diverse valuable bioactive compounds, there are no reports on applying different concentrations of fractions from *N. oceanica* for immune stimulation. This study aimed to assess the immune-boosting effect of several *N. oceanica* fractions obtained through marine biorefinery concepts.

Materials and Methods

Four different concentrations (0.5, 0.25, 0.1, and 0.01 mg mL^{-1}) of crude lysate (CL), crude polysaccharides (CP), and sulphated polysaccharides-rich (SPF) fractions obtained from *Nannochloropsis oceanica* were tested in European seabass *Dicentrarchus labrax* head-kidney primary cells (1 x 10^7 cell mL^{-1}) to assess cell viability and myeloperoxidase activity. Afterwards, CL, CP, and BGF were used at 0.5 and 0.01 mg mL^{-1} concentrations for 24h to assess their effects on cell viability, myeloperoxidase activity and respiratory burst (RB) of head-kidney primary cells of European seabass after stimulation with inactivated *Photobacterium damselae piscicida* for 4h.

Results

Results showed that the cell viability of European seabass head-kidney primary cells ranged from 70% to >100%, depending on the fraction and concentrations. Higher cell viability was exhibited when cells were exposed to CL and SPF, regardless of the concentrations. In addition, cell viability was higher at 0.5 mg mL^{-1} compared with other concentrations. Regarding myeloperoxidase activity, results revealed that cells at 0.5 mg ml^{-1} and 0.01 mg mL^{-1} tended to present higher myeloperoxidase activity, depending on the fraction (P>0.05).

RB was measured in European seabass head-kidney primary cells after 4h of exposure to inactivated bacteria. The results indicated that RB was not affected by the fractions, as no significant differences were detected, regardless of the concentration (ranging between 0.44 ± 0.20 and 0.73 ± 0.29 nmol O2•-, P>0.05).

Conclusions

The results support the idea that *Nannochloropsis oceanica* presents bioactive compounds with the ability to induce immune responses in European seabass head-kidney primary cells. Further analysis focusing on ATP and nitric oxide production, total antioxidant capacity, and gene expression will be assessed in European seabass head-kidney primary cells stimulated with inactivated bacteria to determine whether *N. oceanica* fractions exert a consistent immune modulatory effect.

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Acknowledgements
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IMPROVING GROWTH BY BIOTECHNOLOGICAL TREATMENTS OF VEGETABLE INGREDIENTS IN AQUAFEEDS FOR *Seriola dumerili*: INSIGHTS ON INTESTINAL FUNCTION, IMMUNE RESPONSE AND MICROBIOTA

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Introduction

In response to the increasing demand for food worldwide, the aquaculture sector should explore the biological and socioeconomic potential of new species in order to expand the industry. In this sense, the greater amberjack (*Seriola dumerili*) is a good candidate due to its high growth rates and consumer acceptance. However, as a carnivorous species, it has high protein requirements, mostly provided by fishmeal. Thus, it is necessary to develop new formulations based on plant sources and nutraceutical compounds that allow good growth rates as well as the sustainable development of the sector. However, these ingredients can cause negative effects on the digestibility and bioavailability of nutrients, as well as on intestinal functionality and health. Therefore, the aim of this study was to improve the absorption and digestibility of diets with a high content of plant ingredients and its supplementation with nutraceuticals derived from micro- and macroalgae, pre-treating them biotechnologically by enzymatic hydrolysis. For this purpose, growth parameters, and intestinal function and health were examined by electrophysiological techniques, gene expression of immune-related genes, and microbiota characterization.

Material and methods

Juveniles of *S. dumerili* with 6.34 ± 0.01 initial mean body mass were randomly distributed in 9 tanks of 400L (25 fish/tank) at the experimental facilities of *Servicios Centrales de Investigación en Cultivos Marinos* (SCI-CM, University of Cádiz). After a two-weeks acclimatation period, fish were fed *ad libitum* for 69-days with 3 experimental diets, in triplicate: i) CTRL, similar to commercial feeds with 60% total protein (>92 % from animal origin); ii) PP, with the replacement of 50% of animal products with plant ingredients; and iii) PP-LB, as PP but supplemented with 3% of macro- and microalgae extracts. All vegetable ingredients we pre-treated biotechnologically by enzymatic hydrolysis. Temperature (22ºC) and salinity (37‰) were constant during all feeding trials. Biometric samplings were performed every 3 weeks to assess growth parameters. At the end of the trial, 12 fish per experimental diet (4 fish/tank) were anaesthetized with a lethal dose of 2-phenoxyethanol and then biometric measurements and intestine samples were taken for further growth, electrophysiology, microbiota, and gene expression analyses.

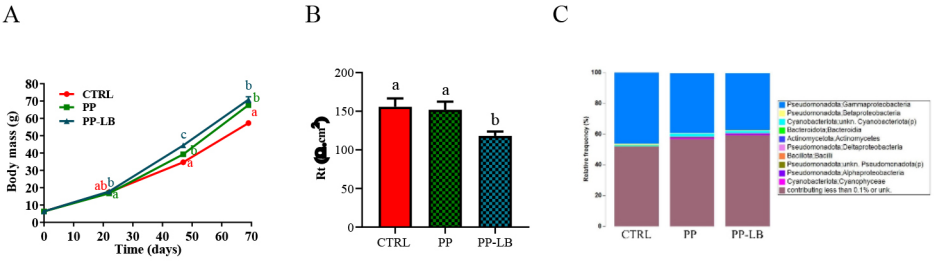

Fig. 1. **A)** Evolution of body mass; **B)** Basal transepithelial resistance (Rt, Ω cm^2) in the anterior intestine; and **C)** Relative frequency (%) at the phylum and class level for each pooled sample of microbiota from the intestinal fluid of *S. dumerili* juveniles fed with the experimental diets (CTRL, PP, and PP-LB). Different letters represent statistically significant differences at a p-value<0.05 resulting from the two-way (A, n=3 replicate tanks) and one-way (B, n=11 fish) ANOVA analysis and post hoc Tukey's test.

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Results and discussion

After 69 days of the feeding trial, fish fed PP and PP-LB diets showed a significant increase in feed intake and specific growth rates without differences in feed efficiency. Accordingly, fish fed with the PP and PP-LB grew faster achieving a final body mass of 67.7±0.95 g and 70.8±1.7 g, respectively, compared to control fish (57.3±0.28 g) (Fig.1A). Also, fish fed with the PP diet showed the highest condition factor, reaching values of 1.70 ± 0.01, similarly to other studies in wild and cultured specimens of *S. dumerili* (Fernández-Montero et al., 2018). On the other hand, we also observed the typical increment of intestinal length index in fish fed with plant-based diets. Nevertheless, electrophysiological analyses of the intestine only showed changes (a decrease) in the epithelial resistance (Rt) in fish fed the supplemented diet (PP-LB), which may indicate alterations in intestinal barrier integrity and functionality (Fig.1B).

However, the paracellular permeability, using standard methods with 4kD FITC-dextran and 70 kD RITC-dextran, was unchanged although a non-significant decrease was observed in the permeability of the epithelium to larger molecules (70kD) in PP-LB. Regarding the epithelial intestinal integrity, there was a slightly (non-significant) decrease of *cldn19* in anterior intestine in PP-LB diet but a significant increase of *cldn11* and *cldn12* in medium and posterior intestine, respectively. We also analysed the gene expression levels of six amino acid and peptide transporters of which only *slc15a1* and *slc7a5* changed in anterior intestine increasing in PP and PP-LB diets respectively, while *slc3a1/a2* and *slc15a2/a4* showed, in general, an increase in plant-based diets in medium and posterior regions. Hence, the increase of transporters studied could indicate a major of nutrient bioavailability as a result of the biotechnology treatment. Besides, diet also had an effect on inflammatory and innate immune response as judged by the increase expression of several immune genes in medium (*il12p35a*) and posterior (*il34*, *il12p35a*, *infg*) intestine of fish fed with PP-LB. Likewise, changes in bacterial communities were showed by diet, with the reduction of Proteobacteria, the dominant phylum in this species (Sánchez-Cueto et al., 2023) in both plant-based diets (Fig.1C). Moreover, the class Gammaproteobacteria was reduced in fish fed vegetable diets and accompanied by the emergence of Cyanophyceae and Alphaproteobacteria classes, especially in PP-LB group. In conclusion, the enzymatic pre-treatment of vegetable ingredients improves growth performance in *S. dumerili* without affecting intestinal integrity or immune response, although some changes in these parameters were observed after nutraceutical inclusion (PP-LB diet). Nevertheless, both plant-based diets generate changes in transport processes and microbiota.

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DEVELOPING LESS OR NON-INVASIVE METHODS FOR THE MEASUREMENT OF AN ACUTE STRESS RESPONSE INDUCED BY TRANSPORT IN EUROPEAN SEABASS

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Introduction

Common aquaculture practises constitute a major threat to fish health and welfare, which can affect farming performance and productivity. Fish response to a stressor involves the activation of the hypothalamic-pituitary-interrenal axis (HPI), generating several physiological and metabolic changes. Cortisol, considered the major stress-marker hormone, has been extensively studied in aquaculture to assess the effects of different rearing conditions in fish. This hormone is a reliable acute stress indicator in plasma; however, the blood collection procedure can be a source of stress, becoming necessary to find alternative lower invasive matrices to measure cortisol (Sadoul & Geffory, 2019). Therefore, this study aims to evaluate the physiologic and metabolic effects of an acute stress, as fish transport, in European seabass (*Dicentrarchus labrax*), with a special focus on the correlation of cortisol levels in different matrices (plasma, skin mucus, and water) trying to develop less invasive stress biomarkers.

Material and methods

Fish were distributed in nine 400L tanks and acclimated for one week to an initial stocking density of 4 kg/m^3. Overnight fasted fish were introduced in 10L plastic bags saturated with oxygen (density: 30 kg/m^3) and transported for 4 hours by a transport van. Then, just after finishing transport, half of the transported fish were sampled while the rest were returned to their initial tanks, constituting the recovery group, which was sampled 24 hours later. At each sampling point, non-previously disturbed fish were sampled as controls of transported (Control 1) and recovery groups (Control 2). In all cases, water temperature, oxygen, and pH were measured from the bags or tanks, and extra samples were collected for further analyses. Besides, 12 fish/group were anaesthetized with a lethal dose of 2-phenoxyethanol and then biometric and tissue samples were taken (blood, skin mucus, liver, and white skeletal muscle) for metabolic and cortisol analyses.

Results and discussion

Water quality parameters (O_2, ammonium, nitrite, and nitrate levels) remained within optimal ranges after 4 hours of transport. However, as a typical response in short transport, water acidification was observed (pH=6.9) in transported fish compared with the control (1 and 2) and recovery groups (pH=7.5) which could be the consequence of CO_2 increase. This causes a reduction of haemoglobin-O_2 affinity (Sampaio y Freire, 2016) confirmed by the increase of this haematologic parameter in transported fish. Also, a reduction of haematocrit was observed after transport, which may be related to electrolyte-water balance of the fish blood (Seibel et al., 2021), as observed by changes in plasma osmolality, in part, orchestrated by cortisol. As a primary response to stress, transported fish showed an increase in cortisol levels in plasma, skin mucus and water which corroborates the fact that cortisol is a good marker of acute stress produced by transport in all matrices analysed. In addition, cortisol levels in the plasma correlated positively to skin mucus (Fig. 1A) and water (Fig. 1B) levels which supports the previous studies in fish (Fanouraki et al., 2008; Franco-Martinez et al., 2022) and allows the use of less or non-invasive matrices to evaluate a situation of acute stress in fish, such as transport.

On the other hand, cortisol induces secondary responses related mainly to energy requirements. Accordingly, in plasma, several metabolites such as glucose, triglycerides and lactate increased in transported fish, which could indicate an increased energy mobilisation in order to maintain homeostasis, highlighting the almost total or total re-establishment of these values 24 hours later. Fewer differences were observed in liver and muscle tissues, although lactate and triglyceride levels decreased in transported fish in white skeletal muscle, with the recovery of these levels 24 hours later. To sum up, changes observed in different tissues after fish transportation are the result of the increasing energy demand to cope with the stress situation. Likewise, cortisol has demonstrated to have an important role in the stress response in different matrices, which opens the possibility to use non-invasive methods to measure stress without compromising the animal's welfare.

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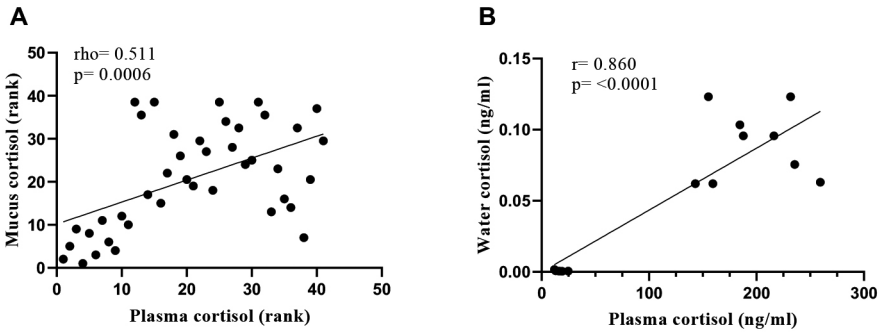

Fig. 1. Correlations between cortisol levels in the plasma and A) skin mucus (Spearman test) or B) water (Pearson's test) after 4-hour transport simulation and recovery. Data correspond to fish from all experimental groups (n=41) (A) and the mean of control and recovery treatments and individual values for transported fish (n=14) (B).

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MICROALGAE SPECIES IS AFFECTING NUTRIENT AND FATTY ACID DIGESTIBILITY IN RAINBOW TROUT *Oncorhynchus mykiss*

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Introduction

Microalgae have already been evaluated in several studies for their application as feed ingredient in aquaculture and due to their high contents of polyunsaturated fatty acids (PUFA) they are considered as valuable lipid source in fish feed (Haas et al., 2016; Sarker et al., 2016). However, a factor limiting their application in aquaculture feeds is their species-specific cell wall which could hinder nutrient accessibility. Processing methods for disruption can improve digestibility and optimize microalgae application (Teuling et al., 2019; Tibbetts et al., 2017). The purpose of this study was to obtain digestibility data for the microalgae species *Isochrysis galbana* and *Tetraselmis chui* treated with different processing methods and to evaluate their potential as a lipid source in feeds for rainbow trout.

Material and Methods

The two microalgae species *Isochrysis galbana* (25.6 % lipid) and *Tetraselmis chui* (13.3 % lipid) were tested in five test diets which consisted of 70 % reference diet and 30 % algae meal as pelleted feed. For each algae species two meals were produced via freeze-drying after harvesting and via freeze-drying and additional homogenization via a centrifugal mill (300 µm). For *T. chui* a third test meal was produced through supplementation of enzymes (mannanase, xylanase and glucanase) before freeze-drying. Titanium dioxide was used as inert marker in the diets for the determination of the apparent digestibility coefficients (ADC). Rainbow trout with ~ 500 g were held in a recirculating aquaculture system in triplicates of trial diets. Faeces were collected via manual stripping 28 h after each feeding event for two weeks during which the fish were fed with 1.5 % of their body weight per day.

Results and Discussion

The ADCs of dry matter and protein were not significantly different between the diets. The crude lipid ADC was lower for the *I. galbana* diets (77.2 %) compared to the *T. chui* diets (87.3 %) and the control diet (91 %). Regarding the digestible lipid content of the diets the control diet showed the highest value (15.3 %) followed by *I. galbana* diets (14.8 %) and *T. chui* diets with the lowest values (12 %). The lipid digestibility of *Isochrysis* was found to be negatively influenced by higher dietary saturated fatty acid (SFA) contents (Caballero et al., 2002) but showed no impairment of feed intake and growth performance in European sea bass (Tibaldi et al., 2015). *T. chui* showed a higher digestibility for nutrients and fatty acids compared to *I. galbana*. Examining total fatty acid groups within algae species, a higher digestibility for monounsaturated fatty acids (MUFA) was observed for *T. chui* followed by PUFA and SFA whereas in *I. galbana* PUFA showed the highest digestibility verifying an efficient accessibility and the potential as applicable lipid source. The homogenization after freeze-drying had no beneficial effect on the digestibility of any nutrient or fatty acid of the microalgae.

Conclusion

Both algae species showed a promising applicability as a multiple nutrient source in aquafeeds for rainbow trout providing important fatty acids and high protein availability. Mechanical or enzyme-treating processing however did not increase the nutrient digestibility of the here used microalgae and sole freeze-drying sufficiently provided nutrient accessibility in this trial. Further research in applying other processing methods to improve digestibility of microalgae is necessary to fully exploit the microalgae potential as well as research in nutrient usability as growth performance in fish.

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FLOATING RAS, THE SOLUTION FOR CLIMATE CHANGE RESILIENT MARINE AQUACULTURE

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Introduction

Climate change is one of the biggest threads to marine aquaculture. Mass mortalities caused by adverse weather conditions, high water temperatures at sea, algae blooms and jellyfish mass invasion have been an ever more frequent news in the relevant seafood and aquaculture newspapers.

To address this issue a floating recirculating aquaculture system: RASxFloater, has been developed by Next Tuna in cooperation with Seafarming Systems AS for the fully closed production of marine species. The specific design of the system has been filed for IP protection with the Norwegian patent authorities and is going to be presented more in detail here.

System requirements

The following design requirements were set:

- Stable floating with wave resistance of min. 1m; suitable for protected areas
- Movable within protected areas to facilitate fish logistics
- Thermal independence from surrounding water temperature
- Possibility for fish transfer through the tank wall
- Size; comparable to standard net pen, feeding into existing value chains
- State of the art RAS system, scalable through replication
- Suitable for precision farming applications

Solution

The developed RASxFloater design is comparable to a 30m diameter, 10m depth net-pen (7'000 m^3). However, instead of a net that keeps the fish, the RASxFloater has a fish tank made of insulated steel and the RAS treatment infrastructure is allocated on the same floating structure (Fig. 1).

The closed characteristic of the system makes it fully independent of environmental influences and as such climate change resilient. In addition, the system has a minimal effect on the environment since all effluent can be treated on land or supply barge and no construction for the implementation is needed.

The novel system with its unique feature of symmetrical allocation of the treatment area and floating support infrastructure around the centre production tank, is constructed and fully mounted in a shipyard and delivered as a plug and play set-up to the fish farmer.

The floating RAS systems has two operational modes:

1. Production mode: the system is connected to a harbour dock, or moored in a sheltered area and receives all essential supplies from land; or barge.
2. Delivery mode: the system is disconnected from land supplies, pulled out of the sheltered/harbour area into the open sea and joint with the delivery net-pen, for safe and stress-free fish transfer and customer delivery (Fig. 1).

During production mode, the floating RAS receives all supplies from land or, if operated in open water, from a supply vessel, including, electricity, oxygen, new (sea) water and delivers all residuals back to land for final effluent water treatment (Fig. 2). For transport, the system is detached from harbour or supply vessel and tugged to destination.

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Fig. 1.: Visualization of the RASxFloater in the transportation mode. (Curtesy of AFRY)

Figure 2: Visualization of a floating production facility in a sheltered port environment (Curtesy of AFRY)

Discussion

Next Tuna wants to present this novel solution for marine aquaculture to the audience and discuss the chosen solutions and their implications with the experts present at the conference.

In addition, new approaches to precision farming and centralized O&M service developed for this new scalable approach to marine aquaculture are presented and submitted to discussion.

The target of Next Tuna is to replace up to 10% of global net-pen aquaculture production by production in floating RAS systems.

A 30 WEEK FEEDING TRIAL SHOWS SUPPLEMENTATION OF ADDITIVE MIXTURES IN PLANT-BASED DIETS IMPROVES GROWTH, MYOGENIC GENE EXPRESSION AND FILLET QUALITY OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

The plant-based protein (PP) can negatively impact growth as well as fillet quality of fish due to the presence of anti-nutritional factors, imbalanced nutrient content, and low digestibility. Consequently, the incorporation of feed additives into PP feed holds promise in enhancing its utilization in fish and improving fillet quality. Thus, the objective of this study was to assess the effects of supplementing PP diets with two different types of additive mixtures on the growth, myogenic gene expression and fillet quality of rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Two thousand rainbow trout (2.22 g) were distributed in four groups with 5 replicates (100 fish/tank) and fed four isonitrogenous (42% CP) and isolipidic (20% lipid) diets namely fishmeal based diet as control (30% fish meal, FM), plant based diet (PP), PP+A1 (PP supplemented with mixture of krill meal, taurine and organic selenium) and PP+A2 (PP supplemented with mixture of proline, hydroxyproline and vitamin C). Fish were fed twice daily and six days a week at apparent satiation for 30 weeks. After end of the feeding trial, growth (weight gain, WG) and feed conversion ratio (FCR) were calculated. The relative expression of myogenic genes (myoblast determination protein 2, *MyoD2*; myogenic factor 5, *Myf5*; and myocyte-specific enhancer-binding factor 2a, *MEF2A*) were measured. The fillet quality parameters like texture profile analysis (TPA: hardness, cohesiveness, springiness, chewiness, and resilience) and fillet color properties (lightness, L^*; chroma, C^*; and hue, h^o) were measured. All the data were subjected to one-way analysis of variance (ANOVA) using General Linear Model in R-programming (RStudio version 2023.06.0).

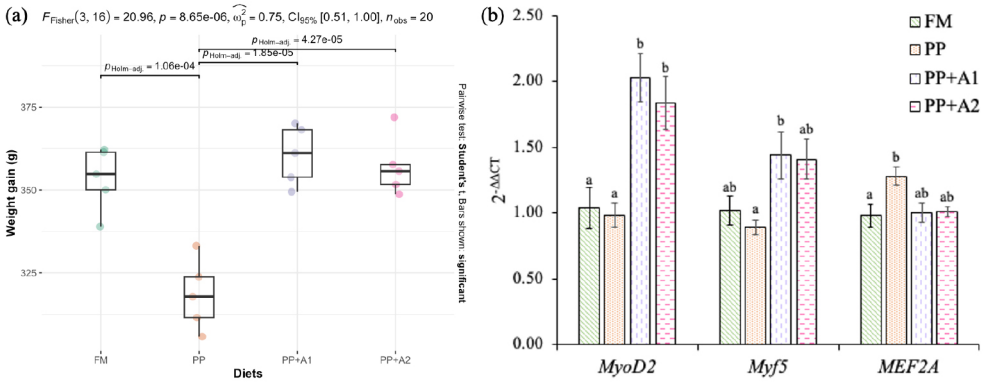

Figure 1: (a) Weight gain and (b) myogenic gene expression of different groups after 30 weeks

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Table 1: Texture profile analysis (TPA) of rainbow trout fillet after 30 weeks

Diets	Hardness (N)	Cohesiveness	Springiness (%)	Chewiness (N)	Resilience
FM	5.51[a]	0.65[ab]	87.1	3.14	0.46
PP	5.11[a]	0.69[b]	87.1	3.03	0.41
PP+A1	7.49[b]	0.59[a]	82.7	3.74	0.39
PP+A2	7.88[b]	0.59[a]	82.3	3.84	0.36
SEM	0.39	0.022	2.07	0.248	0.027
p-value	<0.001	0.013	0.221	0.083	0.08

Results

Results showed that dietary supplementation of additive mixtures in PP diets significantly ($p < 0.05$) improved the growth performance as compared to PP group without additive mixture and comparable to FM group (Figure 1a). Although there was no significant difference in FCR (0.83-0.87), the overall feed intake on PP group was lower than other groups. Hyperplasia related myogenic genes (*MyoD2* and *Myf5*) were significantly ($p < 0.05$) up-regulated in the additive mixture supplemented groups (Figure 1b). Whereas no difference in hypertrophy related gene, MEF2A was observed between additive mixture supplemented groups and other groups. Fish fillet quality was also affected by additive mixture supplementation (Table 1), hardness of both additive mixture supplemented groups was higher than other groups, whereas cohesiveness of these groups were comparable to FM group. Fillet color properties showed that PP+A1 had higher value of C^* and lower value of L^* and h^o as compared to other groups.

Discussions

The supplementation of additive mixtures has been shown to ameliorate the negative impact of PP-based diets and improve the growth of rainbow trout. Furthermore, the up-regulation of myogenic genes (*MyoD2* and *Myf5*) in the groups supplemented with the additive mixture supports its positive impact on growth and nutrient utilization, leading to enhanced muscle mass. The supplementation of additive mixtures has been found to improve fillet quality, particularly in terms of fillet hardness in both supplemented groups. Additionally, the fillet color properties were improved in the PP+A1 group compared to other groups. While previous studies have shown that the complete substitution of fish meal from various species does not have a negative impact on fillet quality during short-term culture periods (Kaushik et al., 1995), long-term feeding of a fish meal-free diet has been found to significantly reduce growth, as well as color properties and sensory quality in rainbow trout (De Francesco et al., 2004). In summary, the supplementation of additive mixtures in PP-based diets positively impacts the growth, muscle development, and fillet quality of rainbow trout.

GEOSMIN AND 2-METHYLISOBORNEOL FATE IN GREATER AMBERJACK RAS FARMING

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Introduction

The presence of off-flavour compounds, produced by various microorganisms, in Recirculating aquaculture systems (RASs) represents a major risk for reducing the quality of farmed fish. Despite being nontoxic in the concentrations detected in RAS systems and fish tissue, they pose a serious threat to the profitability of RAS operator as tainted fish causes customers dissatisfaction. Even at very low levels (< 20 ng/L), the accumulation in fish tissue can reach hundreds of ng/kg of fish (Houle et al., 2011). It has been shown, that humans can detect off-flavors in concentrations close to 250 ng/kg of fish (Lindholm-Lehto et al., 2020). In this study, we investigated the occurrence of two off-flavors, geosmin and 2-methylisoborneal (2-MIB), responsible for earthy and musty taste. The study was performed in a full-scale land-based RAS (located in Germany) during cultivation of greater amberjack (Seriola dumerili). The fate of the off-flavors in the system was examined on two occasions before and after feeding at various locations in the system.

Materials and methods

The study was carried out in a RAS working with artificial seawater and less than 1% of the water renewal rate per day. Seawater was prepared by mixing tap water with a commercial salt mixture (Sequasaal). The RAS comprised of two drum filters with a 100 and 60 mm screen panels and a protein skimmer to remove large and fine particulate waste, respectively. The protein skimmer was operated with ozone (dosing interval between 170-280 mV) to enhance the removal of fine solids, reduce bacteria load, and oxidize part of the total ammonia and nitrite. The system held a total biomass of 4 Metric Tons (MT), and fish were fed with 7 mm Seriola Protec (Skrettiing, France) at a feeding rate of 0.9% of the body weight (BW). Water parameters were the following: Temperature 21ºC, pH 7.6 - 7.8, Oxygen 95-100%, Salinity 20 PSU, Total ammonia <0.5 g-N/l, Nitrite <0.1 mg-N/l, Nitrate 30-70 mg_N/l and Residual Ozone <0.001 mg Cl^-/l. Sampling of RAS water was performed before and after feeding, at 6 locations of an industrial RAS (2500 m^3), i.e. Tank 1 (T1), Tank 2 (T2), after drum filter 1 (ADF1), inside protein skimmer (ISK), after biofilter (ABF) and after drum filter 2 (ADF2). First set of samples was collected 1 hour before feeding in the morning, whereas the second set of samples was collected after feeding in the middle of the day. The samples were taken twice from inside the protein skimmer with ozonation being on and off. All samples were collected in triplicates. A GC-MS/MS method was developed to measure geosmin and 2-methylisoborneol in RAS water. Water samples (0.7 L each) were concentrated by dichloromethane extraction alongside a stable isotope labelled geosmin internal standard (geosmin-d3). Extracts were analyzed by an Agilent 7890A gas chromatograph coupled with an Agilent 7000 triple quadrupole mass spectrometer fitted with an EI source and collision cell was used (Agilent Technologies, Santa Clara, CA, USA). An Agilent DB-5MS UI GC-column (30 m × 0.25 mm x 0.25 µm) was used in analysis. The carrier gas was high purity helium at constant flow (1.2 mL/min). The detection limit was determined through calibration curve slope method and was 1.8 and 2.9 ng/L for Geosmin and MIB, respectively with a linear range of 1-100 ng/L (Correlation coefficient of 0.9997 and 0.9994 for GSM and MIB).

Results

RAS water tested at various locations in the system showed to contain low amounts of geosmin, with an average of 4.8 ng/L before feeding and 4.9 ng/L after feeding. No “hot spots” for geosmin and 2-MIB were identified in the system.

The sensory threshold is believed to be influenced by differences in sensory evaluation person to person, sensory characteristic of the fish species and presence of other off-flavors that could mask the earthy flavor (Lindholm-Lehto and Vielma, 2019). Nevertheless, Petersen et al. (2011) attested that tainting of the fish by geosmin occurs when its concentration in water exceeds 10 ng/L (study performed on rainbow trout). Sensory analysis of the harvested fish confirmed that the geosmin levels were low enough to not cause off-flover issues. Concentrations of off-flavour compounds in the RAS allowed for a spatial mapping and are provided in Table 1.

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Table 1 : Concentrations of off-flavour compounds before and after feeding at different RAS locations

GSM			
Mapping before feeding		**Mapping after feeding**	
Sample Name	**Concentration (ng/L)**	**Sample Name**	**Concentration (ng/L)**
T1-bf	4.83±0.25	T1-af	4.92±0.19
T2-bf	4.67±0.67	T2-af	4.96±0.41
ADF1-bf	5.09±0.48	ADF1-af	4.94±0.17
ISK-noO3-bf	4.79±0.33	ISK-noO3-af	4.94±0.30
ISK-wO3-bf	nd	ISK-wO3-af	4.58±0.62
ABF-bf	4.85±0.35	ABF-af	5.77±1.92
ADF2-bf	4.77±0.41	ADF2-af	4.36±0.47
MIB			
Sample name	**Concentration (ng/L)**	**Sample name**	**Concentration (ng/L)**
T1-bf	<LOD	T1-af	<LOD
T2-bf	<LOD	T2-af	<LOD
ADF1-bf	<LOD	ADF1-af	<LOD
ISK-noO3-bf	<LOD	ISK-noO3-af	<LOD
ISK-wO3-bf	<LOD	ISK-wO3-af	<LOD
ABF-bf	<LOD	ABF-af	<LOD
ADF2-bf	<LOD	ADF2-af	<LOD

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DISAPPEARING SPAT ON NEW ZEALAND'S GREENSHELL™ MUSSEL (*Perna canaliculus*) FARMS, MAGNITUDE, CAUSES, AND POSSIBLE SOLUTIONS

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The Greenshell™ mussel (*Perna canaliculus*) industry is New Zealand's largest and most valuable aquaculture industry, and yet despite its success, the industry remains extremely inefficient at converting seed mussels ("spat") into market-ready adults. The cause of this inefficiency is largely due to high spat losses that often take place early in the production cycle, shortly after seeding out (i.e., within the first 3 months of production). However, until recently, little was known about the magnitude of spat losses across the industry, and the potential causes of these losses. In turn, this lack of knowledge made it difficult to begin to develop any practical solutions aimed at addressing the problem and reducing spat losses. This presentation will summarize the findings of several studies which were undertaken to 1) begin to quantify the magnitude of spat losses both on individual farms and on an industry-wide basis, and 2) to start to identify some likely causes of the problem. The presentation will demonstrate that, despite considerable research effort, the exact causes of spat losses remain poorly understood. However, this presentation will also outline the results from initial studies that show that land and/or sea-based nursery culture (i.e., growing spat to larger sizes prior to seeding) may be a promising approach to finally solving the problem for production.

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UNVEILING THE FOOD PREFERENCES OF COMMON CARP IN FISHPONDS: EXPLORATION OF DIET COMPOSITION OVER GROWING SEASON

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Introduction

European fishponds have a centuries-old history that has played a pivotal role in shaping both local economies and the environment. The central figure in European pond aquaculture is the common carp. Pond aquaculture primarily employs the semi-intensive method, wherein carp rely on both natural prey and supplemental feed (usually cereals). Common carp are known to be omnivorous, consuming a diverse array of food items. This study undertakes a comprehensive exploration of the diet consumed by common carp in Czech fishponds under semi-intensive management.

Material and methods

We did a survey in 3 experimental ponds (surface area: 0.16 ha; average depth 80 cm) in South Bohemia (Czechia). All ponds were similarly stocked with the same size-class common carps (2 years-old age cohort; mean total length: 267.9 ± 15.2 mm; mean weight: 337.2 ±56.6 g) corresponding to 938 ind. ha^{-1} and 316 kg ha^{-1}. Following established practices, fish were fed with cereals since May (Füllner, 2015). Fish were sampled monthly to monitor growth and to collect samples of the digestive tract content (the non-lethal method was used; see Faina, 1983). Furthermore, the fish stock index and food conversion ratio were calculated. Physical-chemical environmental parameters and food items (zooplankton, zoobenthos) were monitored and sampled monthly.

Results and discussion

The total biomass gain yielded 1,420 kg ha^{-1} per pond, with the food conversion ratio reaching 2.83. Common carp consumed a wide range of food items, yet their diet changed throughout the growing season. The common carp consumed primarily zoobenthos at the beginning of the growing season (April–June), and cereals did not contribute significantly to carp nutrition. Nevertheless, the contribution of both cereals and zooplankton to carp diet increased from the mid-summer (June/July). Crustaceans (cladocerans and copepods), therefore, played a more substantial role in the carp's diet during the latter half of the growing season as well as cereals.

Acknowledgement

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THE EFFECT OF INSECT-BASED EXTRUDED DIETS ON GROWTH PERFORMANCE SHELL DEVELOPMENT AND CAPTIVE HUSBANDRY OF SIDE NECK TURTLE (*Emydura subglobosa*)

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Introduction

This study provides empirical data on the balancing, production and quality of freshwater turtle diets containing partially defatted *H. illucens* meal (BSFL) as a fish meal replacement. The BSFL was applied as 7.5 and 15% of the diet substituting 25 and 50% of fish meal, respectively.

Materials and methods

Three diets were calculated: control (CON), consisting of 30% FM and 0% BSFL; H75, consisting of 22.5% FM and 7.5% BSFL; and H150, consisting of 15% FM and 15% BSFL. Feeds were prepared by extrusion processing with a single-screw warm extruder. Experimental feeds were analysed in terms of pellet length, width, expansion, water binding capacity and volume increase. The growth trial was carried out using 27 six-month-old *E. subglobosa* juveniles with an average body weight of 45.7 g (weight range from 42.6 to 49.4 g). The animals were same-age full siblings hatched in the Laboratory of Inland Fisheries and Aquaculture of Poznan University of Life Sciences. They were randomly distributed into 9 rectangular tanks (3 tanks per treatment). A total of 9 turtles were used per treatment. The experiment was 70-days-long.

Results

Post-extrusion tests showed that feed technological parameters are dependent on the BSFL meal share in terms of pellet length, expansion rate, volume increase and water binding capacity. The obtained experimental feeds were well accepted by the animals. In the 70-day-long experiment, no turtle mortality or diet-related issues, as well as differences were recorded in turtle shell development and growth performance among the treatments. However, BSFL meal application increased feed intake when 7.5% BSFL meal was used and decreased the feed conversion ratio for the 15% BSFL-containing treatment. For the first time, it was empirically proven that red-bellied short-necked turtle (*Emydura subglobosa*) efficiently utilizes BSFL meal in up to 15% of the diet. Moreover, the possibility of decreasing total marine resource use by 55.8% in turtle husbandry was recorded, mainly due to 57.4% lower fish meal use per kg of turtle weight gain.

Conclusions

This study proves that partially defatted *H. illucens* meal is a suitable novel feed component for extruded aquafeed production. The obtained results of turtle growth performance and feed conversion indicate that it is well accepted by *E. subglobosa* and its components well utilized. It was empirically proven that the dietary application of *H. illucens* increases the environmental sustainability of *E. subglobosa* husbandry.

INDICATORS FOR SUSTAINABLE PRODUCTION METHODS IN ATLANTIC SALMON FARMING

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Introduction

During the last ten years a wide range of concepts have been developed for facilitating the grow-out phase of Atlantic salmon (*Salmo salar*) farming. In addition to traditional sea-based, open, flexible net pens, current available production methods cover land-based, submerged, semi-closed, closed, and offshore. There are large variations in solutions within each category, with some concepts crossing categories, and there is not complete agreement on where to draw the lines between categories.

Drivers for the development of novel concepts include challenges related sea lice, escapes, effects on wild salmon, fish welfare, climate, and environment. In Norway, production capacity is regulated through the traffic light system which is based on number of sea lice. The various concepts have different solutions for dealing with the challenges of the industry, however they often introduce new concerns such as material use, energy consumption, operational and structural challenges. The development licenses scheme in Norway from 2015 was a significant driver for development of new concepts (Moe Føre et al., 2022). A requirement for being awarded licenses was that the solution was novel and required significant investments (Osmundsen et al., 2022).

Ongoing processes for possible new license schemes are likely to have strict requirements for technology and area use to solve important environmental challenges. Knowledge about the effects of technology and operation on sustainability can become very important in future license schemes. However, uncertainty is still related to effects of novel concepts on environment, economic, and social sustainability.

An ambition of significant increase in Norwegian production in the next decades calls for more knowledge about what effects this growth can have on sustainability. This includes consideration of effects on local environments, climate, social and economic impacts on local, regional, and national levels. For example, land-based production is expected to have higher energy and material consumption that traditional pens, but is also expected to solve challenges related to sea lice, pollution and effects on wild salmon. Therefore, if most of the growth is realized by land-based facilities the total footprint of the industry would be different than if most of the growth was made in traditional pens.

The project "Increased knowledge about effects on climate, nature, and environment from different production methods for salmon", funded by FHF (Norwegian Seafood Research Fund, grant #901833), seeks to enlighten this topic. This presentation focuses on the topic of work package 1 in the project, which is to document and analyse indicators for sustainability for all three dimensions of sustainability – environmental, economic, and social.

Methods

This study investigates indicators for evaluating the sustainability of different production methods for salmon farming. Considering each of the six production methods; traditional/open, land-based, submerged, semi-closed, closed, and offshore, the goal is to map and define relevant indicators for all three sustainability dimensions. The research question is what indicators are the most relevant for evaluating sustainability of production methods, what is the knowledge status, and what are the research gaps?

Initially, document analysis is performed to describe the status from existing literature on effects of salmon farming. This covers research literature, standards, regulations and sustainability reports from companies. Workshops with industry partners are then held to discuss their perception of the practicalities and usefulness of indicators, and what are the most important in their opinion with respect to the challenges of the industry and achieving sustainable farming of salmon. This step is supplemented with assessments in the project group to arrive at a selection of indicators that are more effective and comprehensive, hence having a higher utility than other indicators. Finally, interviews and discussions with key resource individuals both in industry and research will enable clarification and quality assurance of the work. In addition, a questionnaire is sent to a selection of industry partners ahead of the workshops.

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Results & Discussion

There is need for a common set of indicators to assess the sustainability of aquaculture and production in new systems. Our work presents an overview of all current indicators for environmental, social, and economic sustainability and a recommendation of the indicators that are most relevant to provide a just assessment of overall sustainability. The indicators will also be weighted to reflect current regulations and national and global sustainability targets.

A wide range of indicators are used in different relations to describe aspects related to sustainability in salmon farming. Several indicators have large impacts on the companies, for example, by being used by authorities in regulating production or in reporting to investors affecting the attractiveness of the company. Uncertainty, precision, and comparability of indicators are important topics determining the trust to and legitimacy in addition to the usefulness – either in terms of directing operation in the desired direction or for gathering information on development. An example of uncertainty is when counting sea lice in a pen. Counting all 200 000 fish frequently is currently not an option, therefore there is statistical uncertainty related to the sample. Precision can be considered in terms of how well the indicator represents the more complex reality it is meant to describe, for example, how well does mortality describes fish welfare? The concern of comparability is related to the degree to which an indicator can be used to make useful comparisons between systems and productions. For example, if indicators are formulated in ways that open for "creative accounting" this can result in data making comparisons futile.

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THE ASTRAL TECHNOLOGY USER GUIDE – A KNOWLEDGE DEVELOPMENT AND CAPACITY BUILDING TOOL FOR AQUACULTURE TRAINING COURSES AND APPRENTICESHIPS

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The Horizon 2020 All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture (ASTRAL) project focuses on supporting and promoting integrated multi-trophic aquaculture (IMTA) farming and sustainable aquaculture production across the Atlantic. The development of new and improved technological innovations, as well as knowledge sharing and capacity building, are priority areas within the project. A combined output from the ASTRAL human capital development plan and technology work packages, is the *ASTRAL Technology User Guide for Training Courses and Apprenticeships* (Smith et al, 2022), which provides a broad overview of the state of the technology landscape available for aquaculture.

The ASTRAL technology user guide provides information on a range of technologies suited to all levels of IMTA farming and aquaculture, from cost-effective solutions for small scale farms to more advanced and data-intensive systems appropriate for larger commercial and research operations wanting to increase efficiency in farm management and optimise production. While there are technology applications in many aspects of aquaculture, this guide focuses on the use of technology for water quality, biomass measurements and monitoring, and touches briefly on the relevant parameters, sensors, associated analytics, and considerations associated with their use and application. The guide is split into several sections including: the sensors and technology used for four different monitoring topics, namely physico-chemical water quality parameters and sensing methods, aquaculture stock and biomass estimation sensors, threat detection, and environmental variables; and the considerations in terms of instrument choice, operational environment, and system design.

Bearing in mind the unique requirements of each site, farming system, and business model, this guide provides an overview of principal water quality parameters, biomass estimation methods, and sensor technologies commonly used to measure and monitor these variables. Examples are provided of relevant commercial solutions, in addition to pertinent ASTRAL-specific technological developments and research topics. It is our hope that this guide will provide an approachable and informative reference source for aquaculture-related training courses.

Acknowledgements
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ficoEst – A TOOL TO ESTIMATE THE BODY COMPOSITION OF FARMED FISH

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Introduction

Estimating the body composition of fish is an important step in fish nutrition, as it is the basis to calculate nutrient retention efficiencies and utilization rates. In fish nutrition studies, nutrient retention can be used to assess the nutritional quality of diets, but also to support the definition of nutrient requirements and the estimation of nutrient waste outputs. From the point of view of fish farmers, knowing the body composition of fish more regularly throughout the production cycle can help in the search for more effective feeding strategies and also to have a better quality-control of their products.

Usually, body composition of fish is estimated through analytical methods, such as the ones described by the Association of Official Analytical Chemists (AOAC). Despite being reliable and robust methods to estimate the body composition of fish, these analytical methods are not always a viable option to estimate the body composition of fish, since they are time-consuming and expensive. This translates into a limitation of the number of samples collected for body composition analysis, which can hinder a detailed analysis of nutrient flux in fish.

Here we present ficoEst, a public web-tool for researchers and fish farmers to estimate the body composition of farmed fish (https://webtools.sparos.pt/ficoest/). ficoEst uses calibrated and validated mathematical models to provide estimates on the body composition of different fish species (i.e., gilthead seabream, European seabass, meagre, rainbow trout, Atlantic salmon and Nile tilapia). ficoEst can be seen as a complementary tool to support studies on fish nutrition or to increase information collected at the farm level, whenever analytical methods are not a viable option.

Data collection and model development

Data on the whole-body composition of fish were collected for the abovementioned fish species. All data was processed into a standard format and analyzed. The outcomes of the data analysis process were crucial in providing a solid foundation for the model development phase, e.g., for identifying the key explanatory variables.

Different types of models and calibration methods were developed and tested, aiming to select the best methods in estimating the body composition of fish. All developed models fall into one of the following three families:

- BC1: models that consider only the body weight of fish as input;
- BC2: models that consider the body weight and water percentage of fish as inputs;
- BC3: models that consider the body weight, water and ash percentage of fish as inputs.

To select the best combination of model and calibration method (e.g., least squares, Huber loss minimization, mixed-effects, seemingly unrelated regressions), per family, we used cross-validation, where different error metrics were evaluated (e.g., MAPE, RMSE, AE). After selecting the model and calibration method to use per family, the models were calibrated and validated for each species. Figure 1 shows the model validation results for the BC3 model (similar analysis was conducted or BC1 and BC2 models, but not presented here).

Figure 1. BC3 model validation results. The "error - PREDICTIONS" is associated with the model performance and is calculated as the mean absolute percentage error (MAPE): $MAPE\ (\%) = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{P_i - O_i}{O_i}\right|$. The "error - OBSERVATIONS" is associated with the average coefficient of variation in the data used to validate the model and is calculated as: $PE_{observations}\ (\%) = \frac{100}{n}\sum_{i=1}^{n}\left(\frac{sd_value_i}{mean_value_i}\right)$.

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Tool implementation

All modules that compose the ficoEst tool were implemented in R. The back-end engine consists of different functions used to: compile, process, and plot data; evaluate, calibrate, validate and run models; and generate reports. The front-end (i.e., user-interface) consists of functions used to create input controls, and informational and navigational elements. Figure 2 shows the user-interface. To get estimates on the crude protein, crude lipids, water, ash, phosphorus and energy content of fish, users need to enter data on the body weight, and/or water and ash content of fish (depending on the model selected).

Acknowledgements

This work is part of project 47175_FICA, supported by Portugal and the European Union through FEDER/ERDF, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020.

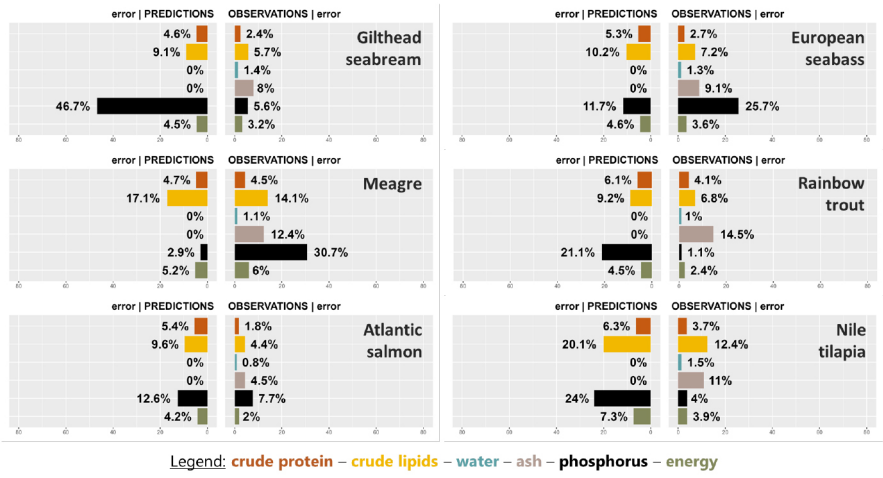

Figure 1. BC3 model validation results. The "error - PREDICTIONS" is associated with the model performance and is calculated as the mean absolute percentage error (MAPE): $MAPE\ (\%) = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{P_i - O_i}{O_i}\right|$. The "error - OBSERVATIONS" is associated with the average coefficient of variation in the data used to validate the model and is calculated as:

$$PE_{observations}\ (\%) = \frac{100}{n}\sum_{i=1}^{n}\left(\frac{sd_value_i}{mean_value_i}\right).$$

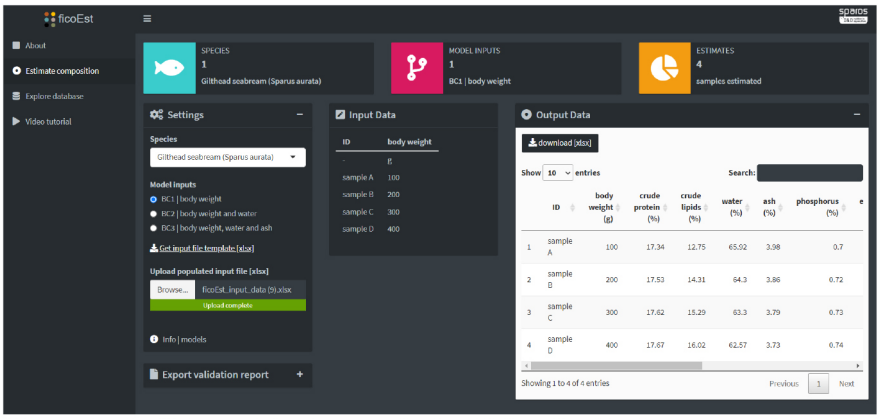

Figure 2. ficoEst user-interface, available at https://webtools.sparos.pt/ficoest/.

EVOLUTION OF INTESTINAL MICROBIOTA DURING ANTIBIOTIC TREATMENT IN *Sparus aurata* LARVAE FED WITH LIVE FOOD ENRICHED WITH MICROALGAE AND PROBIOTICS FORMULATIONS

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Introduction

Sustainable aquaculture involves monitoring water quality, food safety, and microbiological indicators. However, with the intensification of the production, as a result of the pressure to provide food to a growing world population, with increasing farming densities and with the use of live preys in the early larval stages, the occurrence of disease outbreaks increases (Sanches-Fernandes *et al*, 2022). These outbreaks are normally provoked by bacteria and there's the need to recourse to antibiotics, which may pose some problems of bacterial resistance (Radhakrishnan *et al*, 2023). Probiotics are microorganisms that are administered as a dietary supplement to protect the host against pathogens and improve the immune system (Rohani *et al*, 2022). The aim of this study is to understand how the addition of probiotics in life preys impacts the gut microbiota when antibiotic treatments are performed.

Methods

To test the effect of two algae-based formulations for live feed enrichment (rotifers and artemia) *Sparus aurata* larvae were distributed through nine tanks of 300 L, at an initial density of 76 larvae L^{-1}, in triplicates. Treatments were as follows: RP - RedPepper (Bernaqua™), ALL - a mix of *Nannochlororpsis* sp., *Tisochrysis* sp., *Aurantiochytrium* sp., *Tetraselmis* sp. microalgae with the addition of probiotic and vitamins and POC - a mix of *Nannochlororpsis* sp, *Tisochrysis* sp., *Porphyridium* sp., *Aurantiochytrium* sp., *Tetraselmis* sp. with the addition of probiotic and vitamins (Necton, Portugal). The culture was maintained at 18.0 ± 0.6 ºC, with 6.9 ± 0.4 mg L^{-1} of oxygen and a photoperiod of 14:10 (day/night). Suddenly, at 17 DAH (days after hatching) all tanks had high mortality, and microbiological analysis was carried out on plates with twenty-five larvae collected from each tank, which were homogenized in sterile saline solution and plated on tryptic soy agar medium (TSA) (Lyophilchem, Italy) with 1% (w/v) NaCl for total marine bacteria and on thiosulphate citrate bile salts medium (TCBS) (ITW Reagents, Spain) exclusive for Vibrionaceae, with 0.5% (w/v) NaCl. The plates were incubated at 24°C for 48h and the bacterial colonies (Colony Forming Units (CFU)) were counted. To choose the most effective treatment, antibiograms were performed with four different antibiotics, enrofloxacin (ENR), florfenicol (FFC30), flumequine (UB) and oxytetracycline (OTC), placing the plate to incubate at 24°C for 24h, verifying that the bacteria were sensitive to OTC. The treatment with 30 ppm of oxytetracycline (OTC) for 2 h was administered during 10 days. Microbiological analysis of the larvae's digestive tract was performed at different sampling points throughout the trial (at 3, 15, 24, and 33 DAH) in the three treatments. Larval survival was calculated at the end of the trial (38DAH).

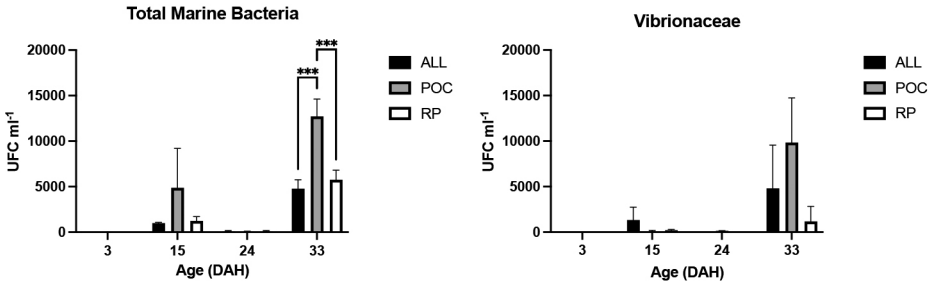

Figure 1 - Mean colony forming units (CFU) of a) Total Marine Bacteria and b) Vibrionaceae bacteria from *Sparus aurata* larvae at 0, 15, 24 and 33 days after hatching (DAH) in different treatments (ALL, POC and RP). Asterisk marks significance between treatments (*** - ANOVA, p<0.001)

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Results and Discussion

At initial analysis (3 DAH) no bacterial growth was observed in TSA and TCBS, as sampling was performed prior to the introduction of live preys. At 15 DAH there was higher growth of total marine bacteria than Vibrionacea, except for the ALL group which had a value similar in both mediums. The ALL group, although it had a lower growth of total bacteria, compared to the POC, was the group that had a higher prevalence of Vibrionacea. At 24 DAH there was no growth of total marine bacteria or Vibrionacea, which is probably related with the OTC treatment performed. Seven days after the treatment (33 DAH) there was a significant increase in total marine bacteria in the POC group compared to the other groups. Besides, there was also a tendency for the POC to present a higher prevalence of Vibrionacea compared to the other groups. In conclusion, the inclusion of probiotics does not appear to have any benefit on the recovery of the gut microbiota after treatment with the OTC antibiotic, however, this did not affect larval survival at 38DAH in the three treatments (ALL - 18.7%; POC - 16.1%; RP - 15.5%), corresponding to weaning time.

Acknowledgments

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EVOLUTION OF MONOGENEA INFECTION WITH THE USE OF ALGAE IN A iRAS SYSTEM OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Integrated Recirculating Aquaculture Systems (iRAS) is a closed system that reuse the water of production based on the use of mechanical and biological filters to remove the toxic compounds produced by the animal, such as ammonia. This system, besides being more costly-effective, allow a better control of parameters such as temperature, salinity, water supply, among others (Sujita, 2021). Although RAS generally reduces disease outbreaks, but rearing conditions can sometimes provide a favorable environment for the reproduction of opportunistic pathogens (Balami, 2019). This study aims to evaluate the impact of macroalgae production on the parasite load in *Sparus aurata*, on an iRAS system.

Methods

The trial was carried out in two independent iRAS systems at the Aquaculture Research Station of Olhão (EPPO): 1) with algae (SA) and 2) without algae (SB). Each system consisted of four fish tanks, nine algae or water tanks, and one filtration zone composed of a mechanical filter and a biological filter (Fig.1). The initial density on the fish tanks was 12 kg/m^3 and the water temperature was around 24±1ºC. During the experimental period, fish from each system tanks A (FA3, FA4) and B (FB1, FB2) were periodically sampled for the observation of parasites. For that, the first two branchial arches on the left side were visualized under an optical microscope (Leica ICC50W) at 0 (T0), at 26 (T26) and 55 (T55) days after the beginning of the experiment. Monogenea eggs occurrence was monitored weekly (T4, T11, T18, T25, T32, T39, T46, T53) by placing quadruplicates of cotton with 11.2 cm each, during five days, in the fish tanks from system A (FA) and B (FB), in the tanks with (SA) and without algae (SB), and filters of both systems (A and B) and the total number of eggs was counted under a magnifying glass, Nikon SMZ1000.

Results and Discussion

Throughout the trial, the occurrence of monogenea eggs was analyzed in the different tanks. No eggs were ever found in the SA and SB tanks (Fig.2a). In the fish tanks (Fig.2b), five days after the beginning of the trial there was a significantly higher number of eggs in the FA4 than FB2, but at the following sampling point, there was an increase in the FB2 tank and a significant decrease of eggs in the FA4 tank remaining stable throughout the trial. The occurrence of eggs in the tank filters (Fig.2c) was lower than the presence of eggs in the fish tanks. From the first observation, there was a decrease in the presence of eggs, and on the remaining days, the quantity of eggs visualized was low.

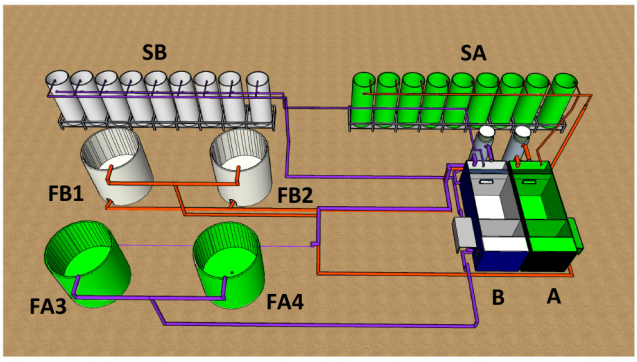

Figure 1 - Schematic representation of the RAS system (by Ivo Monteiro)

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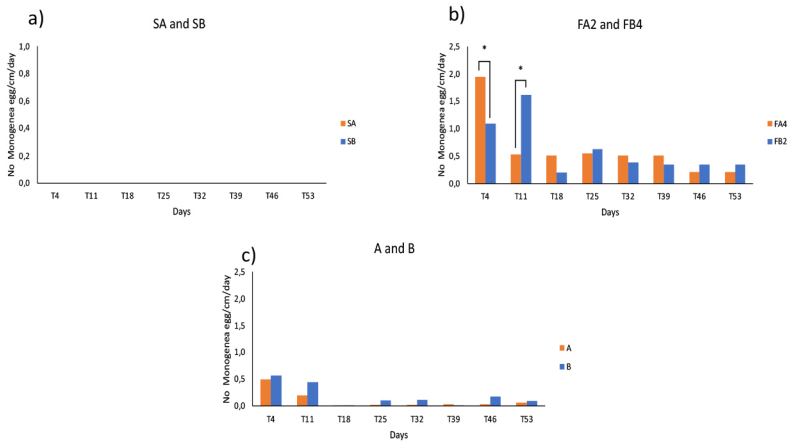

Figure 1 – Mean number of monogenean eggs per centimeter per day, collected by the cotton in **a)** algae tank (SA) and algae-free tank (SB), **b)** filters belonging to the system with algae (A) and to the system without algae (B) and **c)** fish tanks of algae-free system (FB2) and fish tank of algae system (FB4) observed at different times of the trial (T4, T11, T18, T25, T32, T39, T46, and T53). Asterisks reprersent significant diferences between systems (t-test - p-value<0,05)

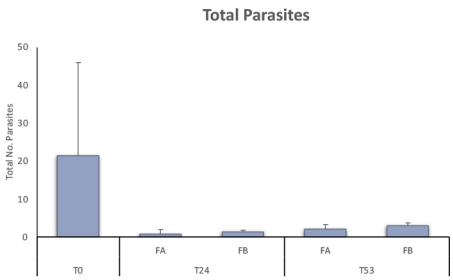

Figure 2 – Mean of total number of monogenea at time 0 and in the fish tanks of system A (FA) and fish tanks of system B (FB) at time 24 (T24) and 53 (T53) days after the beginning of the trial

Concerning the total number of parasites (Fig.3) observed in the fish, the initial point had a higher parasitic intensity per fish corresponding to the mean monogenea quantity of fish before distribution to the system tanks, decreasing in the following two points. At T24 the mean occurrence of monogenea was similar in both systems (A and B), with a tendency to increase at T53. In conclusion, the presence of algae in the iRAS system does not affect the appearance of monogeanean eggs.

Acknowledgments

The present work was financed by the project ATLAZUL (ALG-01-0247-FEDER- 069971), SAUDE&AQUA (MAR-02.05.01-FEAMP-0009), AQUARAS (MAR-02.01.02-FEAMP-0223), INTEGRATE (INTERREG Atlantic Area EAPA_232/2016) and VERTICAL ALGAS (C644915664-00000026).

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DISSOLVED OXYGEN INFLUENCE ON THE OCCURRENCE OF ECTOPARASITES IN MEAGRE (*Argyrosomus regius*)

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Introduction

With the intensification of aquaculture, and consequently the increase on the farming densities, there's also an increase on the appearance of epizootics, resulting from the imbalance of the conditions of the surrounding environment which greatly impact the profitability of the production (Jerônimo et al, 2022). Thus, for a successful aquaculture production, one of the most important parameters to consider is the water quality of the systems as it can have effects on survival and growth, as well as on the overall fish immunity and resistance capacity (Waruiru et al, 2020). Another important factor for an efficient production is the concentration of dissolved oxygen in the water column. The optimal levels for this parameter are species-specific, dependent on the temperature and any changes may have an impact on the produced fish welfare (Akhter et al, 2021). The aim of this work is to evaluate the impact of different oxygen levels on external parasite prevalence in meagre farming.

Methods

The experiment was conducted at the Aquaculture Research Station at Olhão (EPPO) with 250-350g meagre, *Argyrosomus regius*, distributed in triplicate, with a density of 9 Kg/m^3 per tank under natural temperature conditions, over three different dissolved oxygen levels: DO1 - low oxygen (2.5 - 3.0 mg/L); DO2 - medium oxygen levels (4.0 - 5.0 mg/L); DO3 - high oxygen levels (6.0 - 7.5 mg/L). The trial had a duration of 26 days and at the end, 6 fish per tank were sacrificed for observation of external parasites. The first two gill arches on the left side were removed and observed under an optical microscope (Leica ICC50W) and the number of parasites observed was recorded and identified.

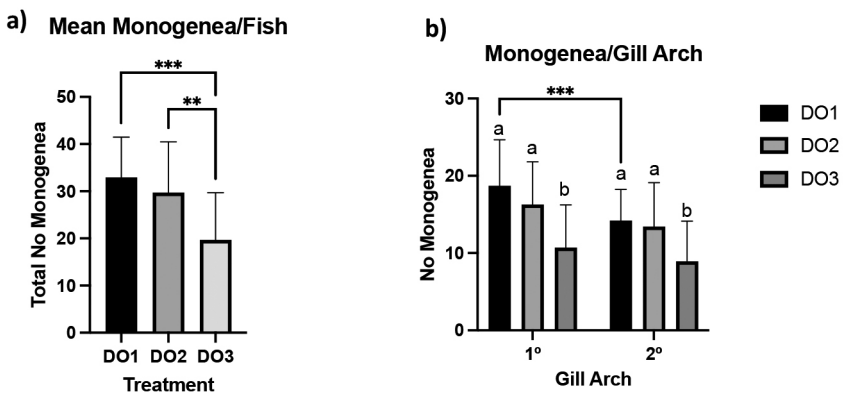

Figure 1 - a) Mean number of monogenea per fish and **b)** number of monogenea per gill arch present in meagre (*Argyrosomus regius*) exposed to three different dissolved oxygen levels: DO1 - low level (2.5 - 3.0 mg/L); DO2 - medium oxygen levels (4.0 - 5.0 mg/L); and DO3 - high oxygen levels (6.0 - 7.5 mg/L). Asterisks and *a* and *b* mark significant between treatments (** - 2-way ANOVA, P≤ 0.002 and *** - 2-way ANOVA, p<0.001)

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Results and Discussion

Observation of the gills revealed the presence of monogenea parasites. In general, the mean number of parasites (**Fig.1a**) was significantly lower in the DO3 treatment compared to the other treatments, with a tendency for DO1 to be higher than DO2. Analyzing the parasitic occurrence in two gill arches separately (**Fig.1b**) we found that DO1 had a greater effect on the occurrence of monogenea presenting statistically significant differences with DO3. DO3 was significantly lower in the intensity of monogenea per arch than DO1 and DO2. In the DO1 treatment, we observed significant differences in the occurrence of monogenea between brachial arches. Despite the presence of parasites in the gills, growth, feed intake and survival were not affected as described by Barata *et al* (2023). In conclusion, higher levels of dissolved oxygen in the water contribute to reduce the occurrence of external parasites, contributing to better welfare conditions.

Acknowledgments

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MYXOBACTERIA AS OFF-FLAVOR PRODUCERS IN RECIRCULATING AQUACULTURE SYSTEMS - ISOLATION AND INFLUENCE ON NUTRIENTS ON OFF-FLAVOR GENERATION

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Introduction

Earthy and moldy off-flavors in aquaculture products reduce consumer preferences for farmed fish and consequently, the off-flavors also cause economic loss for RAS farmers (Abd El-Hack et al., 2022). Finding solutions to this reduce the presence of these compounds is essential, both for economic reasons as well as for the reputation of the entire aquaculture industry. Such off-flavors have historically been affiliated with the compounds geosmin and 2-MIB. These microbiologically produced compounds have mainly been attributed to Cyanobacteria and *Streptomyces*, but as gene sequence databases recently have expanded, molecular studies now indicate that the more obscure Myxobacteria may be the leading bacterial group responsible for earthy off-flavors in aquaculture (Lukassen et al., 2022, Lukassen et al., 2019). For the first time, we succeeded in isolating these bacteria from RAS, enabling their production of geosmin, 2-MIB and other off-flavors to be studied in details by GC-MS. After cultivation in growth media with a variable nutrient composition, production of geosmin and 2-MIB by selected Myxobacteria was characterized to estimate which compounds might control off-flavor production in RAS. These findings provide valuable information to the aquaculture sector to optimize their practice to combat off-flavor issues produced by these bacteria.

Material and Methods

Samples from different compartments of two different Recirculating Aquaculture Systems (RAS) in Denmark were used for the isolation of the bacteria. Isolated strains were identified through 16S rRNA gene amplicon sequencing and subsequently whole genome sequenced using Nanopore. The isolates were cultivated in growth media composed of varying levels of relevant nutrients (nitrogenous compounds, phosphorus, carbon), and volatile organic compounds (VOCs) were extracted using stir bar sorptive extraction (SBSE). VOCs were analyzed through Gas Chromatography-Mass Spectrometry (GC-MS). Production of VOCs in the different media were normalized to cellular production by enumeration of cells through fluorescence microscopy.

Results and Discussion

For the first time, isolation of Myxobacteria from RAS was successful. This bacterial group has the largest genomes among prokaryotes, ranging from 9-16 Mb, which might explain their notoriously slow growth. The slow growth, in combination with their slime-production and swarming colonies, makes isolation and purification of isolates especially hard. Utilizing baiting techniques for both predatory and saprophytic Myxobacteria, three different strains were isolated and purified. Results from 16S rRNA gene amplicon sequencing identified two of the isolates as belonging to the genera *Myxococcus* and *Corallococcus,* with high sequence identity similarity to species *Myxococcus virescens* and *Corallococcus exiguus*. The closest relative to the third isolate was found to be the genus *Pseudenhygromyxa.* Growth of the isolates in rich media, e.g., containing high levels of all considered nutrients, produced insignificant levels of 2-MIB. However, under the same conditions, geosmin was produced in levels >1200 ng/L. For reference, the human odor threshold for geosmin in water is about 5 ng/L (Srinivasan and Sorial, 2011). The high geosmin production supports that these bacteria may have a significant contribution to off-flavours in RAS-reared products. Results from other cultivation conditions are currently undergoing data analysis but will be presented at the conference.

Conclusion

Myxobacteria are prolific producers of the prominent off-flavor compound geosmin in RAS. Strategies to combat the production of off-flavor in these systems can only be developed through knowledge regarding the production of the specific bacterial producer in question. The present study provides further knowledge of how the nutritional characteristics of the rearing water will influence geosmin production in these bacteria.

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GENOMIC ANALYSIS OF A DOWNGRADING TRAIT IN ATLANTIC SALMON USING FIELD DATA

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Introduction

Downgrading traits are economically important for the Atlantic salmon industry because they are directly related to the price of the products. Their prevalence is typically low, and it is therefore difficult to obtain data in a normal sib trait testing regime for genetic studies and thus obtain breeding values for selection as part of a breeding programme. In this study, we focus on melanin spots in the fillets, which is the most common fillet quality problem (Nordberg, 2018). Melanin spots is estimated to be the cause of 9–67% price losses, depending on the intensity and size of the discoloration (Färber, 2017).

There are only a few reported estimates of heritability of melanin spots in salmon fillets; they typically indicate only low genetic variation for melanin, and the heritability estimates were coupled with high standard errors ($h^2 = 0.025 \pm 0.019$ in a study where 32 % of fish had melanin on at least one of their fillets; Kettunen et al., 2020) and $h^2 = 0.01 – 0.02$ in a study where ca 15% of fish had melanin spots (Mørkøre et al., 2015). We hypothesise that these low heritability estimates were a result of the poor data quality, in particular the low overall frequency and unbalanced data with regard to family origin. In this task we set up a system where we sampled production fish at a processing site of MOWI and used this fish for a genetic study, once we had obtained enough data. These fish are related to the nucleus fish and each batch has a limited number of families. The analysis included estimation of heritability, and a genome-wide association study. We compared the parameters obtained from a quantitative analysis assuming a normal distribution of the trait to a probit analysis, to take account of the binary nature of the trait.

Materials and Methods

Data were collected at the processing site of Mowi at Eggesbønes, Norway. At the slaughter line, filleted Atlantic salmon were sorted into Superior (no melanin spots) or Melanin (presence of melanin spot in the front part of the fillet under the spine). Four random batches of fish were sampled from cages with known geographical origin, but with unknown sires and dams. There were 1643 fish in total with 45% Superior and 55% Melanin fish. All fish were genotyped with the in-house custom made SNP-chip of MOWI. 63,826 SNPs passed filters and quality control and were used to set up a genomic relationship matrix using the GCTA software of Yang et al. (2011). Genomic estimates of variance components were estimated from a univariate threshold (with, ***!BIN*** and ***!PROBIT*** functions) and linear models implemented in ASREML 4.2 (Gilmour et al., 2015), using sex (2 levels) and batch (4 levels) as fixed effects. A genome wide association analysis was performed using the following linear mixed animal model implemented in GCTA program with the "–mlma-loco" function (Yang et al. 2011) using sex, batch and five principal components showing stratification in the genetic structures.

Table 1. Variance components computed using linear and threshold models. LM= Linear Animal Model; $TM_{sire-dam}$ = Threshold sire-dam model; TM = Threshold animal model; σ_g^2 = Genetic variance; σ_e^2 = Residual Variance; SE = standard error of estimates.

Source	GENOMIC	
Components/Models	LM	TM
$\sigma_g^2 \pm SE$	0.045±0.010	0.484±0.077
$\sigma_e^2 \pm SE$	0.178±0.008	1.000±0.000
$h^2 \pm SE$	0.202±0.040	0.326±0.035

(Continued on next page)

Results and Discussion

The use of data from non-family nucleus fish from a processing site proved successful for variance component estimation of Melanin spots in Atlantic salmon fillets. The genetic variation for melanin accumulation in fillet obtained using genomic information were low to moderate with estimates of 0.202±0.040 and 0.326±0.035 with linear and threshold models, respectively. It should be noted that heritability of case/control traits often are overestimated.

In the genome-wide association study, chromosome *ssa03* had 7 SNPs located in the region between bp 88.3Mbp - 92.4Mbp that surpassed the chromosome-wide significance threshold. On *ssa07*, 9 SNPs located in the region between 37.1Mbp - 45.7Mbp surpassed the chromosome-wide significance threshold. This is the same genomic region where QTL linked to resistance to Pancreas Disease (PD) have also been detected (Hillestad et al., 2020; Aslam et al., 2020). A random sample of 10 fish were analyzed for the detection of PD virus which showed that 7 out of 10 had PD virus. These results indicate a possible genetic link between melanin spots and susceptibility to PD. They also agree with previous suppositions that the prevalence of melanin spots is linked to inflammatory diseases such as PD (Mørkøre et al, 2015).

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AQUACULTURE TECHNOLOGY GOVERNANCE: FROM STREAMLINED EFFICIENCY TO COMPLEX HETEROGENEITY AND IMPERFECT SOLUTIONS?

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Introduction

Norwegian aquaculture has undergone significant growth and development in less than 50 years. This success, however, has come at a cost, such as pollution, sea lice, and escapes, as well as industry costs related to the combat of these problems. For instance, the cost of combating sea lice increased from about NOK1/kg (€0,085/kg) in 2011 to NOK4.25/kg (€0.36/kg) in 2016. For the industry as a whole, this amounted to an increase from NOK4 billion (€338 million) in 2011 and NOK5 billion (€422 million) in 2016 (Abolofia, Asche et al. 2017, Iversen, Hermansen et al. 2017). To tackle these issues, the government facilitated industry innovation through a temporary licensing regime in 2015, known as the development license regime (or development projects). This has led to a range of new production systems based on a mixture of heterogenous and complex technologies. This paper shows:

1. How these new technologies and production systems challenge the existing governance system, and
2. How increased this in turn demands an adaptive governance approach.

Materials and methods

The paper is an empirical paper, based on an inductive multimethod approach. We examine six new and significantly different production systems. Through almost fifty semi-structured interviews with various actors involved in the processes of developing these systems, we gain a deep understanding of how new technologies change the system-to-be-governed and how this, in turn, affects the governing system (Johnsen 2017). Through Thematic Content Analysis (TCA), we identify common themes, while also presenting the voices across all participants (Anderson 2007).

Results

The paper will show *empirically* how new technology increases the "wickedness" of aquaculture governance by increasing the complexity, diversity, uncertainty, and controversies in the aquaculture governance system (Marchant 2011, Marchant, Allenby et al. 2011, Marchant 2020). Due to the increased wickedness, there are no optimal solutions to how to manage these emerging technologies. Moreover, as the development projects always are "work in progress", objectives are not permanent, but dynamic and subject to adjustments based on experience and learning. Consequently, the messiness created by these new technologies slows down regulatory processes, but also challenges the bureaucratic processes related to the development projects (Gaudet and Marchant 2011). To handle the rising complexity, diversity, uncertainty, and controversies; the governing system "muddles through", creating working, but imperfect solutions.

Conclusion

Based on a case study of six technological aquaculture innovations, this article shows how government-initiated technological innovations challenge the aquaculture governance system, and how the governing system must adapt to these changing conditions.

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AQUACULTURE DEVELOPMENTS IN EAST AFRICA THROUGH THE LENS OF TWO EUROPEAN FUNDED HORIZON PROJECTS FOODLAND AND PRAECTICE

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FOODLAND and PrAEctiCe are European funded Horizon projects focusing on food production and farming in Africa. FOODLAND began in September 2020 and will run until August 2024 aiming to enhance the diversity of food production and consumption in six African countries displaying different stages of the nutrition transition. To this end, FOODLAND will create a network of 14 local Food Hubs that will aggregate relevant actors and serve as injection points for the introduction of innovations. FOODLAND has identified specific objectives addressing the organizational, technological, and nutritional needs of the local African food systems: 1. To detect behaviour and preferences of consumers and producers, in order to customize innovations to local sensitivities; 2. To develop and implement organizational innovations, aimed at boosting coordination among food operators; 3. To develop, test, and validate (open) technological innovations in the laboratory and in the field; 4. To disseminate knowledge of solutions towards malnutrition reduction and innovations.

PrAEctiCe began in November 2022 and is a 42-month project that will provide a novel agroecology indicator set for East Africa, aimed at helping smallholder farmers in their agroecological transition. The project goes beyond the existing indicator frameworks by putting the "concept into action" with a decision support tool for agroecology advisors supporting the selection of the best suited combination of agroecological farming practices in a local context.

These projects give strong attention to activities pertaining to aquaculture. FOODLAND is developing aquaculture technologies for urban and peri-urban areas to ensure the production is brought closer to the markets resulting in a shorter distribution chain that can be more competitive with imported products. The Aquaculture Working Group, based in Kenya, Uganda, Tanzania, and Tunisia, are innovating in several different areas to develop technologies and techniques that will support and develop local aquaculture practices. New feeds are being investigated to reduce the reliance on fishmeal heavy, imported feeds, new and updated protocols for the production of local species are being created, recirculating technologies that can be reproduced by small-scale farmers for fingerling production are being assessed and research on integrated agri-aquaculture production systems, using wastewater from aquaculture to grow crops, is currently ongoing.

The PrAEctiCe project will establish three living labs focusing on circular water-energy-nutrient systems of integrated aqua-agriculture. Living lab one builds on a previous Horizon 2020 project "VicInAqua" and is a recirculating aquaculture system run on municipal wastewater filtered using a membrane bioreactor, the system will be upgraded to include grow-out ponds and wastewater from the fish production systems will be used to irrigate crop production. Living lab two is an aquaponics system, integrating fish production with a range of crops. The third living lab will utilize a pond culture system integrated with poultry and vegetables, the poultry waste will be used to fertilize the ponds and the wastewater from the ponds will support in the irrigation of the crops. Each of these systems will be adapted to an East African environment and showcase available technologies and opportunities that will be replicable by local farmers.

Each project highlights the different directions into which aquaculture is developing in the different East African countries, emphasizing the technologies, techniques, and systems that are of the highest interest and benefits to the different regions. These differences allow for many exciting areas of research and development across and between the partners involved while also bringing into focus the primary commonality between both projects which is the necessity for all systems developed to be replicable by local farmers at different scales, small-scale, subsistence farming as well as larger, commercial scale endeavours.

Acknowledgement
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TESTING THE DISINFECTION CAPACITIES OF A HYDRO CAVITATION PROTOTYPE IN A RECIRCULATING AQUACULTURE SYSTEM AND THE EFFECTS ON WATER QUALITY PARAMETERS, AND GROWTH PERFORMANCE OF EUROPEAN SEABASS *Dicentrarchus labrax*

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Introduction

To overcome the challenges posed by unstable water conditions in traditional marine hatcheries due to climate change and human activities, this project aims to revolutionize the concept by implementing recirculating principles to minimize reliance on natural water resources. A crucial element of recirculating systems is effective water disinfection and treatment, ensuring a suitable environment for farmed species and reflecting on their health, welfare, and product quality. This study evaluates the impact of a prototype hydro cavitation device on water quality parameters, growth, and feed utilization performance in European seabass (*Dicentrarchus labrax*).

Material and methods

The experimental trial consisted of two phases. Phase 1 focused on testing the hydro cavitation device's impact on biofilter maturation and related water quality parameters. Phase 2 involved a growth trial with European seabass at the hatchery stage to assess the extent to which growth and feed utilization performance were potentially affected by using the aforementioned equipment.

The experiment was conducted in a recirculating aquaculture system (RAS) comprising 12 culture tanks of 130 liters each. The system included a protein skimmer, UV sterilization, ozone sterilization, and a biological filter.

Phase 1 lasted for six weeks, during which the system was prepared and managed by monitoring water quality and providing carbon and nitrogen sources to stimulate bacteria development.

In Phase 2, the growth trial spanned four weeks. Four different disinfection setups were tested: WK1 (hydro cavitation + UV), WK2 (UV disinfection), WK3 (hydro cavitation), and WK4 (no disinfection). Multiple samplings were also performed during each week to monitor the water quality, estimate the total numbers of bacteria (TVC) as well as health checks of fish. The aim was to evaluate the potential impact of hydro cavitation technology on water quality and fish growth performance. European seabass of approximately 5g were randomly allocated, with a starting number of 30 fish per tank in seven experimental tanks.

Table 1. Growth performance of European seabass after four weeks of trial.

	T2	T3	T4	T5	T8	T10
Initial weight (g)	5.07	5.00	5.00	5.07	5.00	4.87
Final weight (g)	11.50	10.90	10.90	11.33	11.55	11.03
WG	6.43	5.90	5.90	6.27	6.55	6.17
Feed intake (kg)	151.00	151.00	149.00	151.00	151.00	151.00
FCR	0.78	0.85	0.84	0.80	0.79	0.85
SGR	2.93	2.78	2.78	2.88	2.99	2.92
SFR	2.29	2.37	2.34	2.31	2.38	2.48
Survival %	100.00	100.00	100.00	100.00	96.67	96.67

(Continued on next page)

Results

The results of the biofilter maturation experiments showed the conversion of NH_4 to NO_2 starting on day 15. The total ammonia concentration initially increased to 1mg/l, but then sharply declined to 0.25mg/l. NO_3- levels exhibited a spike between days 3-4, reaching 100mg/l, which decreased to 0mg/l from day 5 until day 14. A peak of 10mg/l was observed on day 14, followed by wide fluctuations ranging between 10 and 100mg/l from day 17 onwards until day 40.

In phase 2, the overall growth performance followed a similar trend across all tanks. Tank 2 had the lowest feed conversion ratio (FCR), while tank 3 and 10 had the highest. Specific growth rate (SGR) was lowest in tanks 3 and 4, and specific feed rate (SFR) was highest in tank 10 and lowest in tank 2. The survival rate showed no mortalities in tanks 2, 3, 4, and 5, with only one mortality each in tanks 8 and 10.

The water quality profile (temperature, pH, dissolved oxygen, and CO_2) remained stable throughout the four-week experiment, unaffected by the different disinfection setups. The CO_2 levels increased by 0.7mg/l compared to phase 1 (average value 0.2mg/l) due to the fish respiration. There were no significant differences in NH_4 and NO_2 levels among the disinfection setups, but significantly higher NO_3 levels were observed during UV disinfection compared to HC+UV.

Overall, the results indicate that the hydro cavitation device, in combination with UV disinfection, did not significantly impact water stability, growth, or water quality parameters (except for NO_3^-) during the four-week experiment.

The bacterial counting was performed 24h after the incubation and the results showed a significantly higher value of CFU/ml before the disinfection in WK1 compared to the other weeks. However, a sharp decline and a consequent raise were recorded in the following weeks. The samples collected after the disinfection system showed an exponential increase of CFU/ml over the weeks without a significant difference of WK4 compared to WK3. WK1 and WK2 were not considered due to the absence of CFU/ml recorded.

The overall survival rate of 98.9% recorded during phase two of the trial showed the absence of severe negative effect on fish health. However, the most severe clinical sign were found in the animals sampled in absence of disinfection were the congestion and hyperplasia of the gills was indicative of a water quality impairment.

Acknowledgement

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TECHNOLOGICAL INNOVATIONS FOR A CIRCULATING AND DURABLE ECONOMY IN THE AQUACULTURE VALUE CHAIN IN TUNISIA

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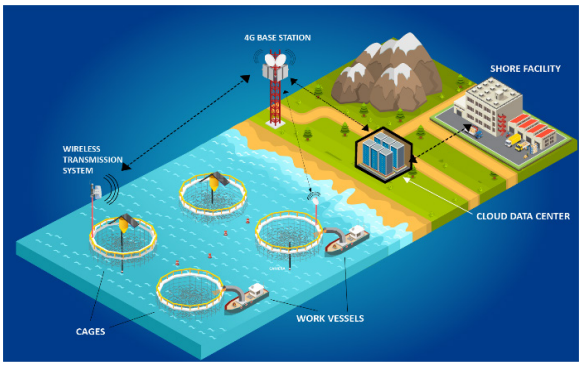
Introduction

The current project, part of Switchmed phase two, is built upon the results of the project's first phase, which was dedicated to characterizing the Tunisian aquaculture value chain. This second phase aims to support further the sustainable development of the aquaculture sector, in line with the Tunisian aquaculture development strategy for the sustainable expansion of this sector. In the last decade, Tunisian aquaculture demonstrated a significant production increase, mainly from intensified offshore marine finfish culture. However, one of the key aquaculture performance indicators, the Feed Conversion Rate (FCR), remains high. High FCR can negatively effect the economic and environmental sustainability of the aquaculture sector. Farmers can improve FCR by regularly adjusting feed quantities to avoid excessive or under-feeding, considering the actual biomass and daily conditions. Over recent years, the aquaculture sector has developed innovative technologies and processes to improve farming efficiency. Adopting appropriate SMART technologies can reduce fish feed losses, reducing the amount of feed being dispersed into the water and, thus, the environmental impact of aquaculture. Many of these systems have high investment costs and are unaffordable to many farmers. More affordable options do exist but they lack demonstration in a production system. This pilot project aims to demonstrate affordable and innovative technologies' advantages in optimizing feed conversion ratio by conducting an experimental campaign in actual production conditions. The Integration of these innovative technologies is accompanied by a training and awareness campaign. The campagin is open to all aquaculture and institutional players who wish to participate and benefit from the expertise of the project partners.

Materials and methods

A combination of SWOT analysis and a cost-benefit approach was used to select the most suitable technologies for improving feed conversion efficiency. The technologies selected for this study include: 1) offshore individual automatic fish feeder, 2) AI software, 3) 4G antenna, 4) underwater camera, and 5) power station based on solar energy. The two parameters that the AI software is based on are feed loss and fish swimming behaviour. Also, a pilot site (Aquafarm) selection process for the trial of selected technologies was carried out based on a decision matrix on key parameters and site suitability. Then, at the selected offshore site, 14 km offshore the coast of Monastir, four different farming scenarios were designed for the experimental campaign: 1) One cage including all the technologies selected, 2) One cage with only the automated feeder and the power station, 3) One cage with an underwater camera, AI and the power station, 4) A control cage with none of the selected technologies. The online training covering the aquaculture value chain's main aspects was created using video presentations on PowerPoint, and the infield training was designed to share practical experience to use these SMART technologies at the offshore site.



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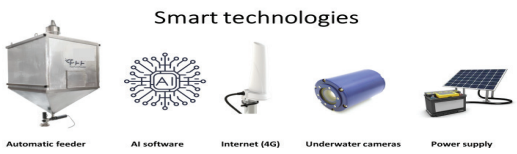

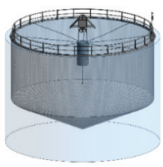

Discussion

The logic of integrating five technologies at the selected farm site in four scenarios aims to assess how using three different combinations of technologies during the experimental campaign influences the FCR and fish feed losses. In particular, the combination of automatic feeder, AI, and camera (scenario 1), compared to scenario 2, in which only the automatic feeder is employed, aims to establish if AI improves the feeder's performance in delivering feed more efficiently. Similarly, scenario 3, where only camera and AI are employed, plus the canon traditional feed administration method, compared to scenario 1 (full fledge), to determine which of the two administration techniques (i.e., canon or automatic feeding) is more efficient. Finally, the control cage, where no smart technologies are employed, to determine how efficient is the current canon feeding strategy compared to scenarios 1, 2 and 3. According to recent statistical data, the average FCR in Tunisia is currently about 2.3. for seabass, meaning that to obtain the commercial size of 400gr, about 920g of feed is needed at the cost of 4 Tunisian dinars (TD) or 1.2 euros per fish. Assuming that the use of Smart technologies improves feeding efficiency, reducing the amount of feed loss and, at the same time, the FCR to 1.3, this would have two main benefits for farms: 1) a decrease in the cost of production per fish of 44% 2) an reducing its environmental impact. Potentially, on an economy of scale for offshore aquaculture, this could be an incentive to adopt technologies, at affordable cost, to monitor feed and fish management, and this shift can potentially disrupt the whole offshore aquaculture industry towards a more profitable and environmentally sustainable finfish farming practice at local and international level. To this extent, online and in-field training aims to increase social awareness about the potential of the introduction of these novel technologies into the offshore aquaculture industry.

THE POTENTIAL OF HIGH INCLUSIONS OF INSECT PROTEINS FOR SUSTAINABLE AQUACULTURE: A STUDY ON GROWTH PERFORMANCE, FEED UTILIZATION AND HEALTH OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

The sustainability of aquaculture has been a key topic since the beginning of the millennium. Over the years, efforts have been made, especially for carnivorous species, to achieve nutritional efficiency in the sector by lowering the fish-in-fish-out ratio of feeds. And by finding sustainable and cost-effective terrestrial ingredients that can meet the necessities of carnivorous species, while still maintaining the quality of the final product[1]. In recent years, many plant-based ingredients have been proven as viable alternative ingredients for aquaculture feeds, with soybean meal (SBM) becoming one of the most used alternatives for the partial replacement of fishmeal (FM) in commercial aquafeeds. However, SBM use comes with challenges. Significant inclusions of SBM in fish diets may negatively impact fish health due to intrinsic anti-nutritional factors, which might induce inflammatory responses in fish's distal intestine[2]. This issue can be mitigated by using sophisticated processing techniques, that come at considerable cost[3,4]. Additionally, replacing FM with SBM may not produce feeds that provide reliable all-round nutrition for carnivorous fish species, as earlier studies had indicated. Furthermore, practices associated with soy production, such as deforestation and pesticide use are environmentally problematic. One promising alternative to SBM are insect meals (IM). As a component of their natural diet, insects provide a sound balance of amino acids, lipids, vitamins and minerals for salmonids[5], such as rainbow trout (*Oncorhynchus mykiss*). Moreover, insect production can utilise food waste: forming part of the circular economy. Plus, the utilisation of normally wasted products makes insect production economically competitive.

This study evaluated the effect of substituting up to 100% of SBM protein with Black soldier fly (*Hermetia illucens*) meal (BSFM) with and without addition of 0.3% guar gum, with respect to growth performance, feed utilization and health of rainbow trout.

Materials and Methods

A dose-response study was conducted with a homogeneous group of juvenile rainbow trout (initial body weight: 135.8 ± 15.3g), randomly distributed in twenty 0.33m^3 circular tanks. Ten balanced experimental diets were formulated to be isonitrogenous (45%) and isolipidic (28%) by replacing SBM with increasing levels [0% (control), 25%, 50%, 75%, and 100%] of BSFM, with and without addition of guar gum (0.3%). The fish were fed *ad libitum* by hand for a period of 69 days (6 days a week) and growth performance, feed utilization efficiency, and nutrient digestibility were evaluated.

Results

At the end of the experiment, all groups showed a linear positive correlation between the increasing replacement of SBM with BSFM with all measured fish performance parameters [final body weight (FBW), specific growth rate (SGR), total feed intake (TFI) and feed conversion ratio (FCR)]. The addiction of 0.3% guar gum had no effects on fish performance.

Conclusion

The results suggest that the inclusion of high amounts of BSFM in rainbow trout diets can be a viable option to replace plant-based ingredients in aquafeeds, with respect to important performance parameters. However, although there is growing evidence of the benefits of insect components in aquafeeds, further research is needed to fully understand the potential advantages and limitations of including insect protein in aquafeeds.

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VIRUS-INDUCED INTERFERENCE AS A MEANS FOR ACCELERATING FITNESS-BASED SELECTION OF CYPRINID HERPESVIRUS 3 SINGLE NUCLEOTIDE VARIANTS *in vitro* AND *in vivo*

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Introduction

Cyprinid herpesvirus 3 (CyHV-3) is the archetype of fish alloherpesviruses. It causes severe economic loss within the carp culture industry worldwide. Genomic and biological comparisons of CyHV-3 strains have revealed a negative correlation among strains, suggesting the existence of genovariants conferring advantages *in vitro* but reduced fitness *in vivo*, and *vice versa*. We identified such genovariants of the CyHV-3 ORF131 gene. This gene is essential for viral growth in cell culture and encodes a 429 amino acid type 1 membrane protein. We demonstrated by mutagenesis that the genetic determinant of the phenotypic trait related to ORF131 depends on a single nucleotide polymorphism (SNP) (C225791T mutation) that results in codon 183 encoding either an alanine (183A) or a threonine (183T) residue. Understanding the key factors that determine how purifying (negative) selection operates on herpesvirus genomes would provide useful insights into the evolution of these viruses. In the present study, pairs of viruses differing only by the C225791T SNP were generated and compared for fitness *in vitro* and *in vivo* by infection with single viruses or co-infection with both viruses. This study illustrates how the host-virus interactions and the fundamental biological properties of some viruses and their hosts may have a profound impact on the degree of diversity that arises within viral populations.

Materials and methods

Common carp brain (CCB) cells were cultured. A total of seven CyHV-3 strains from various geographic origins were used and indirect immunofluorescence staining was done. Multiple DNA sequence alignments were made using MAFFT online version 7 and then processed using MEGA X software. CyHV-3 strain FL was isolated in Belgium from a fish that died from CyHV-3 infection and used to produce the FL BAC plasmid. FL EGFP rec ORF131-A (or) -T and FL mCherry rec ORF131-A (or) -T were produced by transfecting the FL BAC plasmid into CCB cells. The recombinant strain was cloned by three successive steps of plaque picking. All recombinant strains were confirmed by monitoring SacI restriction fragment length polymorphism (RFLP) and full-length genome sequencing. Growth curves, plaque size assay and syncytial plaque assay were investigated. Live cell images were collected using Incucyte to ensure that cells detected as double positive represented cells co-infected by EGFP and mCherry recombinants. *In vivo* infection was carried out either by immersion of uninfected fish in water containing virus or by cohabitation of uninfected fish with infected fish. The experiments, maintenance and care of fish complied with the guidelines of the European Convention CETS 123. Viral genome copies were quantified by real-time TaqMan qPCR. Fish were analyzed using an IVIS Spectrum *in vivo* imaging system.

Results

The results demonstrate that among the strains studied, FL, Cavoy and T strains were the most fit in cell culture but the least virulent *in vivo*. The opposite was the case for the M3, I, E and GZ11-SC strains. The strains with the 183A genovariant formed syncytia, whereas the strains with the 183T genovariant did not. This suggests that the ORF131 183A genovariant is responsible for syncytial plaque formation. The experiment involving simultaneous or delayed infection (one genovariant, then infection with another after) of monolayer revealed that the primary infection of monolayer reduces its ability to be superinfected by a second virus and that this phenomenon increases with the length of delay between the first and second infection. The ORF131 183T genovariant confers higher fitness *in vivo* than the 183A genovariant but in the case of *in vitro* it is vice-versa.

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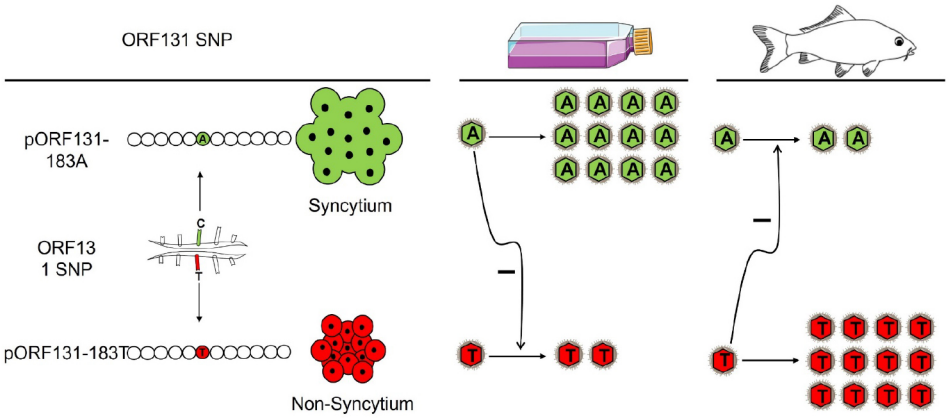

Figure: Schematic representation of research findings

Conclusion

The present study indicates that CyHV-3 may have an intrinsic ability to actively contribute to the purifying selection of less fit variants by stimulation of superinfection inhibition at both the cellular and the host levels. However, more widely, our observations demonstrate how the fundamental biology of some (perhaps many) viruses and their hosts may have a profound impact on the degree of diversity that arises within viral populations.

DISEASE MITIGATION: A POSITIVE AND NEGATIVE APPROACH

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Disease remains a major bottleneck for the expansion of aquaculture. This is particularly true as aquaculture intensifies and production stressors negatively impact the animal, increasing disease susceptibility and denting the profits of producers. This has created the need for prophylactic disease management, through the use of functional feed. There is often a misconception that additives can solve all problems, and these unrealistic expectations have resulted in skepticism about feed additives.

This is particularly true when combatting bacterial disease. There are two types of bacteria, which differ in membrane structure. Gram-negative bacteria have an outermost layer of lipopolysaccharides (LPS) whilst the external layer in Gram-positives is comprised of peptidoglycans. Both types contain pathogens and not surprisingly, different management approaches are needed to combat them. In tilapia this is particularly relevant, as the fish are exposed to many bacterial pathogens, including *Aeromonas hydrophila*, *Edwardsiella tarda*, *Francisella noatunensis* etc. (G-ve) and *Streptococcus* spp. (G+ve).

Organic acids and their salts have been used extensively in aquaculture and are a 'go-to' additive to combat fish disease. It is often thought organic acids act to reduce the pH in the gut, but this is not always true unless a very high dosage is used. More important is to explore antimicrobial properties where lower doses might work but are highly formulation specific. For example, an enhanced acidifier (Biotronic® PX Top3) was found to be more effective than similar products, despite having a 4x lower inclusion rate (0.5 kg/t vs 2.0 kg/t). After an eight-week feeding period, tilapia (initial weight = 10.96 ± 0.02g) were exposed to an *Aeromonas hydrophila* challenge via IP injection (7.5 x 10^5 CFU/ fish). After monitoring for 20 days, highest survival was seen in the Biotronic® treatment (85.7%), followed by the competitor products (50-64.58%) and lowest in the control (43.75%). These data demonstrate the value that organic acids can bring in the fight against G-ve pathogens, but also highlights the importance of product formulation.

On the other hand, phytogenics have not been used so widely, but their benefits are particularly interesting against Gram-positive pathogens. To demonstrate this, tilapia fingerlings (initial weight = 10.63 ± 0.01g) were randomly split into two treatments and stocked into 12 tanks (n = 6). Fish were fed either a control diet, or one supplemented with a phytogenic feed additive (PFA, Digestarom® P.E.P. MGE) at 0.2 kg/ t. After a 57-day growth trial, where the PFA significantly improved biomass, weight gain and FCR ($p < 0.05$) fish were exposed to an *S. agalactiae* challenge via immersion (5 x 10^6 CFU ml^{-1} for 1 hr). Following a 15-day monitoring period, a survival of 52.5% was seen in the control treatment. This compared to 70.0% survival in the PFA treatment, a real term increase of 33.3%. This demonstrates that phytogenics can be a useful tool in the continual fight against *Streptococcus* spp.

These studies demonstrate that in order to see the value of feed additives, the additive type and active components should be carefully selected for the specific field challenge. In addition, they are not 'silver bullets', and their use should be complemented with other management considerations, including biosecurity, vaccinations, water quality etc.

TRIPLOIDIZATION AS A FERTILITY REDUCTION TOOL IN PIKEPERCH S*ander lucioperca* AND VOLGA PIKEPERCH *Sander volgensis* HYBRID PRODUCTION

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Introduction

Triploidization is a common technique of chromosome set manipulation that promises benefits associated with reduced fertility or sterility in aquaculture by the incompatibility in homologous chromosome pairing during meiosis I (Lee 2018.). Production of sterile fish finds its use when sexual maturation affects growth and development as in salmon, loach, or catfish production (Janhunen 2016., Park 2006.). Triploidization can reduce aggressive behavior through altered hormonal and metabolic homeostasis, which can also be applied to fishes of the Percidae family (Garner et al. 2008). Also, sterile triploid fish are used as surrogate hosts in primordial germ cell transplantation. The production of pikeperch with diminished fecundity but with acceptable vigor is particularly specific and complicated because pikeperch is considered a highly stress-sensitive fish, and the triploid fish in general appear to be more susceptible to stress (Fraser 2012.). Interspecific hybridization is a commercially used genetic tool in aquaculture to improve disease resistance and environmental tolerance in many species (Jiang 2022.) Nevertheless, thus far the issue of how triploidization would affect the gamete development of originally fertile hybrids was not evaluated.

Material and Methods

Hybrid triploid pikeperch was produced by hybridization of pikeperch (*Sander lucioperca*) females with Volga pikeperch males (*Sander volgensis*) and triploidized by inhibiting the first polar body using hydrostatic pressure, 8000 PSI, for 10 min. Larval ploidy level was determined by flow cytometry and chromosome analysis (Káldy 2021). The trial included a hybrid triploid group and three controls, hybrid diploid, pikeperch diploid, and its triploid form. Fish were kept in a recirculation system under natural light at a temperature of 16-22 °C for 330 DPH. Gonads of juvenile fish were fixed in a modified Davidson solution and analyzed using conventional histological techniques, staining with hematoxylin and eosin (Latendresse 2002.).

Results and discussion

Chromosome analysis showed that the triploidization procedure was successful with a 100% rate, in both pressure-treated groups. There were no detected individuals with diploid or mosaic chromosomal structures. In females of both diploid groups, successive development was recorded from the oogonia stage until the cortical alveoli stage, while in males, development from spermatogonia to spermatids was recorded. Among hybrid triploids, there were also two types of gonads. Besides the primordial germ cells - spermatogonia, males showed spermatocytes and spermatids in one of the 5 samples. Hybrid triploids with phenotypically female gonads developed both oocytes (to the same stage as diploids) and spermatids. These fish did not develop normal female gonads but formed an intersex structure. Males, on the other hand, at this stage of development still did not show structural disorders. Triploid pikeperch had uniformed gonads with predominant PPGC and occasional primary oocyte.

From the results of this research, it can be concluded that the gonads of the pikeperch and its hybrid with the Volga pikeperch, in both sexes, develop without a difference, and it seems that they could sexually mature. In the case of triploid pikeperch, a large number of germ cells that did not enter meiosis, with sporadic primary oocytes, and spermatocytes in 100% of the analyzed samples, can justify the conclusion that triploids are infertile due to phenotypically bisexual gonads. While triploid hybrids developed two types of gonads a) pro-male - which did not deviate much morphologically from diploid juvenile testes, neither in shape nor in size, so at this stage of development it cannot be claimed that they are infertile; b) pro-female - due to the large number of spermatocytes and oocytes in the gonad at this stage of development, it can be concluded that they are intersex, and most likely functionally infertile. Further investigation in older age groups of fish is required to confirm these indications.

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Acknowledgments
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INFLUENCE OF A TEMPERATURE DROP FROM 15 TO 8 °C BEFORE OR AFTER WINTER SIGNAL ON EARLY SEXUAL MATURATION IN ATLANTIC SALMON (*Salmo salar* L.) POSTSMOLT

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Introduction

The drive to produce larger Atlantic salmon smolts and postsmolts on land, has intensified the production protocols resulting in increased number of postsmolts maturing early. Early maturation represents both an economic and welfare challenge for producers. Causes for early maturation are multiple, including growth rate, energy surplus, photoperiod and temperature among others. The switch in daylength from winter to spring is the cue that entrains onset of sexual maturation (Fjelldal et al., 2018). However, high temperatures are regarded as the most important factor for early maturation (Melo et al., 2014; Pino Martinez et al., 2023) and has shown to stimulate the proses under different photoperiods (Imsland et al., 2014; Martinez et al., 2023). In these studies, high temperature has induced maturation both when kept stable high during the trial (12,15 and 18 °C), and when it has been increased up to 16 °C together with daylength after winter signal. The present study, in contrast, aimed to assess if dropping water temperature from 15 to 8 °C during or after a winter signal can arrest the development of early sexual maturation in Atlantic Salmon postsmolts.

Materials and methods

900 parr were transferred to eight freshwater tanks at the flow-through facilities of the Department of Biological Sciences (University of Bergen, Norway). Four temperature regimes were established in duplicates (Fig 1A). The first group were held at 15 °C throughout the trial (15), as this temperature is shown to promote early sexual maturation in male salmon (Martinez et al., 2023) The second group started at 15 °C and lowered to 8°C after a 5-week LD12:12 winter signal (15-8L). The third group were started at 15°C and lowered to 8 °C at the start of the winter signal (15-8E). The fourth group were held at 8 °C through the experiment (8), as this temperature is shown to inhibit maturation. We performed nine samplings, collecting n= 6 males per tank per sampling. Body and gonad weight were measured and gonadosomatic index was calculated as GSI (%) = *Gonad W (g)*100/Body W (g)* and used to assess maturation status (Pino Martinez et al., 2023). A Fisher's Exact Test for Count Data used to find differences in proportion of maturing males between treatments. Statistical analyses were performed in RStudio.

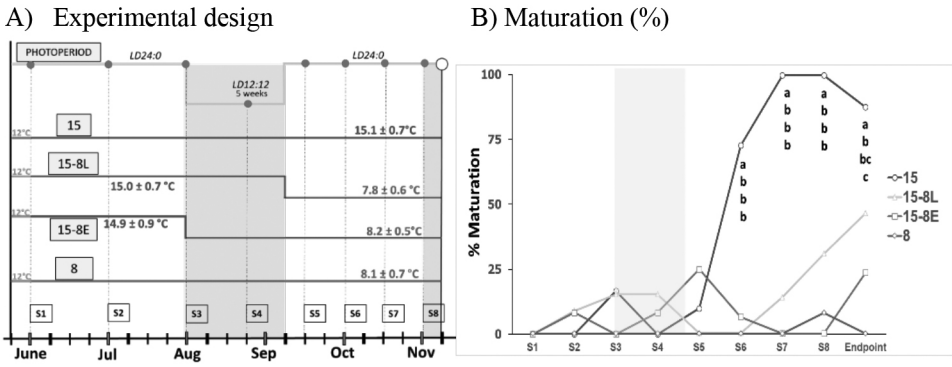

Figure 1 A) Experimental design with four experimental groups (15, 15-8L, 15-8E, 8), light regime, temperatures, and sampling points. B) Maturation (%) per experimental group and sampling. The period of winter signal is marked in gray.

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Results

Maturing males were observed in the three 15°C groups from sampling 2, prior and during the winter signal. After the winter signal, a highly synchronized onset of maturation occurred in all males in group 15, and also commenced in an increasing number of individuals in 15-8L and 15-8E (Fig 1B). Hardly any sign of maturation was observed in the group held at 8 °C throughout the experiment. Some individuals maturing in 15-8L and 15-8E displayed very high GSI and running milt, in contrast to those in 15. Interestingly, other males in 15-8L and 15-8E had a relatively low GSI (i.e. 0.29%), but a white foamy testis appearance typical of spermiogenesis (differentiated spermatozoa).

Discussion and conclusions

Temperature determined the life history strategy of the experimental groups, with all males at 15°C maturing at the end, and none at 8 °C. All groups treated at 15°C had some parr maturing, and the maturation seen prior to sampling 6 was all driven only by the high temperature experienced before the LD 12:12 winter signal. The drop in temperature to 8 °C before or after LD 12:12 caused a significant reduction of maturation. However, the increasing number of males found maturing in 15-8L and 15-8E suggests that the decision to mature had been made before the winter signal, and that the drop in temperature did not reverse but only delayed the process in many individuals. Furthermore, the presence of testes in spemiogenic phase but with low GSI suggests that those fish may have irreversibly committed to maturation early, and the swich to cold water only interrupted the proliferative stage and induced meiotic and spermiogenic phases.

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DO ELECTROMAGENETIC FIELDS AFFECT THE GROWTH, METABOLISM AND BEHAVIOR OF EUROPEAN SEABASS?

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Introduction

Harnessing energy from the wind is becoming an increasingly important research field, particularly in offshore environments. In addition, combining energy production with fish farming under common infrastructures has been highlighted as a priority at a European level. A challenge in that regard is that the effects of electromagnetic fields (EMF) generated by the wind turbines on the farmed species are largely unknown. Yet, studies on several species indicate that the presence of strong EMF may affect, among others, the embryonic development, circadian rhythm, metabolism, orientation, and migration (Formicki et al., 2019; Newton and Kajiura, 2020) of fish. In this study we investigate effects of EMF of industrial intensity on the growth performance, metabolism, and behavior of an important Mediterranean aquaculture fish, the European seabass (Dicentrarchus labrax).

Materials and methods

A trial was performed in the Recirculating Aquculture System of HCMR (Greece) where juvenile European seabass of approximately 200 g were subjected to an EMF of 20 mT for three months. Growth performance was monitored monthly (weight measurements) while towards the end of the trial an intermittent flow respirometer was used (N = 15 per group) to determine the Standard Metabolic Rate. Additional were performed on E. seabass fingerlings (approximately 2 g) using a T-maze setup in order to investigate behavioral effects relating to swimming and directional orientation. These tests were performed with the aid of a Helmholtz coil (Fig 1.) which generates a uniform EMF at a strength of 10mT.

Results

There were no significant differences between the control and the EMF groups in growth performance of the fish. Both groups exhibited normal growth and increased in weight by 150 g over the trial period. Similarly, results from respirometry suggest that the EMF did not significantly affect their basal metabolic requirements; SMR showed minimal differences between groups exhibiting values between 85.1 – 88.4 mg kg^{-1} h^{-1}. However, the initial results using the T-maze (Fig 1.) indicate that the EMF may significantly affect some behavioral traits in E. seabass. In particular, application of EMF appeared to increase the prevalence of 'freezing behavior' (assigned to fish that remain immobile) from 15% to 25% in the tested fish. Moreover, preliminary analysis indicates that while the control group showed uniform distribution towards the south-north axis, the EMF may exhibit a preference for the magnetic South.

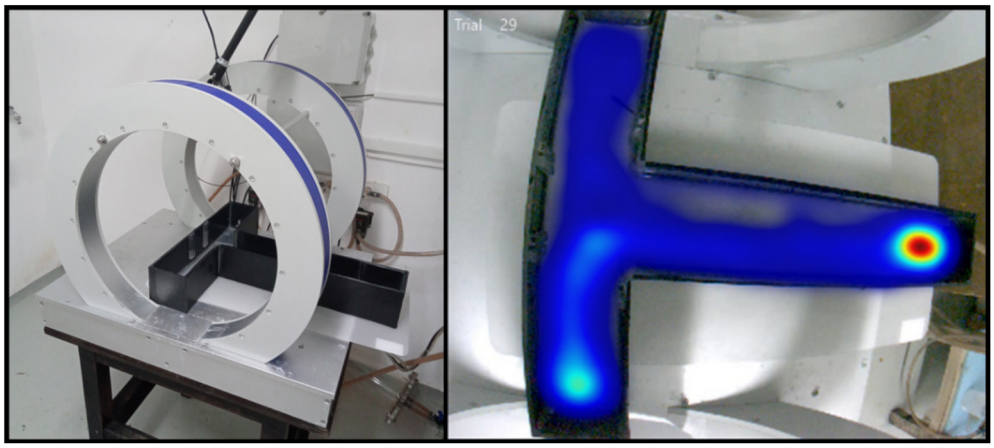

Fig. 1. Left: The Helmholtz coil and T-maze setup used for the behavioral analysis, Right: Heatmap showing the movements of an individual fish in the T-maze, red indicates higher frequency.

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Conclusion

In this study we investigated the effects of EMF on several biological aspects of an important aquaculture species. Our results indicate that under industrial EMF intensity, the growth performance and basal metabolism of E. seabass are not significantly affected. In turn, this is a promising outcome for promotion of multi-use platforms combining fish farming with wind energy production. However, preliminary analysis indicates possible effects on the behavior of young fish which requires further investigation before management and policy recommendations can be made.

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NORTHERN EUROPE'S SUITABILITY FOR OFFSHORE EUROPEAN FLAT OYSTER (*Ostrea edulis*) RESTORATIVE AQUACULTURE

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Introduction

Oyster beds and reefs provide essential ecosystem functions within coastal and offshore ecosystems around the world, providing structural complexity leading to numerous ecosystem services, as well as contributing to coastal economies for centuries (Beck et al., 2011). As such, they are the focus of several flat oyster nature-based solution projects, restorative flat oyster aquaculture projects (Carranza & Zu Ermgassen, 2020) and a multitude of habitat creation or restoration projects in almost all countries border the North Sea or the English Channel (https://noraeurope.eu).

Methods

A DEB-IBM (Dynamic Energy Budget - Individual Based Model) population model was coupled with the LARVAE&CO larvae dispersal model. The DEB-IBM is a bioenergetics-based population model (Stechele et al., 2023). The LARVAE&CO model is an IBM that simulates egg and larval dispersal. This work establishes a population model for flat oyster population dynamics and dispersal and applies the model to the English Channel and the North Sea to indicate suitability for offshore flat oyster habitat restoration and restorative aquaculture.

Suitable locations for restorative aquaculture are indicated by quantifying suitability indicators including population increase, fitness, reproductive potential, self-recruitment and sediment suitability.

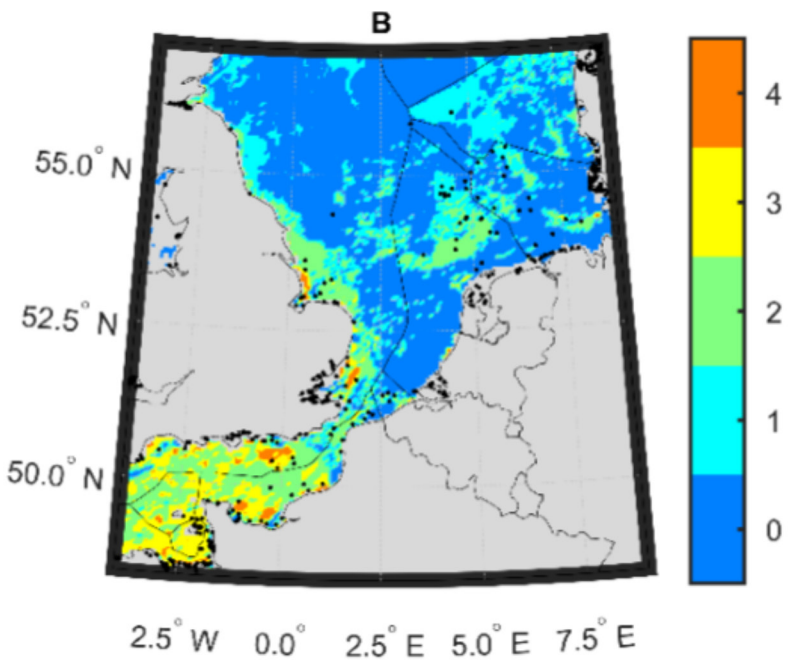

Figure: Suitability for flat oyster restorative aquaculture and habitat restoration

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Results

Restoration hotspots (total suitability indicator score = 4) are located in the Wash (UK), all along the coast of East England and the outer Thames estuary (UK), in the offshore mid Channel south of the Island of Wight, around the Isle of Wight (UK), all along the coast of Dorset and Devon (UK), all along the coast from Saint-Brieuc (FR) to Le Mont-Saint-Michel (FR), Côte Fleurie (FR) all along the coast of Haute Normandy from Fécamp to Le Tréport (FR), around Dunkerque (FR), in the Northern parts of the Scheldt Estuary (NL), off the coast of Noord-Holland (NL), in and around both the Western and Eastern Frysian Islands (DE) and around Helgoland (DE).

Suitability indicators scores can be linked to the occurrence of historical beds.

Conclusion

The English Channel is highly suitable for flat oyster restorative aquaculture (both nearshore and offshore). In offshore locations of the North Sea, we do not expect to see high population increases due to colocation of flat oyster aquaculture and restoration, and restorative aquaculture efforts should be scaled up, to boost larval production and recruitment. To increase success of flat oyster habitat restoration in the offshore environment, we suggest the implementation of a basin-wide coordinated restoration effort that promotes the connectivity between natural oyster beds, restoration sites, oyster NID developments and aquaculture sites.

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SIMULATING NUTRIENT FLUXES AND CARBON BUDGETS FOR AQUACULTURE: MUSSEL AND RAINBOW TROUT FARMING IN THE BALTIC SEA

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Introduction

The links between human footprint and ecosystem is becoming clearer, leading to the development of management tools that incorporate both scientific and socioeconomic information into decision making processes. The valuation of ecosystem services provided by human activities such as aquaculture becomes more prominently present in legislative context and may have the power to change public's, often negative perception of aquaculture. Nevertheless, there is still a significant knowledge gap in systematically quantifying these ecosystem services. Dynamic Energy Budget (DEB) models offer the possibility to quantify nutrient emissions or eutrophication mitigation (N and P) of aquaculture production in dynamic environments, and coupled to spatial biogeochemical datasets, DEB models can provide information regarding spatial variability of these ecosystem services.

Bivalves removing suspended solids from the water that are excreted by finfish, is a classical example of IMTA (reference !) where nutrients are recycled to the benefit of the water quality. Quantification of the nutrient fluxes in IMTA setups remains a challenge. Thanks to a DEB extension that allows for quantification of product formation, such as shells, it is now also possible to quantify carbon cycles through farms.

Methods

Dynamic Energy Budget models provide a generic framework to estimate mass and energy balances. They can be applied to all living organisms and all ecological scales from cells to ecosystems. Applying DEB models to aquaculture relevant species enables quantification of several relevant variables including growth, reproduction, and physiological processes such as feeding, ingestion, assimilation, respiration and (pseudo-)faeces production.

Nutrient fluxes through finfish and mussel farms in the whole Baltic Sea region were quantified using a DEB modelling framework (Kotta et al. 2023). DEB models for the rainbow trout and the Baltic mussel were parameterised and validated using in situ observation of the metabolic performance (e.g., respiration, growth, and reproduction) over a salinity gradient. Standardised fish and mussel farms where introduced and spatial distribution of nutrient uptake and nutrient emissions of these farms were evaluated.

Results

Finfish farms (standardized to 265 tons of wet weight production) on average emit 6500 kg N and 520 kg P to the water column during a production cycle. Mussel farms (standardized to 24 tons of wet weight production) remove on average 210 kg N and 25 kg P from the water column. Spatial variability of nutrient emission and incorporation of both finfish and mussel farms (Figure 1) is large in the Baltic. Nevertheless, simulations demonstrated that despite suboptimal mussel growth conditions, mussel farming has the potential to fully compensate for the discharge of nutrients from finfish farms and may thus represent a solution to sustainable finfish farming in the Baltic Sea region. Besides eutrophication mitigation, simulating filtration, incorporation and biocalcification enabled complete estimation of carbon budgets of shellfish farms.

Conclusion

DEB is a generic framework that enables quantification of ecosystem services related to nutrient and carbon fluxes in dynamic environment such as the Baltic Sea. It has the power to become an important tool in nutrient or carbon budgeting for single activities and can be used to quantify nutrient fluxes through IMTA setups.

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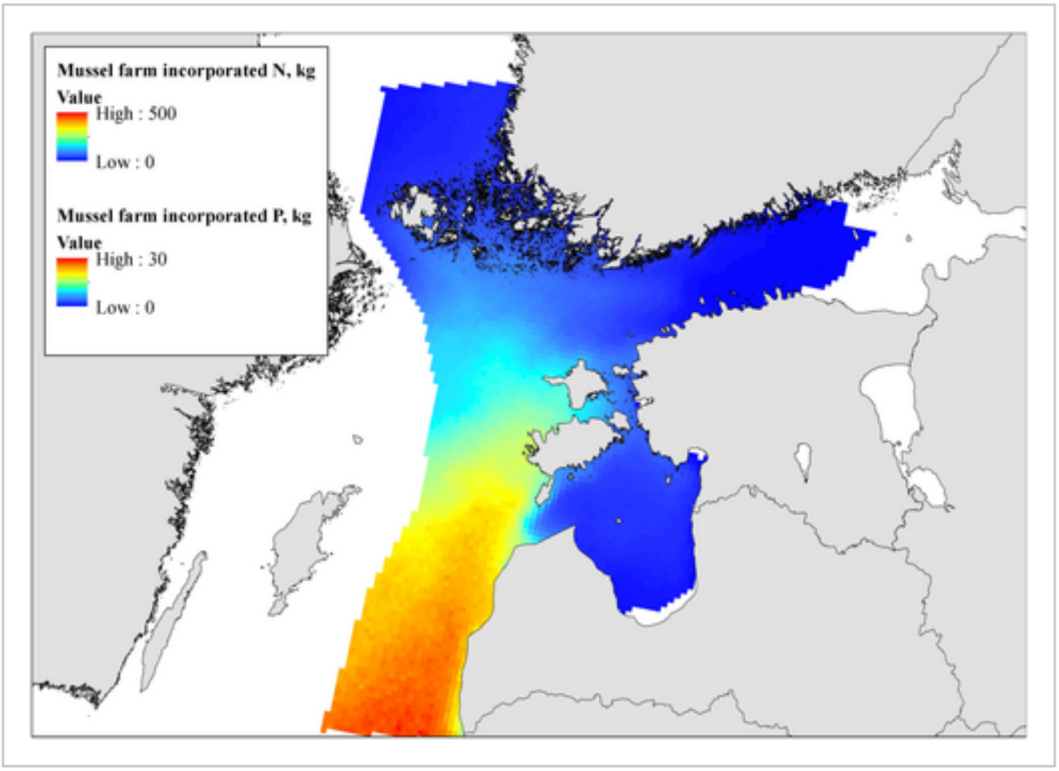

Figure 1: Spatial variability in the total nitrogen (N) and phosphorus (P) (kg) incorporated by a mussel farm per cultivation cycle.

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UBIQUITINATION IN RESPONSE TO INFECTIOUS SALMON ANAEMIA VIRUS AND INFECTIOUS PANCREATIC NECROSIS VIRUS IN ATLANTIC SALMON (*Salmo salar)*

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Introduction

Atlantic salmon (*Salmo salar*) is one of the most globally economically important marine aquaculture species[1]. Improving our understanding of the teleost immune response to pathogens could help combat the high disease burden in the aquaculture sector. Ubiquitination is an essential post-translational modification, known to play critical roles in the initiation, regulation, and termination of the innate immune system in mammalian species[2]. Ubiquitin is present in all eukaryotic cells and is highly evolutionary conserved suggesting that its role in immunity may be conserved in other vertebrates[3]. Despite evidence of many virus-inducible ubiquitination-related genes in fish[4] and the observed up-regulation of the ubiquitination pathway in infected fish[5], the role of ubiquitination in response to infection is still poorly understood. To identify the network of ubiquitin-related responses to viral infection this study combines analysis of the ubiquitinated proteome and gene expression of an Atlantic salmon cell line post-viral infection, using mass spectroscopy and RNA sequencing.

Materials and Methods

Salmon Head Kidney cells (SHK-1) were inoculated with Infectious salmon anaemia virus (ISAV) or Infectious pancreatic necrosis virus (IPNV). Samples were collected at 24 and 48 hours post-infection (hpi), along with time-matched controls (4 biological replicates per condition). For proteomics, cells were lysed by freeze-thaw and ubiquitinated proteins were enriched using HaloTagged Ubiquilin. Ubiquitinated protein content was measured via western blot, then submitted for Mass spectroscopy analysis. Mass spectroscopy was performed with data-dependent scanning, selection based on the host and viral proteome. RNA was extracted using TRIzol, and polyA RNA-seq libraries prepared using standard Illumina protocols and sequenced on a Novaseq 6000 as 150PE reads. Low-quality reads were removed, and gene expression was estimated using Kallisto[6] and the Atlantic salmon reference transcriptome (Ssal_v3.1, GCA_905237065.2)[7].

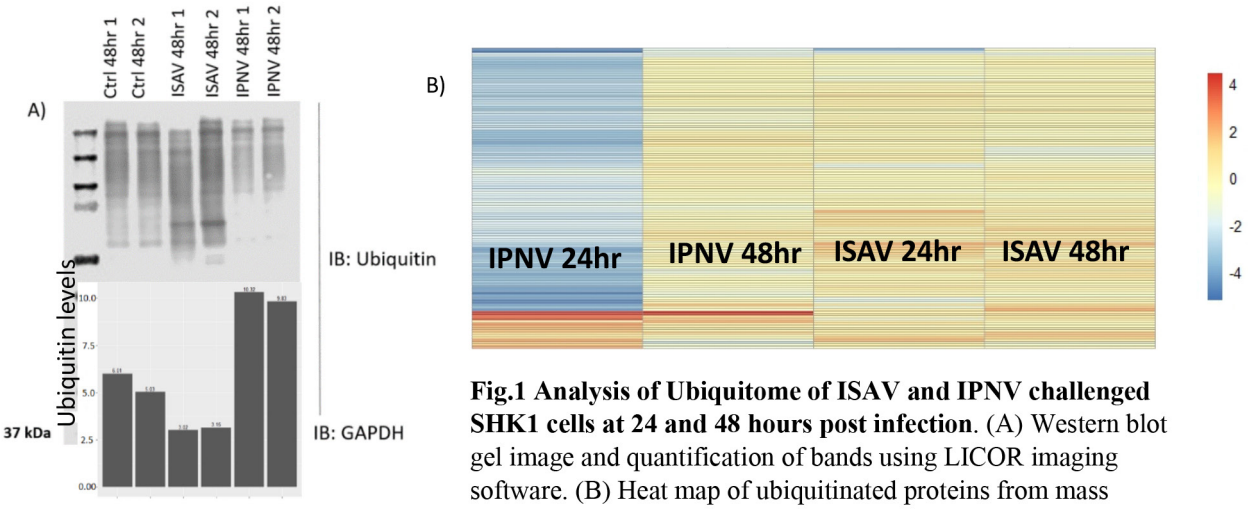

Fig.1 Analysis of Ubiquitome of ISAV and IPNV challenged SHK1 cells at 24 and 48 hours post infection. (A) Western blot gel image and quantification of bands using LICOR imaging software. (B) Heat map of ubiquitinated proteins from mass spectroscopy data (p value <0.05).

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Results

Infection of SHK-1 cells with ISAV induced a global up-regulation of ubiquitin, whilst IPNV infection induced an overall decrease in cellular ubiquitin (Figure 1A). This is consistent with the mass spectroscopy data, where the total ubiquitination profile of ISAV samples incurred a net positive fold change, whilst IPNV induced an overall negative fold change in ubiquitinated proteins, especially at 24 hpi (Figure 1B). A small number of proteins (4-50 depending on the condition) showed significant differences in ubiquitination in response to infection, including several relevant immune genes. For example, a TRIM ubiquitin ligase, TRIM25-like protein, showed significantly increased ubiquitination in response to ISAV at 24 hpi. Interestingly, all viral proteins were identified in the mass spectrometry data, and both the viral polymerase basic protein 2 and Matrix Protein 1 of ISAV contained di-glycine remnants consistent with ubiquitination.

RNA sequencing of the same samples revealed a massive up-regulation of gene expression in response to IPNV, both at 24 hpi and 48 hpi, while the response to ISAV was considerably more restricted (1033 vs 157 differentially expressed genes at 24 hpi).

Conclusion

Our results demonstrate a differential regulation of ubiquitination upon viral infection of different viruses in Atlantic salmon cells. We have found a TRIM25-like protein amongst the proteins with increased ubiquitination in response to ISAV. However, IPNV infection mainly led to decreased levels of ubiquitination in a small number of proteins. This lack of ubiquitination response contrasts with the large dysregulation of gene expression, and may suggest IPNV has the ability to regulate ubiquitination to enable infection. Additionally, multiple viral proteins contained ubiquitination sites upon digestion, namely ISAV viral polymerase basic protein 2 and matrix protein 1.

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CHALLENGED IN ELECTROCHEMICAL "BIOFILTRATION" - USAGE OF CAPACITIVE DEIONIZATION (CDI) IN RAS

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Capacitive deionization (CDI) is an environmentally sustainable technology that utilizes low electric voltage to adsorb ions onto electrical double layer region around high surface area electrodes through electric double layer capacitor mechanism at the electrodes-solution interface that is known to be energy-efficient and environmentally sustainable. Capacitive deionization (CDI) is an upcoming technology, positioned to transform the field of cost-effective, low carbon footprint water desalination. The technology has also been examined with municipal wastewater for removing and recovery of nitrogenous and even off flavor compounds. This also gives the option for fish purging and aquaponics applications. It is possible to engineer capacitive electrodes to target specific charged molecules in the input water stream. Our test in RAS water was the first attempt with salmon to exam its usability as a potential bypass biofilter and possible purging applications.

The capacitive deionization system used was from Stockholm Water Technology AB (Sweden) model STROM. Six individual 1000 L MicroRASs (Landing Aquaculture, the Netherlands) at Nofima Sunndalsøra were used for the experiment with Atlantic salmon. It was designed to have a long-term exposure (60 days) of Atlantic salmon to two different organic loads in a RAS experiment defined by using 2 drum filter mesh sizes. The water treatment used 100 µm drum filter for dirty water and the clean water was cleaned using 20 µm drum filters. Technically this should create an environment with 2 different water bodies that do not harm the fish but should facilitate off flavor compound production in the dirty treatment. The portable CDI unit was used 8 hours in each tank every 20 days. Water was pumped out of the fish tank, and treated for 19 cycles in 8 hours using the STROM system. Clean treated deionized water was sent back to the RAS system and effluent (waste, loaded with ions) water was discarded. During the experiment, 494 L of water was flown through the CDI systems resulting in 342 L being recirculated back to the tank generating only 152 L of reject water. Main components analyzed were nitrogen compounds (total ammonia, nitrite, and nitrate). Off-flavor compounds analysis is still ongoing. Water samples were collected in the beginning of the 8 hours treatment period from the fish tank, deionized water- and effluent water outflow. The same was done at the last cycle after 8 hours. Morning and evening fish tank values of the same molecules were compared to test the effect of continuous CDI usage over 8 hours in the RAS systems.

The results concerning the different nitrogen compounds showed that using CDI technology in aquaculture system needs more tuning of the CDI unit treatment variables, like used Voltage on the electrodes. The unit was adjusted for salinification and could do more to reduce the low concentrations of nitrogenous products of the fish and bacterial metabolism. Performance of the CDI system changes over the day so that the TAN removal tends to be better after running for longer hours. Something unexpected was that in all measurements of nitrite concentration after the CDI unit increased, in the treated and effluent water. Nitrate showed the most promising removal efficiency. Mass balancing of the system was not possible since there was always mixing of inflowing and outflowing water during deionization cycles. We will present the development of the testing procedures and learning experiences by doing different experiments. The latest experiments done at the University of Aarhus with Rainbow trout demonstrate finally that the CDI can remove higher N compound concentration much better than lower. Also, it could be shown that at least under low stocking densities the CDI unit can be used as a biofilter substitute.

Since this was after our knowledge the first try to use the system in salmon RAS application, the CDI unit still has a high potential as an ion removal tool. It could be off flavor purging or especially accumulating of nutrients for aquaponic systems. This used voltage could have led to some reactions for oxidizing Ammonia into Nitrite or reduction of Nitrates into Nitrites at the electrode surface. It could also lead to hydrolysis of bacteria and release of off-flavor compounds which we still try to investigate. In summary we will present results for using a CDI system as something like a "electrical biofiltration" unit and present attempts for further applications based on our findings. As the first experiment of its kind for salmon RAS applications, CDI holds significant potential as an ion removal tool for tasks like off-flavor purging and nutrient accumulation in aquaponic systems.

Nordforsk project link
https://www.nordforsk.org/sites/default/files/inline-images/jxDvsrx8eflDLTWMaPR4X6ABC1kSxOidfdKUH5YbYSzhAMr0FZ.pdf

THE CLEAR-CUT FUZZY BASIS OF BIOSECURITY. KNOWLEDGE AND RISK MANAGEMENT IN PRACTICE IN NORWEGIAN SALMON FARMING

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Introduction and research question

As we speak, more or less well-founded biosecurity plans are being created in aquaculture companies across Europe. New regulations require companies to control biosecurity in their production and area. Thus, company managements, biologists, and operational personnel are making plans on how to achieve and maintain biosecurity in their production and activities. In the operational end, the plans must be interpreted and played out by the personnel. Since plans are not always easy to comply with, the operational personnel have a major responsibility to make the good intentions embedded in the plans come into action.

That is why we in this study ask: *What are the personnel's considerations about routines and knowledge when biosecurity management is developed and played out?*

Method

This presentation will discuss results from an interview study of how biosecurity is handled in practice by biological, managerial, and technical personnel at Norwegian salmon RAS hatcheries and smolt transport. 18 persons involved in hatcheries and 11 persons in the well boat segment were interviewed in 2022.

The study is a part of the "Smittekontroll"/"Infection control" research project, funded by the Norwegian Seafood Research Fund (901734) and aimed at suggesting measures for predictable water treatment and disinfection in recirculating aquaculture systems (RAS) salmon hatcheries and smolt transport. The project studies microflora and technology, in addition to this study of operational routines.

Definition and earlier research

Biosecurity involves a set of management measures to reduce the risk of transmission, development and spread of infectious diseases, between populations, production zones, vessels, sites, and enterprises (Lillehaug et al. 2015). Diseases have been on the agenda for the aquaculture industry since its commercialization in the 1960s. Disease spread has been reduced through technological, organizational, and biological advances such as vaccines, disinfection systems, hygiene routines, and combat zones for diseases.

For increased biosecurity, one must identify risk factors, and use the risk assessments to make biosecurity plans (Lillehaug et al. 2015). Yet, further improvement has been hampered by limited knowledge, cost considerations, technology design, and increased professional and operational complexity (Larsen et al. 2020).

Transport of live animals is one of the most prominent risk factors for the spread of infectious diseases, and in aquaculture, wellboats are a significant route of infection (e.g. Murray et al., 2002). Nevertheless, there is still limited knowledge about infection in and from wellboats. Biosecurity on wellboats is supported by e.g. technical standards, cleaning practices, and hygienic treatment of intake and discharge water from the vessels, but the practices are not compliant (Larsen et al 2020). It has previously been described that wellboats operate with small margins which can lead to shortcuts (Fenstad et al. 2008).

There has been a significant development with the use of RAS, which require new practices and knowledge about biosecurity and disinfection routines at hatcheries. Different facilities have different practices for production and disinfection, resulting in different microbiota composition at plants (Dahle et al., 2021; Lazado & Good, 2021). In addition, there are major differences in mortality between hatchery facilities, giving opportunities for improvement, e.g. through routines and technology (Tørud et al., 2019). Interview studies at RAS facilities, among others, have shown that employees consider some operational routines to be unwavering due to the plant's design, but that many hatcheries still can improve their biosecurity routines (Tørud and Størkersen 2021).

Larsen et al. (2020) urge for more knowledge so it can be possible to establish a best practice and biosecurity analysis for RAS facilities and wellboats. As we see, both biological and organizational research is needed.

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Discussion and conclusion

Our study has gone into the transport and RAS segment, where the research gaps are deep, which influence the biosecurity management.

The interviewed personnel in this study describe how they work to manage biosecurity. They have an array of descriptions of how measures are made, used, and collaborated around, and how biosecurity relates to the job they are there to perform. Some consider routines and procedures to be clear, and they perform their work without doubts. Still, the majority express concerns around their biosecurity measures, and find the knowledge base to be uncertain and the intention behind biosecurity plans difficult to meet.

Altogether, the interview findings illustrate how biosecurity management is based on biological knowledge, but dependent on knowledge-strength, technology, investments, production routines, regulatory demands, habits, and other organizational conditions. In that sense, biosecurity is similar to other values that organizations need to control – like safety, quality, and ethics. Biosecurity also includes several sciences, like biology and natural sciences, that interact with a line of organizational conditions. This requires scrutiny and attention moving forward.

The increased use of the term biosecurity illustrates an awareness of this dependency in the aquaculture industry. Currently, many think of biosecurity as health studies, but it is also an organizational study object. Some of the biological foundations of biosecurity are well-researched and agreed upon, while others are either unknown, uncertain, or debated, causing trouble for risk assessments and the trustworthiness of biosecurity plans. To manage problems of diseases, one needs more biological knowledge, but also to utilize it in management and regulations, in a practical and enforceable way.

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QUANTIFICATION OF THE NORWEGIAN FEED SYSTEM USING MATERIAL FLOW ANALYSIS – A COMPARISON OF SALMON AQUACULTURE AND LIVESTOCK PRODUCTION

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Introduction

Atlantic salmon (*Salmo salar*) is one of the most important export commodities in Norway. Future growth in the industry is expected, and global demand for Norwegian produced salmon is rising. Salmon is generally accepted as a sustainable source of protein, and several studies state that the carbon footprint of salmon is lower than for example beef, pork and poultry (Hilborn et al., 2018; Nijdam et al., 2012)aquaculture, and capture fisheries that measured four metrics of environmental impact (energy use, greenhouse-gas emissions, release of nutrients, and acidifying compounds. A majority of the carbon footprint of the salmon from farm to harvest is accounted for by the feed (Ziegler et al., 2021). Feed for salmon produced in Norway has global and complex supply chains, with 92% of all ingredients imported in 2020 (Aas et al., 2022). An average for the last 10 years show that around 45% of ingredients in compound feed for livestock production in Norway were imported (Landbruksdirektoratet, 2023). These global value chains can be vulnerable for political shifts, conflicts, epidemics as well as climate change. There is a national goal to increase the Norwegian self-sufficiency degree, and producing sustainable feed resources in Norway is a key component to succeed here. The SusFeed project, financed by the Norwegian Research Council (grant #326825) aims to increase raw material for feed produced in Norway and investigates the potential and sustainability of novel feed ingredients such as insects, microalgae, and grass fibres.

To investigate and assess the sustainability and robustness of the feed system, a holistic perspective on the feed system is needed. We aim to quantify the current supply chains of feed in Norway using Material Flow Analysis (MFA) (Brunner and Rechberger, 2016). MFA is useful methodology to assess the resource efficiency of a system. A quantification and visualisation of the feed system with a MFA gives a deeper understanding of the current feed system, on the amounts of mass, protein and energy currently used and how new ingredients can increase the sustainability of the feed system.

Methods

The quantification of the current feed system is done based on the MFA methodology. The system boundaries are from feed ingredient to edible product of animal in Norway in 2020. Separate systems were made for salmon, cattle, pork, and poultry. The system was quantified for total mass of feed, protein, and energy in the feed. The MFA is based on numbers on total feed consumption and animal production from official statistics data sources (Fiskeridirektoratet, 2023; Landbruksdirektoratet, 2023; SSB, 2023). Feed composition is based on average diets from the major feed producers for salmon (Aas et al., 2022) and livestock (Animalia, 2023). Rest raw materials are calculated using conversion factors. Other inputs than feed and waste streams have not been quantified for this work. The systems are visualized using Sankey diagram in Python with the use of the plotly package (Plotly Graphing Libraries, n.d.).

Results & Discussion

In a world with increasingly higher demand of food and animal-based proteins, sustainable supply chains are important to reduce negative environmental, social, and economic impacts. It is important that new ingredients fulfil the animal's nutritional requirement, is produced in a sustainable manner and that resources are used efficiently. Therefore, knowledge on the current feed system can help to identify where improvements can be done. Using MFA to quantify and visualize the feed system gives a great overview of the current resource utilization and efficiency. Our model also considers a protein and energy layer as well as the mass layer, giving an even more profound understanding of the system. This methodology is also useful to assess the suitability of new resources in terms of required amounts of mass, protein, and energy.

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The visualisations show both how much ingredients are imported, and the degree of resource utilization based on feed ingredients into the system, compared to the amount of the edible product as output of the system. However, our work is based on the national feed system, which means our findings is not valid for individual producers, but our results do give indications of the current resource use and efficiency in the Norwegian feed system. New ingredients will change both the mass of ingredients, and the feed system as whole. We also identify that the degree of utilization of rest raw materials have great impacts on the efficiency of the system.

Further work in the Susfeed project will analyse the resilience of new feed vale chains and the environmental impact of novel feed ingredients using a life cycle assessment and the socio-economic impacts using input-output modelling.

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START-UP AND PERFORMANCE TEST OF TWO NITRIFICATION BIOREACTORS IN A COMMERCIAL SMOLT RECIRCULATING AQUACULTURE FACILITY IN FINNMARK, NORWAY

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Aim

The performance and welfare of Atlantic salmon (*Salmo salar*) in land-based recirculating aquaculture systems (RAS) depends on a well matured microbiota of the nitrification biofilter (Dahle et al., 2023). To achieve cash flow in land-based operations as early as possible after start-up, the time needed for nitrification biofilter maturation should be short. The "Core-RAS" (AKVA group, Norway) includes the nitrification bioreactor(s) for ammonia removal by autotrophic microbial nitrification. While performance of mature biofilters and maturation of lab-scale nitrification bioreactors is well documented, data on the maturation of a juvenile filter of commercial scale is lacking. To assist project managers and RAS operators with these challenges, we tested a protocol that allows achieving complete nitrification within 6 weeks of biofilter maturation. We will share our recommendations to minimize maturation time.

Materials and Methods

The 2 RAS tested are identical and part of a smolt production facility in Finnmark, Norway. The removal of solids is achieved by 2 mechanical drum filters, the nitrification by a combination of 1 moving bed bioreactor (30 m³ bio-media × 800²/m³ ~25,000 m²), 6 fixed bed bioreactors (FBBR) (~16 m³ bio-media × 800²/m³ × 6 ~157,000 m²). Ozone is used to control turbidity and degassing of CO_2 is achieved in a degassing tank, located above the FBBR. The system is illustrated in Figure 1. To allow for inoculation of biofilters with high numbers of nitrifying microorganisms adapted to target salinity once commissioning was completed, 2 pre-cultivation systems were set-up. Each pre-cultivation system consisted of 1 Intermediate Bulk Containers (IBC), 1 submersible heater + regulator (500W, Aqua Medic), and 1 shared aeration system (Aqua Forte V60). To supply nutrients, a premix was added (adapted from Navada et al., 2020): to supply ammonia oxidizing bacteria (AOB) in with ammonia, ammonium chloride (40 g/IBC) was added initially. To kick start nitrite-oxidizing bacteria (NOB), sodium nitrite (30 g/IBC) was added initially. To provide for phosphate, trisodium phosphate (10 g/IBC) was added initially. To control pH (target 8.5), alkalinity was added in form of sodium bicarbonate (250 g/IBC). Salinity was set to 3 ppt using sodium chloride. To accelerate microbial growth, the water temperature was set to 27°C. To inoculate nitrifying microorganisms, organic substrate (100g/IBC) was added.

Maturation of the biofilters: the bio-media (PP, 800 m^2/m^3) were first soaked in water for 4 weeks and then introduced in the FBBRs. Both RASs where then filled with fresh water and saltwater was added to set salinity to 11ppt. Circulation over the biofilters was started using the main RAS-pumps. To feed AOB and NOB, ammonium chloride (14 kg/RAS) and sodium nitrite (10kg/RAS) were added to set levels of 5 mg/l NH_4^+-N and 3 mg/l NO_2^--N. To supply phosphate, 5 kg Na_3PO_4 were added to set a ratio of 0.2 g P per g NH_4^+-N. The operating temperature was set to 24°C. Per day, 50% (500L) of the starter cultivate from each IBC was added to each RAS into moving bed. The IBC was then refilled with water, ammonium chloride, sodium nitrite, trisodium phosphate and sodium bicarbonate to create conditions as during pre-cultivation. Levels of NH_4^+-N, NO_2^--N, ortho-phosphate were measured daily using spectrophotometry (Hach Lange DR3900), and alkalinity using drop count test kits (Hach Lange dct). pH, temperature, salinity, and oxygen levels were measured daily (Hach Lange HQ2200).

Results

Pre-maturation in IBC: on day 1, NH_4^+-N was set to 10 mg/l. On day 5, NH_4^+-N levels were 0.1 mg/l in both tanks, and it was decided to reset NH_4^+-N to 30mg/l daily. The daily removal rate averaged ~2 $g/m^3/d$. Maturation of the biofilters: the removal of NH_4^+-N was observed in RAS 1 on day 11 and in RAS 2 on day 16. Removal of NO_2^--N observed in RAS 1 on day 31 and in RAS 2 on day 37. Levels of NH_4^+-N were reset to 3 mg/l daily. A nitrification rate of 0.09 $g/m^2/d$ was documented on day 42 (Figure 2).

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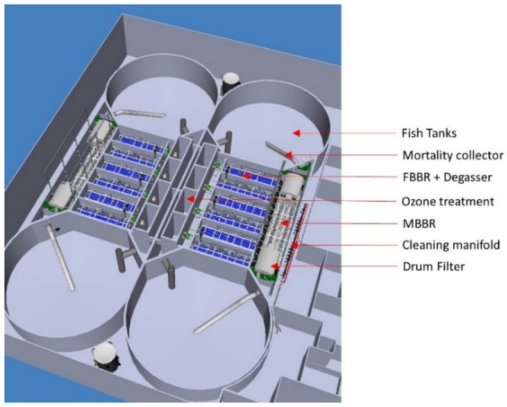

Figure 1: 3D model of the 2 identical RAS and main functional components.

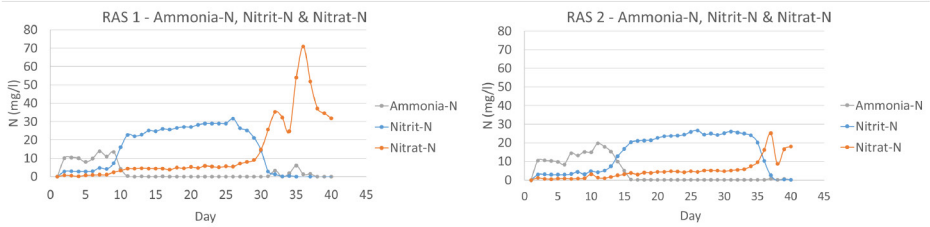

Figure 2: Levels of Ammonia-, Nitrite-, and Nitrate-Nitrogen in RAS 1 and 2 during Start-up.

Discussion and Conclusions

This study demonstrates that the maturation protocol applied, allows to achieve full nitrification within 6 weeks. Levels of NH_4^+-N (Kolarevic et al., 2013) and NO_2^--N (Gutiérrez et al., 2019) were well below the tolerance levels for Atlantic salmon, allowing for stocking of juveniles 6 weeks after starting the maturation of the biofilters. The results enable project managers and farm operators to integrate the maturation period within a clear timeframe of a project. Because adequate housing conditions for the fish are established within 6 weeks, fish welfare is accounted for prior to stocking. This sets the ground for better growth performance and fewer losses, so economic returns can be achieved earlier.

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OVERVIEW: HOW CAN FISH RESEARCH BE IMPROVED ACCORDING TO THE 3R – GUIDELINES

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Introduction

The implementation of the 3R principle (Reduce, Refine and Replace of animal experiments) established by Russel and Burch, is of growing importance in fish research. Nevertheless, the number of experiments on fish has increased tremendously. In 2019, more than 2.5 million fish were used in experimental projects in the European Union and Norway of which 20% were zebrafish and 80% were „other fish species“ (Eur-Lex 2022). Overall, fish are the second most used animal group after mice (52.5%), accounting for 24.6% (2,559,532 animals). Within the “other fishes” group, animals were mainly used for the following categories: animal diseases and disorders (709,198 fish), preservation of the species (207,051fish), protection of the natural environment in the interests of the health or welfare of human beings or animals (205,317 fish), regulatory use (102,802 fish), as well as 126,053 fish for animal welfare experiments (Eur-Lex 2022). Due to these numbers, efforts are present to reduce them and to achieve more animal welfare, especially in the aquaculture sector. For several years, researchers have therefore been developing alternative experimental methods, which were highlighted in the review article by Grunow et al. (2023).

Materials and methods

To illustrate the necessity of implementing the 3R concept in fish research, on the one hand, the statistics of the European Commission were evaluated to gain insight into the amount of animals, especially fish, used for research. On the other hand, we provide an overview of established alternative methods that have been published and are thus accessible to everyone, showing good solutions to promote fish welfare and implement the 3Rs concept in fish research (Grunow et al., 2023).

Results

In order to reduce the number of animals, several studies have proposed the use of DNA extracted from the mucus layers of the skin and mouth and from faeces, but also the use of eDNA (environmental DNA) from water. This method can be used not only for genetic analysis but also for determination of enzyme and hormone concentrations. This technique does not require direct in vivo intervention and is therefore more animal-friendly. The use of eDNA is also completely non-invasive and can even be used to analyse populations in larger waterbodies without the need to remove individual animals.

Under the aspect of „refinement“, the focus is on species-appropriate husbandry and the improvement of animal experiments. Thus, biotic and abiotic parameters must be determined in the keeping, breeding as well as rearing of fish, which combine the wellbeing of the animals with the best possible growth. An example of an abiotic parameter is the light regime, which must be used in a very species-specific manner. Furthermore, the fish species must also be considered when euthanizing them. Various studies show how different species respond to anaesthetics and the concentration used. In addition, the depth of anesthesia needs be studied so that the fish is not simply paralysed by the use of, for example, a muscle relaxant such MS222 as an anaesthetic and end up fully conscious.

Another non-lethal method is gastric lavage, which can be performed for trophodynamic analyses instead of whole gastrointestinal preparations. This method has been used very successfully not only with large predatory fish, but also in very small fish species. In addition, there are now numerous computer vision techniques for image analysis that are freely available on the web and can be used, for example, to analyse swimming behaviour or size/growth, but also to identify species down to the individual (based on body pigmentation and other features (e.g., scars)) of a shoal. In many cases, this alternative method could eliminate the use of markers, especially since many studies show that the intervention can lead directly or indirectly to death or that it can result in behavioural changes in the animals.

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For the replacement of animal experiments, cell models are a good basis. Up to date, around 900 fish cell lines from 200 fish species are listed at the cellosaurus database (https://www.cellosaurus.org/). These cell lines are suitable for basic research, e.g. investigation of the effect on climate changes (temperature increase), but also for applied research. As an example for applied research, the successes in ecotoxicology can be mentioned, as since 2019 the fish cell line RTgill-W1 from rainbow trout can be used for ecotoxicological screening instead of in vivo tests - ISO standard 21115:2019. Also in fish virology, cell culture from fish are a very often-used model system.

Discussion

Progress toward implementation of 3R principles has been significant in recent decades. Research on alternative experimental models and methods continues to be developed and expanded so that even fewer experiments will need to be performed on fish in the future. It is hoped that by publicizing these numerous alternatives to in vivo studies, animal numbers will decrease in future.

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EFFECTS OF NEONICOTINOID INSECTICIDE AND ITS ACTIVE SUBSTANCE ON ANTIOXIDANT BIOMARKERS OF EARLY LIFE STAGES OF GRASS CARP (*Ctenopharyngodon idella*)

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Introduction

Neonicotinoids are synthetically produced compounds, originated from nicotine, they have been launched on the market since 1990s. Neonicotinoids are highly effective against a wide range of pests. They accounted for nearly 23% of the global insecticide market in 2016 (Morrissey et al. 2015; Casida 2018; Klingelhöfer et al. 2022). Neonicotinoids are classified as systemic insecticides and neurotoxins acting on the central nervous system of organisms (Wang et al. 2018). They work in insects and mammals as nicotinic acetylcholine receptor (nAChRs) agonists, especially for the subtype α4β2 (Tomizawa and Casida 2005). Given the low toxicity of neonicotinoids to standard test species, they were not expected to significantly impact the aquatic ecosystem until later studies showed that this assumption was not correct (Morrissey et al., 2015; Sánchez-Bayo et al., 2016).

Flupyradifurone is the only representative of the new butenolide insecticide group. It received an EU authorisation in 2015 and has been classified by the Insecticide Resistance Action Committee (IRAC) as Group 4 - NAChR agonists. Group 4 includes nicotine, neonicotinoids and sulfoximines, in addition to butenolides. All the insecticides from this group principally share the same binding site on the nicotinic acetylcholine receptors (NAChRs) and therefore are considered to share the same mode of action. Sub-classification is based on structural differences of the insecticide molecules (IRAC, 2023). However, PAN Europe (2016) counters that, although the structure of flupyradifurone (and sulfoxaflor) is different, they are still neonicotinoid insecticides. For this reason, flupyradifurone should be treated accordingly by the regulator, considering its systemic and the harm it could cause to non-target organisms. Studies most often deal with the effect of flupyradifurone on bees, bumblebees, or other pollinators. Several studies also follow up on the effectiveness of flupyradifuron against pests. Only a few authors have so far researched the effect of flupyradifurone on non-target aquatic organisms. Most often, these are studies that compare the effects of pure flupyradifurone and other neonicotinoid substances.

Material and methods

The pure insecticide flupyradifurone (99 %) and the insecticide Sivanto Prime, which contains 17.1 % of the active ingredients flupyradifurone, were chosen to assess the effect of neonicotinoids on grass carp. Used concentrations of flupyradifurone were determined as 0.1; and 1 % of median lethal concentration (LC50) for grass carp larvae, stated by Zhong et al. (2021). Concentrations 0.21 and 2.1 $mg.l^{-1}$ were used. Contentrations of Sivanto Prime used in trial were determined to concentrations were chosen to match the flupyradifurone content of the product (17.1 %). Concentrations 1.23 and 12.3 $mg.l^{-1}$ were used. Each concentration was performed in duplicate.

The Fish, Early-life Stage Toxicity Test on grass carp was performed according to OECD methodology no. 210 (OECD, 2013). The test started 6 hours after eggs fertilization (hpf) and lasted for 28 days. Mortality was monitored every 24 hours when the bath was changed. Larval growth and development were assessed every 7 days. At the end of the test, 6 fish were collected from each test group to assess the effect of the used substances on the oxidative stress and antioxidant biomarkers of the individuals. Biochemical analysis of proteins, lipid peroxidation (LPO), glutathione S-transferase (GST), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and acetylcholinesterase (AChE) was performed on the larvae samples.

Results

Flupyradifurone and also Sivanto Prime affect levels and concentrations of oxidative stress and antioxidant biomarkers. Significant changes ($p<0.5$) in grass carp larvae were observed in tested groups, especially in larvae exposed to Sivanto Prime.

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Conclusion

The toxicity of neonicotinoids varies for different species. The fact that standard test organisms are relatively resistant to their effects also contributed to their worldwide spread. Only a few studies have compared the effect of active ingredients in pesticide products and pesticide products themselves. However, pesticide formulations may have a very different effect than the active ingredient alone. Although the test substance may not have a direct effect on the mortality of the test organisms used, it may affect the development, growth or immunity of the organism.

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HEALTHY FEED TO HEALTHY AQUATIC FOOD VIA SINO-NORWEGIAN COOPERATION (FEED2FOOD)

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This Sino-Norwegian collaborative project is at the forefront of scientific research in utilizing molecular, physiological and high advanced methodology to quantify the challenges with feed additives in combination with high fat diets (HFD). The overall objective of the Feed2Food project is to investigate the molecular mechanism behind the intestinal damage caused by a common anti-mold feed additive, sodium propionate (SP) under high fat diet conditions. We will also evaluate potential health issues associated with SP-induced translocation of intestinal microflora and microbial lipopolysaccharides (LPS) on fish and human consumers. Our previous study and the published scientific reports have showed that the widely used anti-mold feed additive SP induced intestinal injury in zebrafish under high fat diet conditions. This may impair intestinal barrier to an extent leading to microbial and lipopolysaccharide (LPS) translocation over the intestine. We therefore identified the molecular mechanisms behind the damaging effect. Our study then has focused on monitoring the possible secondary biological hazards that can pose a health challenge for both fish and the consumer. Results from the fish trials conducted in Norway and China with SP added to the feed as a novel ingredient are in progress and expected to be presented at this Aquaculture conference. The outcomes from this project will generate improved knowledge on the comparative effect and mechanisms of specific feed additive and lipid levels following HFD feeding, which is important for the information on potential health hazards to fish and consumers and development of future commercial aquaculture diets. We trust that this is the first of many investigations into possible damaging effects of novel feed stabilizers in the fast-growing aquaculture. This project can benefit all stakeholder groups and is essential for achieving a sustainable aquaculture. The Sino-Norwegian consortium consisting of Norway (NIBIO, NTNU) and China (CAAS, ZJSU), will work together and contribute to food safety through this Sino-Norwegian cooperation. This study is planned to be presented at this conference as an oral presentation.

EVALUATION OF BRAIN FUNCTION FOLLOWING DIFFERENT STUNNING METHODS IN NILE TILAPIA (*Oreochromis niloticus*)

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Background

Nile tilapia (*Oreochromis niloticus*) is an important aquaculture species and was the third most produced species globally in 2020 (FAO, 2022). The most common killing procedures for this species include asphyxiation, exsanguination through gill cutting or decapitation and/or direct evisceration (Lines and Spence, 2011; Robb and Kestin, 2002). To meet requirements of humane slaughter and provide strong animal protection, an efficient stunning prior to killing is crucial (WOAH, 2019). Yet all of the above-mentioned methods are merely killing procedures that do not include any form of stunning and therefore will compromise the welfare of the fish (Robb and Kestin, 2002). For a stunning method to be efficient, it should induce immediate loss of consciousness and the unconscious state should be irreversible, or at least persist long enough for death to ensue before consciousness is regained (WOAH, 2019; EFSA, 2004). Efficiency of stunning methods for Nile tilapia is largely unknown and there is an urgent need for validation and/or development of humane slaughter methods for this important aquaculture species.

Material and methods

Unconsciousness was determined by absence of visually evoked responses (VERs) in the electroencephalogram (EEG) of the Nile tilapia (Figure 1). EEG was obtained by inserting two 21G needles, soldered to 1.5 mm shielded wire electrodes acting as positive and negative electrodes, approximately 1 cm posterior to the eyes and 0.5 cm lateral to each side of the sagittal suture. A 29G needle electrode that served as the ground was placed subcutaneously at the tail. To study VERs, or lack thereof, EEG was measured in a dark room where a strobe light delivered repeated 20:480 ms light:dark episodes. The EEG signal was analyzed using the 13-32 Hz frequency range (Figure 1) as this is associated to conscious, waking states in many animals including fish (Bowman et al., 2019; Verhoeven et al., 2015). The different stunning methods assessed were i) percussive stunning, ii) live chilling, iii) electrical stunning and iv) a combination of electrical stunning followed by throat cut and placement in ice slurry. EEG was recorded before, during (when applicable) and after the stunning procedure. Percussive stunning was achieved using a handheld pneumatic bolt gun and live chilling was carried out by replacing the warm water with ice slurry. For electrical stunning, 1 second stun durations was initially performed to verify that the electrical exposure managed to induce an immediate loss of consciousness (Cook et al. 1995). When this was verified, 30 second stun durations using similar settings were applied to evaluate if the unconscious state could be prolonged by prolonging the stun duration. For the combinational procedure (*i.e.*, combining electrical stunning with throat cutting and placement in ice slurry), a 30 s electrical stun duration was applied before the fish was throat cut and placed in ice slurry.

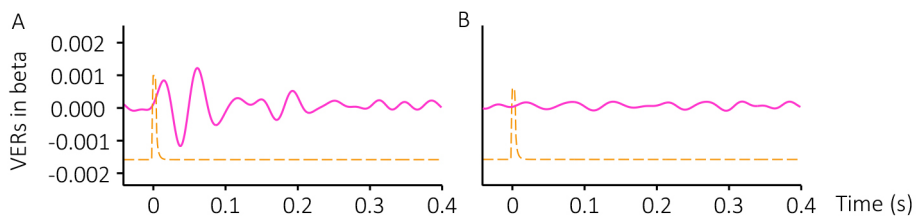

Figure 1. The beta wave frequency of the electroencephalogram (EEG) in a representative fish prior to and post percussive stunning. A) show the EEG before- and B) show the EEG immediately after stunning. The hatched orange line represent the light flash as registered by a custom made solar panel (the y axis is not in scale) and the pink solid line represent the average beta wave frequency of the EEG during one minute. Clear VERs are present prior to stunning whereas no distinguishable pattern in the EEG can be seen after stunning (*i.e.,* the fish is deemed to be unconscious).

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Results

Percussive stunning rendered all fish immediately and irreversibly unconscious. However, this required handling, air exposure and restraining the fish, which, each on its own is stressful. Also live chilling rendered all fish unconscious but had a long induction time (4.92±2.26 min). Electrical stunning for 1 s managed to induce immediate loss of consciousness when an electric field strength of 8.2 V_{RMS} cm^{-1}, a current density of 0.68 A_{RMS} dm^{-2}, a frequency of 50 Hz sinusoidal alternating current (AC) and a water conductivity of 753 μS cm^{-1} was used. When prolonging the stun duration to 30 s using similar electrical parameters (*i.e.,* 8.2 V_{RMS} cm^{-1}, 0.68 A_{RMS} dm^{-2}, 50 Hz sinusoidal AC and 753 μS cm^{-1}), fish lost VERs for at least 30 s. When electrical stunning using similar settings (*i.e.,* for 30 s using 8.2 V_{RMS} cm^{-1}, 0.62 A_{RMS} dm^{-2}, 50 Hz sinusoidal AC and 753 μS cm^{-1}) was followed by throat cutting and ice slurry immersion in two fish, VERs came back after 80 and 96 seconds, respectively. However, when the strength of the exposure was increased (electric field strength and current density of 14.2 V_{RMS} cm^{-1} and 1.07 A_{RMS} dm^{-2}, respectively), VERs did not come back within 30 minutes after the electrical exposure when it was followed by throat cutting and ice slurry immersion.

Conclusion

Here, we show that when combining electrical stunning with throat cutting and ice slurry immersion, an immediate and irreversible loss of VERs can be accomplished. Also percussive stunning using a handheld bolt gun is efficient in rendering the fish immediately and irreversibly unconscious. However, the latter method require handling and could benefit from a pre-stunning procedure, such as electrical stunning. These results show that electrical stunning followed by both percussive stunning or throat cut and immersion in ice slurry have the potential to ensure humane slaughter of Nile tilapia. However, the electrical exposures needed to guarantee that the effect persists long enough for death to ensue is largely depending on the stun-to-kill time of the selected killing procedure. Collectively, our results shows that humane slaughter of Nile tilapia is possible. If the outcome of our study is implemented this may aid in safeguarding the welfare of billions of tilapia at time of slaughter.

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EXPLORING THE POTENTIAL OF DIETARY CARBOHYDRATE ON THE DENITRIFICATION PERFORMANCE OF A MARINE RECIRCULATING SYSTEM USING INTERNAL CARBON SOURCES

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Introduction

Denitrification is a biofiltration process which is commonly applied in RAS to control nitrate concentrations by converting nitrate-nitrogen (NO_3-N) to nitrogen gas. This process requires high amounts of organic carbon, which is commonly supplied by external carbon sources that increase farm operational costs. Faecal waste can be used alternatively as an internal carbon source for denitrification but its insufficient carbon bioavailability often limits NO_3-N removal. The quantity of bioavailable faecal carbon depends on diet digestibility and the respective faecal composition. Lately numerous alternative ingredients have been used in aquafeeds, frequenly resulting in increased indigestible carbohydrate fractions. As such, faeces capacity to act as an internal carbon source for denitrification may vary with diet composition (Meriac et al., 2014). In this respect, the present study aimed to explore the effect of dietary carbohydrate on the denitrification potential of a marine RAS using internal faecal carbon sources.

Materials and methods

In vitro trial: Settleable feaces originating from a preceding feeding trial (Syropoulou et al., 2022) testing six dietary ingredients (shrimp shell meal; SSM, feather meal, insect meal; IM, seaweed, single-cell meal, dried distillers grain with solubles; DDGS) on European seabass were collected. Faecal material was incubated in anoxic batch reactors under isocarbonic conditions for a 14-day period. Abiotic parameters including NO_3-N were monitored daily and faecal samples were obtained every 24 h for the first three days (Day1, 2, 3) and every alternate day onwards. Samples were immediately analyzed for volatile fatty acids (VFAs) to determine the degree of fermentation (Letelier-Gordo et al., 2017). Data was normalized to the Day0 values and was expressed per unit of organic matter (OM) faeces and per unit of feed, respectively.

In vivo trial: Four identical RAS equipped with an up-flow sludge blanket denitrification reactor (DR), were stocked with juvenile European seabass (1.78 ± 0.02 kg/RAS). Fish were fed over a six-week period with two of the aforementioned six diets (IM, DDGS) in duplicate. Feeding level was gradually increased until Week2, when a fixed amount of 50 g feed/RAS/day was established. During the trial, presettled feacal waste produced per system was constantly fed to the respective DR. NO_3-N levels in the outlet of the fish tanks were analyzed weekly, whereas in the inlet and outlet of the DR at the end of the trial over a 24-h scheme. Finally, after the completion of the experiment, both faeces and DR sludge were analyzed for their nutrient composition in order to create nutrient balances on a DR level.

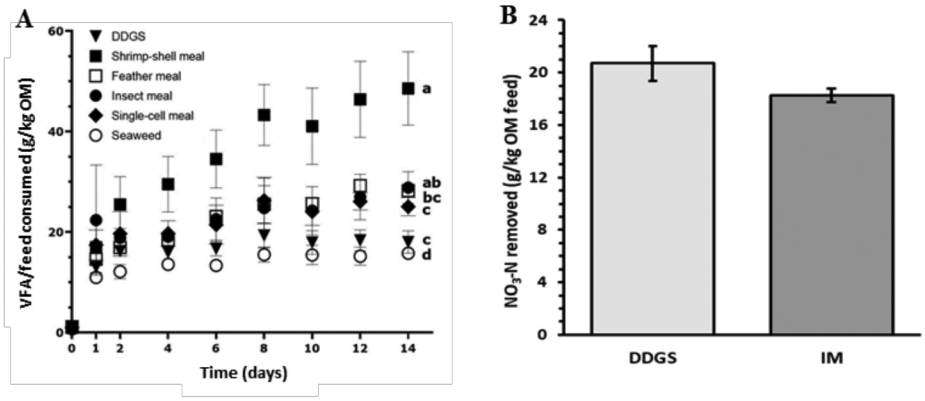

Figure. A) Volatile fatty acid (VFA) production (mean ± SD, n =2) in faecal samples fermented in the *in vitro* trial, with lowercase letters indicating for significant differences ($p<0.05$) as obtained by multiple comparisons (time × diet) over the whole timespan of the experiment. B) Nitrate-nitrogen (NO_3-N) removal (mean ± SD, n =2) for the two diets tested in the *in vivo* trial.

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Results & Discussion

Degree of fermentation, expressed as VFA production per kg OM feed, was influenced by OM digestibility, faecal removal efficiency via settling and carbon bioavailabity of feaces. Even though, fish fed with SSM excreted a low amount of feaces, feacal quality improved faecal removal efficiency and thus more material was available to be fermented per kg OM feed. Additionally, feacal carbon bioavailability was highest for this diet which explains the highest VFA production not only per kg OM feed (Figure A) but also per kg OM faeces. Among the tested diets, IM produced the second highest amount of VFAs whilst DDGS the second lowest. Additionally, the two diets yielded a highly different VFA profile, with propionate being produced only from IM faeces and lactate only from DDGS faeces. Due to the higher amount of VFAs produced in the *in vitro* trial along with the more favorable VFA profile, we hypothesized that IM would act as a more efficient internal carbon source for denitrification in RAS between the two. However, when denitrification efficiency was studied in the *in vivo* trial, no significant differences were found for NO_3-N removal between the two dietary treatment groups ($p>0.05$; Figure B). This discrepancy between trials might be attributed to their different experimental duration, indicating that possibly long-term anaerobic digestion of feacal material may allow for better utilization of feacal carbon. Moreover, nutrient conditions differed upon case, since NO_3-N was soon depleted in the *in vitro* trial, whereas NO_3-N was constantly supplied to the DRs in the RAS setup. This likely resulted in the development of different microbial communities with different capacities to utilize faecal carbon. Additional microbial data will help identify the reason of this inconsistency. In conclusion, the present study showed that batch fermentation of faecal material is not a good indicator for the potential of faeces to act as an internal carbon source for denitrification.

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REARING DIFFERENT COMMON CARP (*Cyprinus carpio*) AGE-CLASSES WITH ALGAE ENRICHED FEED

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Introduction

In common carp (*Cyprinus carpio*) farming, the use of complete feeds is expected to increase in the future. An important component of fish feed is fish meal, which is made from small, less valuable marine fish. As a result of overfishing, the stock size of marine fish is drastically reduced, so the price of fishmeal as well as of artificial fish feed is constantly rising. Partial substitution of fishmeal can be solved by mixing microalgae into the food, which contain the nutrients necessary for fish in the right quantity and quality. In our experiments, common carp fry of different age-classes was reared in ponds. During rearing, we examined and evaluated the effects of feeding cereals, traditional artificial feed and algae-enriched feed on growth of common carp fry and fingerling.

Materials and methods

Experiments were carried out in fry, fingerling and on-growing ponds of Szegedfish Ltd. during the breeding seasons of 2021 and 2022. During samplings, we collected data on body length and weight of the fish.

Conditions for the experiments related to advanced fry rearing
The experiment took place in June 2022. Carp fry were reared in six ponds, the size of which was one hectare each. In two ponds, fish received traditional fry feed, and in four ponds, we fed the fish with algae-enriched fry feed. The algae concentrations in the feeds were 1.0% and 3.0%, respectively. At the end of the fry rearing period, the body length and body weight of 30 fish from each pond were measured.

Conditions for the experiments related to fingerling rearing
Experiments were carried out in two 10-hectare rearing ponds in the 2021 breeding season. We stocked 300,000 advanced fry into both ponds in the first week of July. In the control pond, we fed the fish with traditional rearing food, and in the "algae" pond, we fed the fish with algae-enriched food. The concentration of algae in the food was 3.0%. During rearing, we measured the body length and weight of 50 individuals on two occasions (July 27 and September 8). The data for the control and the "algae" pond were compared with a two-sample t-test ($P < 0.05$).

Conditions for the experiments related to rearing of two-summer old common carp
The experiments were carried out in three 40-hectare ponds in 2022. We stocked 150,000 one-year-old fry in all three ponds, the average weight of which was 65 grams. The fry were fed in the three ponds with cereal feed, traditional formulated feed, and formulated feed enriched with algae (1%), respectively. We only fed formulated feed or algae-enriched feed during the last four weeks of the rearing season. At the beginning and middle of the breeding season, these fish were also fed with cereal grains. During rearing, we measured the body length and weight of 30 individuals on one occasion. The data were compared using one-way analysis of variance (F-test) ($P < 0.05$).

Results and conclusion

Figures 1-4 summarize the results of the evaluation of data recorded during the rearing of different age-groups of common carp in earthen ponds.

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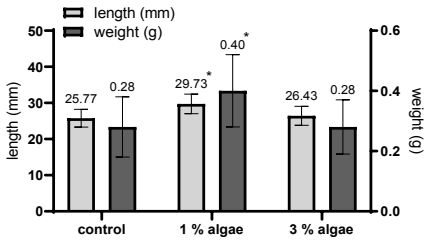

Figure 1: Body length and weight (mean ± SD) of advanced fry in the control and treatment ponds. Data from the "1% algae" pond were significantly higher (ANOVA, Fisher's test, $P < 0.05$)

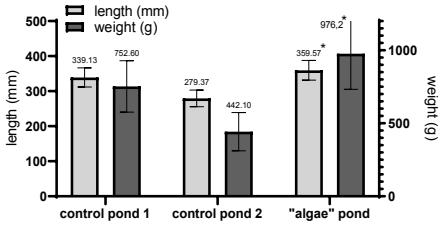

Figure 2: Body length and body weight (mean ± SD) of the two-summer old carp. Both indicators can be considered significantly higher in the group fed with "algae" feed ($P < 0.05$)

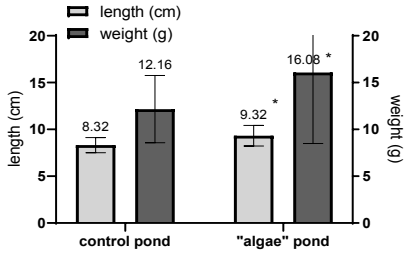

Figure 3: Body length and body weight of fingerlings (mean ± SD) on July 27, 2021. Both indicators can be considered significantly higher for the „algae" pond ($P < 0.05$)

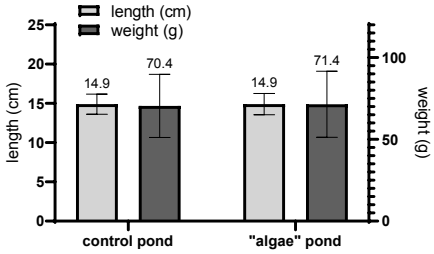

Figure 3: Body length and body weight of fingerlings (mean ± SD) on September 8, 2021. No significant differences between the mean values were detected

Based on our experiments, it can be concluded that feeding algae-enriched feed had positive effects on the growth of all three investigated age groups. At the same time, it should be noted that the tests were carried out in relatively large rearing ponds, which greatly limited the number of replicates that could be used. The results come from single sampling times, so it cannot be ruled out that the measured differences balance out in the later stages of the rearing season. In any case, supplementing fish feed with algae does not have any negative consequences, so it can be a good alternative to replacing fishmeal in the future.

Acknowledgements

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EFFECTS OF INNOVATIVE FEEDS ON THE HOMEOSTASIS OF STURGEON FISH WITH PARTICULAR EMPHASIS ON THE DIGESTIVE SYSTEM

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Introduction

Aquaculture is currently the most rapidly growing sector of animal production and until now, fishmeal has been the main source of protein used in compound feeds. Unfortunately, the high demand for fishmeal has pushed up its price and overfished the natural fish stock. For this reason, alternative sources of protein that can be used in compound feeds are being sought. One such alternative are plants of the genus *Lupinus*, characterised by high environmental tolerance and a favourable amino acid profile (Abraham et al., 2019). Many studies have been carried out to assess the possibility of using lupins in aquatic nutrition with promising results (Szczepański et al., 2022). However, there is currently no literature data on the use of lupins in the feeding of sturgeons. Thus, the aim of this study was to assess the feasibility of using innovative feeds, composed on the basis of lupin meal, in Siberian sturgeon (*Acipenser baerii*) nutrition, while taking into account both the economic and animal welfare aspects of sustainable aquaculture.

Materials and methods

The study was carried out as part of the project no. 0001–6521.1-OR0700001/17/20 founded by Operational Program 'Fisheries and Sea' (2014–2020) financed by the European Maritime and Fisheries Fund.

The feeding experiment lasted 125 days. The fish were divided into four groups. In the reference group (R), the sturgeons were fed with a commercial feed dedicated to the breeding of sturgeon fish, while in the control group (0L), a composed feed with the addition of fish meal as the main protein source. Groups 5L and 10L were experimental groups using composed feeds with inclusion of white lupin meal at levels of 5 and 10%, respectively. After 125 days, the fish were killed, weighed, total length and standard length measured. Samples from liver, anterior and spiral intestine from twelve individuals from each group were collected (n=48) for histological (HE staining) and biochemical () analysis. In livers, the activities of alkaline phosphatase (ALP), acid phosphatase (ACP), superoxide dismutase (SOD) and glutathione peroxidase were examined. In anterior and spiral intestine, the activities of ALP, ACP, amylase, trypsin and lipase were evaluated. The obtained results were statistically analysed.

Results

At the end of the experiment, distinct differences in the mean body weights (BWf) of the fish were observed, with the largest differences ($P < 0.05$) noted in R group compared to 5L and 10L groups (Table 1). Sturgeons in R group had the highest average total length compared (TL) to the other groups ($P < 0.05$) (Table 1). The gastrointestinal tract sections showed no significant histopathological changes. The longest intestinal folds (IF) with the proportionally largest absorptive area were found in the 10L group. The livers of all fish were characterized by severe *steatosis*, with the largest hepatocytes (HA) observed in the 0L group. In anterior intestine, a signifficant decrease in lipase activity (U/mg) was observed in 10L group compared to other groups. In the liver, signifficant decreases in alkaline phosphatase (ALP), acid phosphatase (ACP) and superoxide dismutase (SOD) activities were noted in 5L and 10L groups (Table 1).

Conclusion

Based on the performed analyses, it can be concluded that feeds with lupin meal inclusion are less suitable for feeding young Siberian sturgeon compared to commercial feeds and generally those with fish meal as the main source of protein. However, the lack of histopathological changes and the absence of enzymatic disturbances may suggest the need for further studies using older fish.

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Table 1. Body length and weight, histomorphometric parameters and enzyme activity in the gut and liver of sturgeons.

Parameters	Groups			
	R	0L	5L	10L
Length and body weight of fish, and histomorphometric parameters				
TL (cm)	28,57 ± 3,43 [a]	25,39 ± 2,67 [b]	23,70 ± 2,39 [b]	23,93 ± 2,34 [b]
BWf (g)	65,64 ± 24,36 [a]	47,73 ± 15,90 [ab]	38,81 ± 12,05 [b]	43,24 ± 10,95 [b]
IF (μm)	812,40±226,27 [a]	691,48±193,91 [b]	674,86 ± 149,51 [b]	926,34±217,90 [c]
HA (μm^2)	232,36 ± 55,69 [a]	334,49 ± 127,83 [b]	314,13 ± 104,18 [b]	262,09 ± 88,45 [a]
Enzyme activity in anterior intestine				
ALP (IU mg^{-1})	0,1364 ± 0,059	0,1412 ± 0,055	0,1447 ± 0,052	0,1300 ± 0,051
ACP (IU mg^{-1})	0,0026 ± 0,001	0,0024 ± 0,001	0,0020 ± 0,0005	0,0021 ± 0,0005
Lipase (IU mg^{-1})	0,0285 ± 0,012	0,0307 ± 0,021	0,0405 ± 0,026	0,0053 ± 0,002 [a]
Amylase (IU mg^{-1})	0,0160 ± 0,010 [ab]	0,0339 ± 0,023 [a]	0,0057 ± 0,007 [b]	0,0141 ± 0,009 [ab]
Trypsin (IU mg^{-1})	0,0280 ± 0,023	0,0311 ± 0,013	0,0250 ± 0,023	0,0291 ± 0,019
Enzyme activity in spiral intestine				
ALP (IU mg^{-1})	0,3158 ± 0,160	0,2642 ± 0,109	0,4564 ± 0,236	0,3073 ± 0,107
ACP (IU mg^{-1})	0,0017 ± 0,0004 [a]	0,0025 ± 0,0009 [b]	0,0024 ± 0,0007 [ab]	0,0018 ± 0,0006 [ab]
Lipase (IU mg^{-1})	0,0059 ± 0,006 [a]	0,0264 ± 0,012 [b]	0,0264 ± 0,008 [b]	0,0032 ± 0,001 [a]
Amylase (IU mg^{-1})	0,0184 ± 0,013	0,0146 ± 0,017	0,0080 ± 0,004	0,0167 ± 0,008
Trypsin (IU mg^{-1})	0,0359 ± 0,024	0,0250 ± 0,024	0,0486 ± 0,028	0,0374 ± 0,016
Enzyme activity in liver				
ALP (IU mg^{-1})	0,2427±0,093 [a]	0,1359±0,053 [a]	0,0550±0,013 [b]	0,0500±0,013 [b]
ACP (IU mg^{-1})	0,0031±0,0007 [a]	0,0030±0,0007 [a]	0,0014±0,0003 [b]	0,0016±0,0005 [b]
SOD (IU mg^{-1})	0,0242±0,012 [a]	0,0243±0,006 [a]	0,0043±0,002 [b]	0,0049±0,002 [b]
GPX (IU mg^{-1})	0,2533±0,054 [ab]	0,4499±0,198 [a]	0,2567±0,102 [ab]	0,2142±0,092 [b]

Means followed by different letters in the same row are significantly different ($P < 0.05$).

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GROWTH PERFORMANCE, AMINO ACID AND FATTY ACID COMPOSITION OF GILTHEAD SEABREAM (*Sparus aurata*) FED ALGAE MEAL DIETS

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Introduction

Aquaculture sector is thriving as a response to the rapidly increased demand for seafood products. The sector is using, 18 million tons of wild fish that are targeted for fishmeals and fish oils in aquafeeds (FAO 2020), thus affecting the sustainability of marine ecosystem. This has led the research to focus on environmentally sustainable sources of proteins and lipids as replacements of fishmeal and fish oil. Microalgae biomasses seem to be potential feed ingredients containing sustainable amounts of essential amino acids, essential fatty acids, vitamins, and pigments, that could be produced with a low environmental footprint (Nagappan et al. 2021). This study aims to investigate the effect of dietary inclusion of algae meal on the growth performance, amino acid composition and fatty acid composition of the white muscle of gilthead sea bream (*Sparus aurata*).

Material and methods

The experimental trial was conducted at the Department of Ichthyology and Aquatic Environment, University of Thessaly, in Volos, Greece. Briefly, 360 individuals *S. aurata* (initial mean weight 6.43±0.04g) were distributed randomly to six 250l tanks. Two experimental diets were formulated to be isonitrogenous, isolipidic, and isoenergetic with diet 1 (PA) consisted of 8% *Phaeodactylum tricornutum*, and diet 2 (HA) consisted of 8.23% *Schizochytrium limacinum*. Each diet was assigned to triplicate groups of 60 fish per group. The trial lasted 45 days, after 15 days of acclimatization. The fish were fed three times per day *ad libidum*. Fish were weighted individually at the beginning and end of the experimental trial under anaesthesia. Feed consumption was recorded daily in order to evaluate accurately values for feed utilization.

Results

The results showed that weight gain, feed consumption, specific growth rate and survival did not have statistically significant differences ($p>0.05$) between fish fed the diet consisted of *Phaeodactylum tricornutum* (PA) and the diet of *Schizochytrium limacinum* (HA). Fish fed with PA had statistically significant ($p<0.05$) lower feed conversion ratio and higher protein efficiency ratio (Table 1).

Amino acid composition revealed a strong positive correlation, highly significant ($p<0.05$) between diets and the white muscle of fish in each treatment. Moreover, fatty acids analysis showed a significant decrease ($p<0.05$) of eicosapentaenoic acid (EPA) and an increase ($p<0.05$) of docosahexaenoic acid (DHA) in white muscle of seabream fed the HA diet, compared to the PA treatment. Arachidonic acid (ARA) of white muscle was unaffected ($p>0.05$) between the dietary treatments.

Table 1: Growth performance parameters of gilthead seabream fed the experimental diets.

	PA	HA
Final Weight (g)	26.54±0.34^a	26.62±0.35^a
Weight gain (g)	20.07±0.34^a	20.22±0.35^a
Feed consumption (g/fish)	15.48±0.53^a	17.89±0.78^a
Specific growth rate (SGR, %/day)	3.10±0.02^a	3.13±0.02^a
Feed conversion ratio (FCR)	0.81±0.01^a	0.93±0.01^b
Protein efficiency ratio (PER)	2.92±0.04^b	2.52±0.04^a
Survival (%)	98.88±0.55^a	99.44±0.55^a

Values are presented as means±standard error. Means sharing the same superscript are not significantly different from each other ($P<0.05$).

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Discussion

The results of this study showed that the inclusion of algae meal in *S. aurata* diets did not affect growth performance and amino acid deposition. The lower FCR and higher PER exhibited in fish fed the PA diet denotes a better protein utilization of *P. tricornutum* diet than *S. limacinum* by *S. aurata*. In terms of aquaculture sustainability, the substitution of fish oil with heterotrophically produced microalgae in salmon feeds proved the nutritional feasibility of low trophic level organisms such as algae in aquaculture fish feed (Kousoulaki et al., 2017). In accordance with the results of this work, fishmeal replacement with the microalgae *P. tricornutum* in started diets of *S. aurata* showed that SGR was not affected ($p>0.05$) and there was an increase of saturated fatty acids in fish (Atalah et al. 2007). Moreover, the inclusion of 2.5% *P. tricornutum* in finishing diets of gilthead sea bream did not significantly change growth, feed utilization parameters and muscle fatty acid profile ($p>0.05$) (Ribeiro et al. 2017). Furthermore, the 2.5% inclusion of *Schizochytrium* sp. as lipid source and especially DHA source in microdiets of *S. aurata* had no negative effect on larvae (Ganuza et al. 2008). In contrast to the forementioned authors, Eryalçin & Yildiz (2015) reported that the replacement of fishoil with dried *Schizochytrium* sp. meal had a negative effect on growth of seabream although the DHA was increased.

The differences found in the fatty acid profiles on the muscle tissue of fish are characteristic to the fatty acid's profiles of each algae species as *S. limacinum* is known for being richer in DHA and poorer in EPA compared to the *P. tricornutum* and *S. limacinum* has been used successfully in the diets of seabream and other species, mainly for fishoil replacement. According to Santigosa et al. (2021), the growth performance of seabream fed 3.5% *Schizochytrium* sp. oil as replacement of fishoil was unaffected and fillet fatty acid composition was in accordance with the diet fatty acid composition, suggesting that dietary microalgae could lead to an environmentally friendly final product rich in n-3, n-6 fatty acids. The dietary inclusion of algae meal (*Phaeodactylum tricornutum* and *Schizochytrium limacinum*) does not influence growth performance but there is an effect on FCR, PER, amino acid deposition and fatty acid composition of the white muscle of seabream (*Sparus aurata*) follows the fatty acid and amino acid composition of the two algae suggesting they can be used and produce a final product rich in n-3 and n-6 fatty acids. Algae meal biomass is promising ingredient to move the formulation of sea bream feed to a more sustainable future.

Acknowledgement

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EFFECT OF DIFFERENT INCLUSION RATES OF YEAST *Candida utilis* AS PROTEIN SOURCE IN HYBRID AFRICAN CATFISH *Clarias gariepinus* x *Heterobranchus longifilis* STRESS RESPONSE AND LIPID METABOLISM

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Introduction

As the aquaculture industry continues its decade-long growth, ensuring the supply of sustainable alternative feed ingredients is an urgent need. A promising new protein source is dried cells harvested from cultures of monocellular microorganisms, notably yeast. Yeast production has the advantage of being highly sustainable, requiring little space or resources, and enable valorising agro-industrial side-streams (Sharif et al., 2021). Yeast contains a high protein content, and the amino-acid composition is generally well-adapted to satisfy most nutritional requirements of fish (Agboola et al., 2021). Moreover, yeasts are a good source of prebiotic compounds with benefits for health, digestive function, and general performance (Petit et al., 2019). Therefore, this study aimed to assess the effect of dietary replacement of plant-based ingredients with yeast (*Candida utilis*) on stress response and plasma metabolite profile of hybrid African catfish to evaluate impacts on farming performance, health, and welfare.

Materials and methods:

Fish (initial body weight 77.7±0.25 g) were allocated between 3 isoenergetic and isonitrogenous diet treatments (in triplicate tanks) in freshwater RAS. All diets contained 5% of fishmeal alongside plant-based ingredients (PBI) [soymeal, wheat gluten, and soybean protein concentrate] which were partially substituted with yeast in the other diets: ***Diet 1*** (control, 0% yeast); ***Diet 2*** (10% of yeast corresponding to 12% of PBI replacement); ***Diet 3*** (20% of yeast, 25% PBI replacement). During the feeding trial (10 weeks), feed was delivered to satiety. Water temperature, stocking density, and photoperiod were 27.3°C, 17 $kg.m^{-3}$, and 12:12 L/D. At the end of the trial, 18 fish from each group (N=6 per tank) were sampled, from which 9 were euthanatized (pre-stress) while the other 9 were subjected to acute stress challenge (ACT, 15 min of crowding (800 $kg.m^{-3}$), followed by 45 min of recovery before euthanasia. Plasma cortisol was measured using ELISA, and ions (chloride, potassium, and magnesium) and metabolites related to energy and lipid metabolisms (creatinine enzyme, triglycerides, LDL and HDL) were quantified using Pentra 400. Data were subjected to One-way ANOVA followed by Tukey to compare diets, and to Student's t-test to compare between the two stress states ($p < 0.05$).

Results

Growth performance (final body weight approx. 421 g) showed minor impacts of the various diets, with a slight but not significant increase correlating with increased yeast inclusion levels. Similarly, the ACT only appeared to have very limited effect on the catfish, as shown on the cortisol levels only showing significant increase in Diet 1 and with non-significant reduction of chloride and potassium levels after ACT (except for Diet1). Magnesium showed a large variance in Diets 1 and 2 following the ACT (Fig. 1). On the other hand, metabolites related to lipid metabolism showed significant changes among diets. Triglycerides were significantly higher in Diet 1, both before and after the ACT. Although plasma cholesterol was unaltered (data not shown), the TAG and LDL plasma concentrations significantly decreased while HDL increased with higher inclusion rates of yeast in the diet (Fig. 2).

Discussion and conclusion

Substitution of PBI with yeast did not hinder growth performance. This is encouraging considering that yeast production is more sustainable compared to SM. Catfish is also a prime candidate for alternative protein sources considering their omnivorous diets and efficient abilities to digest a wide range of ingredients. Further, creatinine was found at a lower level in the fish receiving the yeast diets. Elevated plasma creatinine levels are noted to being associated with kidney damage or impaired functions, and these results could point to anti-nutritional factors found in soy ingredients.

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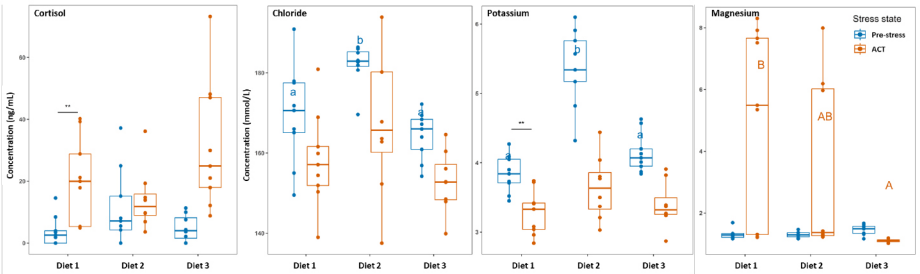

Fig. 1. Plasma cortisol and ion (chloride, potassium, and magnesium) levels. Lower- and upper-case letters denote significant differences among diets for pre-stress and ACT, respectively, while asterisks indicate significant differences between the pre-stress and stressed state within each diet (** denote $p < 0.01$).

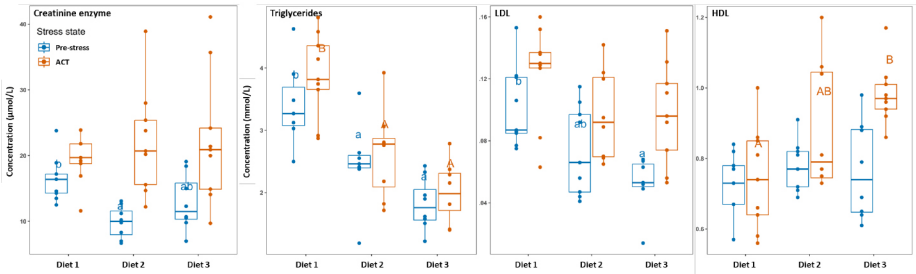

Fig. 2. Plasma levels of creatinine enzyme, triglyceride, LDL, and HDL. Lower- and upper-case letters denote significant differences among diets for pre-stress and ACT, respectively, while asterisks indicate significant differences between the pre-stress and stressed state within each diet (** denote $p < 0.01$).

Triglyceride were higher in the fish fed the Diet 1. This may be because triglycerides are commonly associated with diets high in grain and cereals. Increased dietary yeast elevated plasma HDL while reducing LDL, suggesting that dietary substitution with yeast impacted lipid metabolism and transport mechanisms. Elevation in HDL, a major lipoprotein in fish, could signal improved cardiovascular performance compared to the control diets. Alternatively, shifts in HDL/ LDL proportions have also been linked to oxidative stress, various metabolic disorders, dietary fat levels, or changes in food acquisition (e.g., fasting) (Long et al., 2021; Kjær et al., 2009) and a more targeted approach is needed to elucidate the impacts of yeast substitution in catfish diets and their implication for lipid metabolism.

Cortisol and ion levels showed limited increase after ACT. Since catfish are notoriously rustic, tolerating poor water conditions, it is likely that the stressor type, duration, or intensity may have been insufficient to trigger a marked stress response. Conversely, plasma magnesium levels, a known stress-marker, showed a reduced response to the ACTs with increasing dietary yeast inclusion. However, the results showed strong individual variations (Fig. 1) and it is possible that while most fish were able to handle the ACT with little difficulty, a few were more affected.

The present study not only showed no negative effect of replacing PBI with yeast but was associated with some improved parameters (creatinine enzyme, LDL/HDL ratio), confirming the suitability of this protein source as a feed ingredient in catfish diet. However, changes in lipid metabolism highlights the need of further studies targeting the liver lipid content and profile and molecular mechanisms underlying these changes.

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Acknowledgments

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DEVELOPMENT OF EPIDEMIOLOGICAL MODEL FOR PREDICTING CAGE-LEVEL SALMON LICE (*Lepeophtheirus salmonis*) ABUNDANCE

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Introductions

Salmon lice (*Lepeophtheirus salmonis*) are parasites on salmonid fish and a density-dependent constraint to the sustainable farming of salmonids in open net pens. To control the parasites, fish farmers in Norway are required to count the number of salmon lice in different developmental stages on a subset of the fish each week. Furthermore, they must ensure that the number of adult female lice per fish does not increase beyond a specified threshold level. In this project, we have developed a statistical model with the goal of determining the optimal way to utilize fish farm monitoring data from the AquaCloud system. AquaCloud data platform is a system that has been automatically aggregating data from Norwegian fish farmers since its establishment in 2017 (https://aquacloud.ai). We have focussed on predicting the lice development one week ahead in the absence of any lice treatment. We included data for weeks without treatments and with observations of lice as well as environmental variables (temperature, salinity, oxygen saturation level) in the two weeks preceeding the week to be predicted. This inital dataset included 190,528 observations from 242 farms spanning the years 2017 to 2022. These records represented salmonid farming across most regions of Norway, spanning latitudes from 59 to 71 °N (Figure 1).

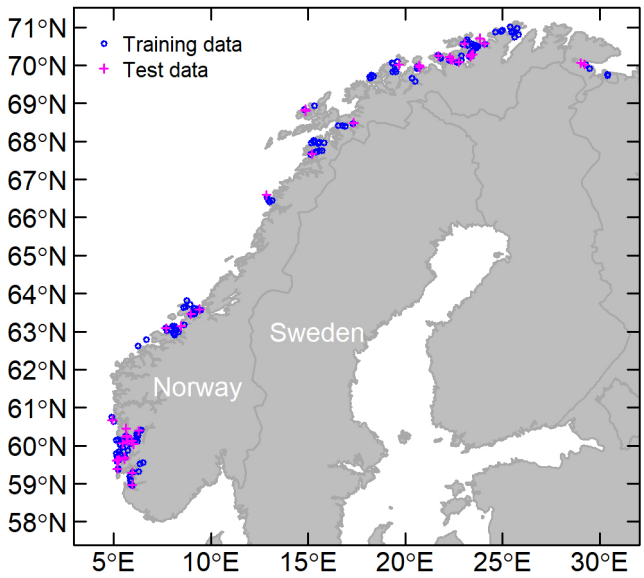

Figure 1. Location of data. The points show the locations of the 201 farms with data for all variables used in the final model. Training data were used to fit the model. Test data were used to assess predictive power.

We used data from a random selection of 80% of the farms to train and fit the model (Training data), and then data from the remaining 20% of the farms (Test data) to see how well the model could predict. Initially, we considered various model formulations, to assess possible nonlinearity in associations and using posterior predictive checks to find an appropriate error structure. These analyses suggest that the following nonlinear random-effects model formulation with a negative binomial error structure fits the data well:

Count data for all three stage groups (A, adult females; O, other motiles; S, sessile) were analysed jointly in one statistical model. The total numbers of lice of each stage on the counted fish in a given cage (subscript C) and week (subscript T) were considered to follow negative binomial distributions (equations 1–3):

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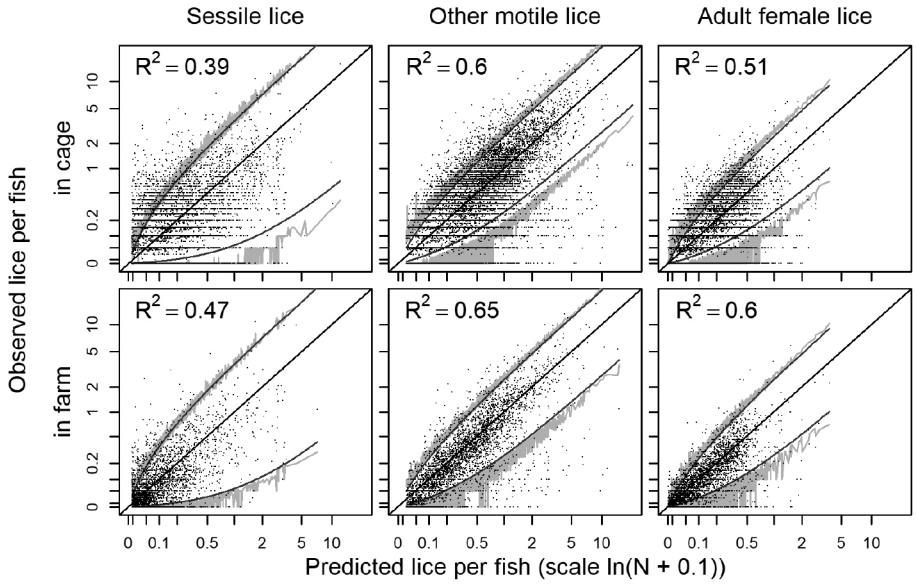
$$A^{COUNT}{}_{C,T} \sim \text{negative binomial}\ (\mu^{A}{}_{C,T} \cdot N^{COUNT}{}_{C,T}, \theta_A) \quad (1)$$

$$O^{COUNT}{}_{C,T} \sim \text{negative binomial}\ (\mu^{O}{}_{C,T} \cdot N^{COUNT}{}_{C,T}, \theta_O) \quad (2)$$

$$S^{COUNT}{}_{C,T} \sim \text{negative binomial}\ (\mu^{S}{}_{C,T} \cdot N^{COUNT}{}_{C,T}, \theta_S) \quad (3)$$

Here, μ^A, μ^O and μ^S are the expected number of lice of each stage per counted fish, N^{COUNT} is the number of fish on which lice were counted and θ_A, θ_O and θ_S are dispersion parameters of the negative binomial distributions (specifically, variance = mean + mean^2 / θ).



Predictions of lice abundance in test data set are shown in Figure 2. Black lines show the 1:1 lines where the observed lice per fish equal the predicted. Blue lines show 90 % prediction intervals for the true lice levels. Grey lines show 90 % prediction intervals for lice counts, also taking into account the counting variability and the uncertainty in the parameter estimates. The widths of the latter intervals are influenced by variation in the number of fish on which lice were counted for each observation event. The model predicted a considerable part of the variation in lice numbers in the test data set, not used in the model selection or model fitting. Specifically, for these "new" data cases, the model predicted 51 % of the cage-level variance and 60 % of the farm-level variance in adult female lice abundance. Corresponding numbers for other motile lice were 60 % and 65 %, and for sessile lice 39 % and 47 %. Prediction intervals were wider for sessile lice than for the other stages (Figure 2).

In conclusion, by using state-of-the-art statistical modelling, the salmon lice prediction model integrates detailed and high-resolved information from a large number of fish farms from large parts of the Norwegian coast. This information enables the development of generic "rules" for how a fish farmer may best use the monitoring data at hand to predict next week's salmon lice abundance. The insights from this model will be integrated to enhance the existing "lice calculator" web application (http://apps.vetinst.no/lusekalkulator/). This app is designed to be user-friendly for the general public and will assist farmers in automatically predicting the expected number of lice for the upcoming week. Hence, fish farmers may move from the subjective experience-based assessment used today to more precisely predicting lice development. Precise predictions in turn enable timely use of lice treatments, avoiding unnecessary treatments at low lice levels but treating outbreaks before they become too severe.

This research was undertaken under the NewTechAqua (New technologies Tools and Strategies for a Sustainable, Resilient and Innovative European Aquaculture) project, which has received funding from the European Union's Horizon 2020 Programme under grant agreement No 862658 (https://www.newtechaqua.eu/).

NANOPLASTICS ARE BIOACCUMULATED IN FISH LIVER AND MUSCLE AND CAUSE DNA DAMAGE AFTER A CHRONIC EXPOSURE

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Introduction

Nanoplastics (NPs), the particle size-fraction under 1000 nm, are potentially one of the most hazardous marine litter, as their physicochemical nanoscale properties allow them to cross biological barriers, including the intestinal wall and the mammalian placental barrier. The detection and quantification of the environmental plastic nanofraction is an ongoing challenge, as the current analytical techniques to detect NPs in complex biological matrices are not fully mature. Nevertheless, the presence of NPs in marine waters has already been confirmed, both in oceanic (Ter Halle et al., 2017) even if its impact is not fully understood. The presence of small plastic particles at the micro- and nanoscales is of growing concern, but nanoplastic has not yet been observed in natural samples. In this study, we examined four size fractions (meso-, large micro-, small micro-, and nanoplastics and in Mediterranean waters (Llorca et al., 2021)including the monomers characterisation by the Kendrick Mass Defect and confirmation and quantification when standards were available. In parallel, the identification of main additives in NPL/MPLs composition, as well organic contaminants adsorbed onto the plastic particles were carried out by analysis of the extracts by LC(C18. Accurately measuring the concentration of NPs is a major challenge, as precise analytical methods are required, accounting for various types of NPs polymers, as well as for a diverse set of complex matrices (soil, sediments, turbid waters and tissues. In the present study, the model fish *Carassius auratus* (goldfish) was exposed for 30 days to polystyrene (PS)-NPs, attempting to mimic an environmentally realistic scenario. The main hypothesis of this study was that fish can take up PS-NPs from the water after a chronic exposure and accumulate them in internal organs, causing alterations in fish health.

Material and methods

Adult fish (11.16 ± 3.23 cm length and 7.07 ± 0.64 g weight) were randomly distributed in the experimental aquariums, and two experimental conditions were considered: 1) Control group (0 µg/L PS-NPs) and 2) the group exposed to PS-NPs (100 µg/L PS-NPs). Fish were exposed to PS-NPs for 30 days, and sampled after that period of time. Erythrocytic nuclear abnormalities (ENAs) were classified into four categories according to Pacheco and Santos (1996).

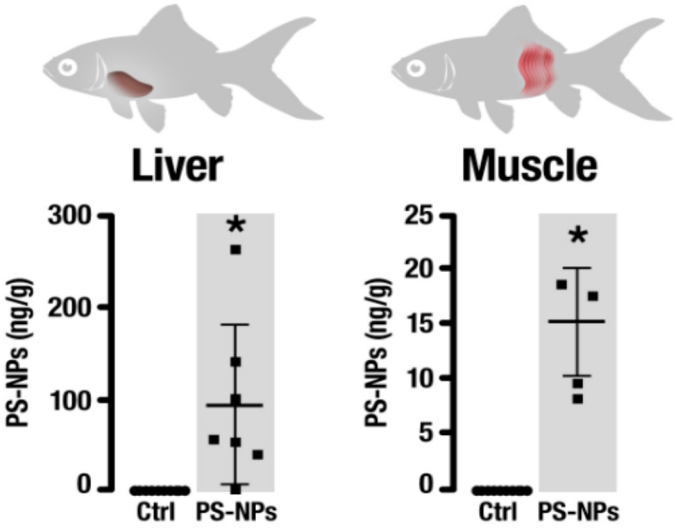

Figure 1. Nanoplastics concentrations in fish liver and muscle.

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Results

PS-NPs were not detected in the gastrointestinal tract of exposed fish. In contrast, PS-NPs were detected in all liver and muscle samples of the exposed fish (Figure 1). In liver, 7 out 9 samples had concentrations levels ranging from 2.23 to 265.89 ng/g liver wet weight; in the other two liver samples PS-NPs were below the limit of detection. In 6 out of 9 muscle samples, PS-NPs were detected at concentrations ranging from 9.58 to 18.64 ng/g of muscle wet weight. The concentrations of PS-NPs were significantly higher in the liver and muscle of fish exposed to PS-NPs for 30 days when compared to the control group. A significant increase in the total number of ENAs, a genotoxicity indicator, was found in the group of fish exposed to PS-NPs, when compared to control. The number of erythrocytes presenting reniform nuclei and micronuclei was significantly higher in fish exposed to PS-NPs than in control fish. However, lobed, and segmented nuclei values were similar in both groups of fish.

Conclusions

The data from this study suggest for the first time that: 1) fish chronically exposed to 44 nm PS-NPs internalize these emergent contaminants from the water. PS-NPs are then distributed in the organism, potentially through blood, and bioaccumulate in internal organs, such as the liver and the muscle. 2) Higher levels of PS-NPs were found in the liver when compared to the muscle. No bioaccumulation was found in the gastrointestinal tract which agrees with the main function of this organ, i.e., absorption. Considering that fish consumption is increasing steadily worldwide, the fact that PS-NPs are bioaccumulated in fish muscle, the edible part of the animal, means that these contaminants may be ingested by humans, presenting a potential threat to human health. 3) PS-NPs induced genotoxicity in fish blood cells, which can escalate to mutagenicity and is related to more serious conditions such as cancer or degenerative conditions.

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IS MORE REALLY MORE? - ASSESSING THE CHEMICAL BOUNDARIES OF AQUAPONICS TO OPTIMISE NUTRIENT SUPPLY VIA AQUAFEEDS

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Introduction

Deficiencies of plant nutrients in aquaponics are usually thought to be caused by nutritional imbalances of aquafeeds, being the main nutrient input into the system, even though studies aiming at optimising feed formulation had only limited success (Seawright et al., 1998). Meanwhile, chemical boundaries that limit the solubility of nutrients in water have not been closer investigated until today, even though known to be essential and assessed a long time ago for nutrient management in hydroponics (Sambo et al., 2019). This leaves aquaponics practitioners with hands tied as it remains unclear whether optimised feed formulation can actually contribute to an efficient management of plant nutrients in aquaponic systems.

The aim of this study was to test the hypothesis whether chemical boundaries determine nutrient concentrations in aquaponics and if results obtained in an aquaponic system are comparable with chemical equilibrium models.

Material and Methods

Three independent RAS (V_{total} = 4500L) were stocked with juvenile Nile tilapia (IBW = 140.7 g±32.0 g) at a stocking density of 15.4 kg m^{-3}. Fish were divided to three tanks per RAS (V_{tank} = 650 L). Isonitrogenous and isocalorific experimental diets containing graded levels of potassium (K) and phosphorus (P) (see table 1) were administered to the fish stock at an average feeding rate of ≈ 2.5% d^{-1} kg^{-1}BW. Water exchange took place at 5% d^{-1}. Quantities of all inputs (feed, water, chemicals) and outputs (water, solids) were recorded. Water and solid samples for element analysis by ICP-OES were taken bi-weekly. The trial lasted for 84 d.

Chemical equilibrium modelling was done using Visual MINTEQ v3.1. In brief, water analysis data, including pH, temperature and obtained element concentrations, were examined for reaching solubility threshold concentrations of amorphous solids.

Results and Discussion

The evaluation of the results is not yet finished as not all sample analysis results were provided by collaborating laboratories until now. Final analysis of all relevant samples is expected to be finished by end of May.

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Table 1: Composition of experimental diets. Data on dry matter basis.

	Low	Medium	High
Crude protein	44.0%	43.1%	42.0%
Digestible energy	15.1 MJ kg^{-1}	14.2 MJ kg^{-1}	14.5 MJ kg^{-1}
Ash	6.8%	9.5%	11.6%
P	6.9 g kg^{-1}	14.0 g kg^{-1}	18.6 g kg^{-1}
K	10.5 g kg^{-1}	13.1 g kg^{-1}	17.0 g kg^{-1}

SHELLFISHERS/FARMERS LOCAL ECOLOGICAL KNOWLEDGE(LEK) ON LOW TROPHIC AQUACULTURE PROMOTE THE CONSERVATION OF THE CRITICALLY ENDANGERED *PINNA NOBILIS* (PinnaSOS project)

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Introduction

The exploitation of aquatic, living resources, in particular mussels, provides work and income to mussel fishermen and farmers, societal cohesion in fishery-sector dependent communities and low cost, valuable protein-rich intake to consumers. Provided that mussel fishery and aquaculture are sustainably practiced, production, financial and ecosystem services are fully met.

Within the context of the EU and Greece co-funded research project (MIS 5052394) PinnaSOS (www.PinnaSOS.upatras.gr) about enhancement of natural recruitment of fan mussel (*Pinna nobilis*), certain preliminary actions took place. Namely, experimental transplantation, extensive sampling, monitoring of any living specimens and artificial breeding of the critically endangered, according to IUCN, bivalve species were conducted by a conventionally staffed research team along with a vastly experienced group of mollusk fishermen and farmers.

This study shows that the 'participatory" approach used in PinnaSOS, presents a way forward to involve and mobilize local stakeholders, increases the sampling efficiency while in parallel promotes credibility and acceptance by society and funding agencies.

Methods

During the transplantation / translocation phase shell-fishers and mussel farmers made available their vessels and technical expertise. Divers, mostly shell-fishers/farmers, removed the selected specimens and farmers' vessels made the transportation from one place to another. As it was vital that the byssus thread of the fan mussel remain as much as possible intact, shellfish-divers went through this with remarkable success. Though strange it may sound, they were partly "co-writers" of the research protocol, and they had significant contribution to the selection of sites, being aware of local physical and meteorological conditions (depth, waving, windy regime). On the other hand, it was a mussel farmer who received the transplanted specimens next to his production unit by ensuring no anchoring or stealing could occur and therefore, bias the results.

Second, during the sampling and monitoring phase, fishermen gave continuous information about the current population existence due to their daily employment. Fishermen tend to make the optimum, both in terms of time and space, sampling, as they leave no sampleable area, unfished. Moreover, the dissemination of knowledge about biota, substrate and vegetation was imminent. In addition to this, farmers gave information and space about the potential settlement of fan mussels on or near their marine infrustructures (ropes, boats, production units, etc). Mussel farmers took part in the manufacture of spat collectors and their expertise was of great help. Finally, a significant endowment to the project was the provision of aquaculture equipment and the day-to-day monitoring of artificially manipulated specimens in situ, as well as a problem-solving approach due to the "adoption" of the project.

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Results and Discussion

Davis & Wagner (2003) emphasized the need for researchers, when using local experts to gain from LEK, to describe, even briefly the selection criteria. In this case, we ranked our experts based (in descending order) on a) experience, b) willingness to collaborate, c) peer influence and d) (quality and safety of) equipment used. The corresponding criteria for farmers were a) willingness to collaborate, b) size of vessel, c) size of production unit and d) farmed species. Using a wider public to crowdsource data is an emerging technique that still needs elaboration. Choosing who to be your "partner" may substantially affect the project's outcomes and the transparency and repeatability of results (Drescher *et al*, 2013).

Even though, that the above-mentioned project is not purely a conservation action, but more of a project trying to solve or mitigate a problem (the mass extinction of fan mussel), proposing possible etiological causes of the incurred disease and to a lesser extent conceptualizing conservation actions and fishery management plans, the framework (participatory process) argued by Buchs et al, 2021) was partly applied. Nonscientific knowledge has the potential to improve understanding of ecosystems and may act complementary with science (Hernandez et al, 2014; Theodorou *et al*, 2022).

Conclusions

To conclude, nowadays, it seems that issues of research staffing is not only how to build a multi-disciplined team with inter-institutional attributes, committed to work for and achieve the common goal. It is also to engage local experts who can decisively contribute with ecological knowledge and technical expertise.

Acknowledgements

This research is part of the project "Innovative Actions For The Monitoring-Recovering–Enhancement of The Natural Recruitment of The Endangered Species (Fan mussel) *Pinna nobilis*", which was funded by the Operational Program for Fisheries and Maritime 2014–2020 (MIS 5052394). This action was co-funded by the European Maritime and Fisheries Fund (EMFF).

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INVASIVE ASCIDIANS, SPONGES & RAPANA WHELKS MANAGEMENT IN CE MEDITEARRANEAN MUSSEL FARMING, GREECE

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Introduction

The aim of the present study is to provide, in the CE Mediterranean, information on species' biofoulings in the Greek mussel farms, and to describe the best management strategies that will reduce the costs of the end-product while controlling biofoulings' populations.

The information provided by the professionals consists of one of the most direct approaches to activate the links between mussel farmers and scientists to upgrade the quality of the information and data provided, especially in data-poor areas such as those of shellfish farms.

Material and Methods

Local Ecological Knowledge on changes in the composition of species biofoulings in their installations, as well as production fluctuations over time retrieved from the operating management of mussel farmers empirical knowledge. Working protocols for questionnaire/interviews design based on Theodorou et al. (2022, 2023).

Results

The major problem in the Greek shellfish farms was ascidians and to a lesser extent the sponges. The surface size covered by a shellfish farming unit was also significantly and negatively correlated with the time of appearance of invasive ascidians, where ascidians appeared earlier in the larger units. The problem was more intensive in farm units covering an area between 2.0 and 2.5 ha, whereas it seemed moderate in units over 5.0 ha. This might be explained by the fact that the low-sized farms have built-in routine technologies (e.g., regular cleaning, etc.) as mitigation measures for the establishment of biofoulings. Ascidians also exhibited the highest intensity during the last 20 years, whereas gastropods were the invasive species with the highest intensity in the recently established/operated farms (since 2015). In terms of the final product, ascidians exhibited the most significant impact, whereas sponges showed a moderately negative impact, with the effect of the reduced amount of flesh being the most important.

The inherent challenges in shellfish farms, where management interventions are costly and difficult to implement, the rapid invasive parasite recolonization, and the potential for off-target effects pose regulatory barriers to many mitigation measures. Our results showed that the cost of farming management rises for ascidians and sponges, mostly impacted by damages to maintenance and labor, and to a lesser extent to fuel. The studied invasive species affected the operational cost of production at a rate of 21%-50% peaking from July to September, whereas the months previous (June) or following (October) were identified as the second most significant temporal influence on production. The greatest impact of the presence of invasive species was focused on repairs—maintenance, labor, fuel, maintenance, and service during the harvest season. Extra labor during May-June due to the invasive ascidians added extreme threats to the profitability of a shellfish farm with additional economic costs.

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Conclusions

Management interventions can result in significant net economic benefits. Monitoring on mussels' production could provide an empirical quantitative estimate of the economic impact of invasive species impacts. Although there are many variations of integrated invasive organism management, the basic principles are combined risk assessment and risk management activities based on five main pillars: a thorough understanding of invasive organism ecology, bioeconomic cost-benefit relationships, continuous monitoring at the right scales, active prevention, and reactive control. These principles could be applied to shellfish farming, with experts' judgment knowledge playing a critical role in a long-term approach to invasive organism management. When it comes to understanding the ecology of invasive organisms, the effects of both bio-pollution and invasive organisms also tend to vary spatially, temporally, and/or demographically. Thus, site-to-site variation factors, such as species' plasticity to adapt to different environmental conditions, could also help to predict and assess the risk in farmed areas exposed to invasive organisms.

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PHYLOGENETIC RELATIONS OF INVASIVE ASCIDIANS IN GREEK MUSSEL FARMS

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Introduction

Ascidians (Ascidiacea) are marine invertebrate filter feeders with high spatial activity and are ranked among the most important biofoulants (Aldred & Clare 2014). Biofouling (i.e., the dynamic process of attachment, accumulation and development of elements of aquatic flora and fauna on any natural or artificial surface) of ascidians in anthropogenic facilities (e.g., aquaculture), often has detrimental economic and ecological impacts (Tsotsios et al. 2023). Especially in shellfish aquaculture (e.g., in mussel farming), the effects of these invasive species are greater than in other forms of mariculture, as the farmed organism itself acts as a substrate for the biofoulants to settle, creating operational problems in production (Tsotsios et al. 2023). Within Greek marine areas, 75 species of ascidians have been recorded (Antoniadou et al. 2016). Most of them originated from Atlantic, whereas a gradual increase in records of invasive species from the Indian Ocean has been observed (Antoniadou et al. 2016).

Farmers consider ascidian biofouling a major etiological agent of detrimental effects for the viability of Greek shellfish aquaculture (Tsotsios et al. 2023). However, whilst ascidians' genetic structure elucidation is crucial towards biofoulants' proper management, such knowledge is not available for Greek marine areas. The aim of this study is to provide the first insights regarding the identification and phylogeny of biofouling ascidian species from Greek mussel culture facilities, using sequence analysis of both mitochondrial and nuclear DNA barcode markers.

Material and Methods

A total of 77 ascidian specimens were collected from four aquaculture mussel farms located in the Aegean and Ionian Seas. Genomic DNA was extracted using the Nucleospin® Tissue Kit (Macherey-Nagel) according to the manufacturer's protocol. Cytochrome oxidase subunit I (COI) and 18S rDNA gene fragments were amplified using primer sets LCO1490 and HCO2198 (Folmer et al. 1994) and 18S1 and 18S4 (Tsagkogeorga et al. 2009), respectively. Polymerase Chain Reactions (PCRs) contained 1X Kapa Taq buffer, 1.5 – 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM for COI and 0.4 μM for 18S rDNA of each primer, 1 U Kapa Taq (Kapa Biosystems) and ca. 20 ng of DNA template, in a total volume of 20 μl. PCR cycling conditions consisted of an initial denaturation step at 94 °C for 3 min (COI) or 4 min (18S), followed by 37 cycles of 30 sec (COI) or 40 sec (18S) at 94 °C, 1 min at 48 °C (COI) or 40 sec at 50 °C (18S), and 1 min at 72 °C, with a final elongation step at 72 °C for 10 min. Amplified products were purified using the commercially available NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel) and sequenced on an AB3500 genetic analyzer (Applied Biosystems).

Blast algorithm was employed for species identification of the studied individuals. Manual editing of newly acquired sequences was performed using SeqMan II software (DNASTAR). Clustal Omega was used for the multiple sequence alignment of the COI and 18S rDNA datasets. Individual data sets of COI and 18S rDNA were finally concatenated for a total evidence analysis. For phylogenetic reconstruction of the combined dataset, Bayesian Inference analysis was performed using MrBayes (v. 3.1.2) while the best-fit model according to the Akaike Information Criterion (AIC) was determined by jModelTest software (v. 0.1.1).

Results &Discussion

All individuals were successfully identified at the species level, using both COI and 18S rDNA gene fragments, (Table 1).

Following manual editing and multiple alignment the final dataset comprised 1472 nucleotides (590 for COI and 882 for 18S rDNA). Bayesian Inference (BI) analysis, based on AIC TPM1uf+G model, resulted in a dendrogram with four distinct and well supported clades (1.00), corresponding to the species level (Fig. 1). The species orders formed three robust monophyletic clades following the phylogenetic relationships within the subphylum of Tunicates (Delsuc et al. 2018). Stolidobranchia included the Styelidae (*Styela plicata*) and Pyuridae (*Microcosmus squamiger*) families, Aplousobranchia the Clavelinidae (*Clavelina oblonga*) and Phlebobranchia the Ascidiidae (*Phallusia mammillata*) families (Fig. 1).

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Phylogenetics and population genetic diversity of invasive ascidians have been sporadically evaluated in eastern Mediterranean based on various molecular markers (e.g., Maltagliati et al. 2014). The present study is the first attempt to genetically identify and elucidate the phylogeny of biofouling ascidians invading Greek mussel farming facilities. Investigation of the spread and genetic composition of non-indigenous-cryptogenic ascidians and practices to deal with infestation in mussel farms is necessary to assess the magnitude of the problem in production activities.

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SHELLFISHERS/FARMERS LOCAL ECOLOGICAL KNOWLEDGE(LEK) ON LOW TROPHIC AQUACULTURE PROMOTE THE CONSERVATION OF THE CRITICALLY ENDANGERED *Pinna nobilis* (PinnaSOS project)

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Introduction

The exploitation of aquatic, living resources, in particular mussels, provides work and income to mussel fishermen and farmers, societal cohesion in fishery-sector dependent communities and low cost, valuable protein-rich intake to consumers. Provided that mussel fishery and aquaculture are sustainably practiced, production, financial and ecosystem services are fully met.

Within the context of the EU and Greece co-funded research project (MIS 5052394) PinnaSOS (www.PinnaSOS.upatras.gr) about enhancement of natural recruitment of fan mussel (*Pinna nobilis*), certain preliminary actions took place. Namely, experimental transplantation, extensive sampling, monitoring of any living specimens and artificial breeding of the critically endangered, according to IUCN, bivalve species were conducted by a conventionally staffed research team along with a vastly experienced group of mollusk fishermen and farmers.

This study shows that the 'participatory" approach used in PinnaSOS, presents a way forward to involve and mobilize local stakeholders, increases the sampling efficiency while in parallel promotes credibility and acceptance by society and funding agencies.

Methods

During the transplantation / translocation phase shell-fishers and mussel farmers made available their vessels and technical expertise. Divers, mostly shell-fishers/farmers, removed the selected specimens and farmers' vessels made the transportation from one place to another. As it was vital that the byssus thread of the fan mussel remain as much as possible intact, shellfish-divers went through this with remarkable success. Though strange it may sound, they were partly "co-writers" of the research protocol, and they had significant contribution to the selection of sites, being aware of local physical and meteorological conditions (depth, waving, windy regime). On the other hand, it was a mussel farmer who received the transplanted specimens next to his production unit by ensuring no anchoring or stealing could occur and therefore, bias the results.

Second, during the sampling and monitoring phase, fishermen gave continuous information about the current population existence due to their daily employment. Fishermen tend to make the optimum, both in terms of time and space, sampling, as they leave no sampleable area, unfished. Moreover, the dissemination of knowledge about biota, substrate and vegetation was imminent. In addition to this, farmers gave information and space about the potential settlement of fan mussels on or near their marine infrustructures (ropes, boats, production units, etc). Mussel farmers took part in the manufacture of spat collectors and their expertise was of great help. Finally, a significant endowment to the project was the provision of aquaculture equipment and the day-to-day monitoring of artificially manipulated specimens in situ, as well as a problem-solving approach due to the "adoption" of the project.

(Continued on next page)

Results and Discussion

Davis & Wagner (2003) emphasized the need for researchers, when using local experts to gain from LEK, to describe, even briefly the selection criteria. In this case, we ranked our experts based (in descending order) on a) experience, b) willingness to collaborate, c) peer influence and d) (quality and safety of) equipment used. The corresponding criteria for farmers were a) willingness to collaborate, b) size of vessel, c) size of production unit and d) farmed species. Using a wider public to crowdsource data is an emerging technique that still needs elaboration. Choosing who to be your "partner" may substantially affect the project's outcomes and the transparency and repeatability of results (Drescher *et al*, 2013).

Even though, that the above-mentioned project is not purely a conservation action, but more of a project trying to solve or mitigate a problem (the mass extinction of fan mussel), proposing possible etiological causes of the incurred disease and to a lesser extent conceptualizing conservation actions and fishery management plans, the framework (participatory process) argued by Buchs et al, 2021) was partly applied. Nonscientific knowledge has the potential to improve understanding of ecosystems and may act complementary with science (Hernandez et al, 2014; Theodorou *et al*, 2022).

Conclusions

To conclude, nowadays, it seems that issues of research staffing is not only how to build a multi-disciplined team with inter-institutional attributes, committed to work for and achieve the common goal. It is also to engage local experts who can decisively contribute with ecological knowledge and technical expertise.

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GENOME WIDE ASSOCIATION STUDIES OF PRODUCTION TRAITS IN RAINBOW TROUT USING WHOLE GENOME SEQUENCING

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Introduction

Rainbow trout is one of the most commonly farmed salmonid species across the world and the most farmed fish species in France. Trout are reared in France for the production of large fillets which are consumed fresh or smoked. Thus, the main objectives of genetic selection are to improve growth and fillet's yield. To assess growth and fillet's quality various traits are measured, such as body weight, carcass and fillet yields or fat content. Though some QTLs have been found in previous experiments (Blay et al., 2021), we still lack precise information about genes and biologicals mechanisms linked to those traits. Our aim was to estimate genetic parameters of production traits in a commercial line of rainbow trout and to detect and accurately localize associated QTLs.

Materials and methods

The stock was established from 3 generations of a commercial selected line of Les Sources de l'Avance breeding company (Aqualande Group, France) whose breeding program started 12 generations ago. The stock was reared under commercial conditions until harvesting. From the 9th, the 10th and the 12th generations, 2198, 1410 and 824 fish were sampled between 503 and 539 days post fecundation. Fish were measured for fork length (FL, cm), body weight (BW, g), carcass yield (CY), head gutted carcass weight (HGCW, g), head gutted carcass yield (HGCY), viscera weight (VW, g), gut yield (GY), fat content in the muscle (FAT, %) recorded using a Fish Torry Fat-meter®.

Fin samples of 3390 phenotyped fish were genotyped for 57,501 SNPs using the Axiom® Trout Genotyping array by the INRAE genotyping platform Gentyane (Clermont-Ferrand, France). All the parents of phenotyped fish were also genotyped with the 57K array (184, 183 and 80 parents of the 9th generation,10th and 12th generations).

After SNPs quality control including filtering out SNPs with minor allele frequency (MAF) < 1%, and SNP call rate < 97%; 31,968 SNPs were retained for the analysis.

The 99 sires of the phenotyped fish in 10th generation were also sequenced with the NovaSeq6000® paired-end technology (Illumina 2×150bp) at INRAE sequencing platform Get-PlaGe (Toulouse, France). We used the nfcore/sarek 2.7.1 pipeline to call variants. We retained 10,520,443 bi-allelic SNPs with MAF > 1%. The imputation of the 32K genotypes of phenotyped fish into 10,520K genotypes was performed using FIMPUTE3 software using pedigree information. After imputation we kept 1,231,034 SNPs with a MAF above 10%, a mendelian error rate below 3% and linkage disequilibrium r^2 between SNPs < 0.9 in sliding 100kb-windows.

Genetic parameters were estimated using BLUPF90 software (Misztal et al., 2014). GWAS was performed for the 8 traits of interest with a Bayesian Sparse Linear Mixed Model (BSLMM) using GEMMA - 0.98.5 software (Zhou et al., 2013).

Results and discussion

Heritability varied from moderate to high values (TABLE 1). The highest heritability was estimated for FAT (0,63 ± 0,03) and the lowest value for FL (0,16 ± 0,02). Production traits were polygenic, the proportion of genetic variance accounted for by the 150 to 500 SNPs with the largest effects ranged from 75 to 95% depending on the trait.

We detected 17 QTLs (TABLE 2) with very strong evidence based on posterior inclusion probability (PIP) of SNPs in BSLMM and associated Bayes Factor (2lnBF > 14). We did not re-detect seven of the ten QTLs found in the previous study based on generations 9th and 10th of the same rainbow trout line (Blay et al., 2021).

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Table 1 Heritability estimates (± standard error) for production traits in rainbow trout

	FL	BW	CY	HGCW	HGCY	VW	GY	FAT
Pedigree based h^2	0.22 (0.03)	0.29 (0.04)	0.59 (0.05)	0.54 (0.05)	0.39 (0.04)	0.61 (0.05)	0.42 (0.05)	0.55 (0.06)
Genomic h^2	0.16 (0.02)	0.21 (0.03)	0.61 (0.03)	0.48 (0.03)	0.36 (0.03)	0.59 (0.03)	0.03 (0.03)	0.63 (0.03)

Table 2 Characteristics of QTLs for production traits in rainbow trout

Trait	Chr	Peak SNP	Position	PIP	2lnBF	QTL Start	QTL End
FL	3	Affx-1237726497	24,399,565	0.21	15.4	24,399,565	24,399,565
FL	22	AQ227110489	7,110,489	0.30	16.3	7,110,489	7,117,869
FL	22	AQ227564448	7,564,448	0.12	14.0	7,547,490	7,567,760
BW	4	AQ0419964635	19,964,635	0.14	14.0	19,964,635	19,964,635
BW	4	AQ0420198141	20,198,141	0.19	14.8	20,197,462	20,198,141
BW	5	AQ0527505234	27,505,234	0.21	15.1	27,505,234	27,505,234
BW	8	AQ0816947480	16,947,480	0.25	15.5	16,947,480	17,177,997
BW	22	AQ227110489	7,110,489	0.55	18.1	6,995,960	7,110,489
HGCW	6	AQ066609263	6,609,263	0.39	14.9	6,547,380	6,638,958
HGCW	20	AQ20439513	439,513	0.70	17.5	439,513	442,488
HGCW	30	AQ306279631	6,279,631	0.34	14.4	6,227,968	6,296,368
HGCY	6	AQ066609263	6,609,263	0.34	14.2	6,608,668	6,609,263
VW	17	AQ1731988589	31,988,589	0.30	14.0	31,988,589	31,992,982
GY	30	AQ308096466	8,096,466	0.40	15.3	8,096,466	8,096,478
CY	18	AQ1827622382	27,622,382	0.32	14.2	27,622,382	27,644,041
CY	8	Affx-88929033	16,330,345	0.25	13.5	16,318,476	16,330,345
FAT	8	Affx-88929033	16,330,345	0.55	16.8	16,330,261	16,330,345

For FL, 3 QTLs were identified, among them the QTL at 7.1 Mb on chr 22 was also detected for BW and entirely located in the *slc39a10* gene. For BW, 4 other QTLs were detected on chr 4, 5 and 8. The QTL on chr 4 (with peak SNP at 20.2 Mb) was located within *rcan2.* The 2 QTLs on chr 8 and 22 were previously identified in the same trout line (Blay et al., 2021). The QTL on chr 8 spanned the region of *map3k7* and *bach2b* genes. For HGCW and HGCY a common QTL was identified on chr 6 in a region where *top3b* and *ppm1f* genes are annotated. All the other QTLs were in intergenic regions.

Conclusion

Thanks to imputation on over 1 million SNPs, we significantly refined the location of 2 QTLs previously identified, as well as we detected 15 new QTLs. These results will be validated in an undergoing study by genotyping the 13th generation of the Aqualande's line to confirm the associations between genotypes and production traits.

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EVALUATING AND SELECTING FISH POLYCULTURES: HAVING THE BIG PICTURE THANKS TO AN INTEGRATIVE ASSESSMENT

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Introduction

Fish polyculture is any production in which at least two fish species are reared in the same rearing system at the same time, with the objective of producing several products with economic value. Such practice is a potentially interesting option for future aquaculture developments by benefiting from the coexistence of taxa and/or interactions among species. Regardless of the systems in which polyculture is applied (i.e. traditionally in ponds or more recently in recirculated aquaculture systems, RAS), it requires compatibility and complementarity occur among the fish species farmed together (Thomas et al., 2021). Thus, fish species to be combined must be able to live in the same production system at the same time, without detrimental interactions (such as predation), benefiting from different resources with little, or, even better, no competition for resources (e.g. trophic, spatial), or developing commensal or mutualistic interactions. These prerequisites for polyculture design were used to identify species to be combined with pikeperch reared in RAS. The aim is to define a strategy to identify the species combinations that best meet the challenges of production and fish welfare for the aquaculture of the future.

Materials and methods

Several polyculture approaches were applied in RAS. Fish combinations were selected on the basis of their compatibility and complementarity. Pikeperch were combined with sterlet, with tench, or with sterlet and tench in a first experimental series and, with common carp, with black-bass, or with common carp and Eurasian perch in a second series. Pikeperch were also reared in monoculture as a reference system. All these experiments were carried out at the Experimental Platform for Aquaculture (UR AFPA, Lorraine University) in France. They were applied in experimental units with a strict control of physico-chemical parameters and a daily fish monitoring. To evaluate polyculture scenarios, several biological traits classified into two categories were measured (Amoussou et al., 2022a): *i)* production data (survival rate, mean final weight, weight heterogeneity, specific growth rate and Fulton Condition Index), and *ii)* welfare data (behavioral traits [agonistic and flight] and physiological traits [cortisol, hematocrit, glucose, serotonin and dopamine]). Multi-trait analyses were then conducted to evaluate the fish polyculture scenarios. Finally, a ranking procedure was also developed to select polyculture scenarios (Amoussou et al., 2022b).

Results

Our experimental works reveal that all studied species were impacted by polyculture, but they are not equally affected. From an aquaculture viewpoint, polyculture could result in beneficial or detrimental impacts on fish production and welfare depending on the species. Our results also demonstrate the value of a multi-traits evaluation based on an integrative analysis to better address the impacts of the polyculture. Differences are measured in the expression of some traits related to fish production or welfare. The application of the ranking procedure makes it possible to consider a variety of stakeholders' expectations, with or without weighting, and thus result in a variable classification of polyculture scenarios.

Discussion and conclusion

Assessment of any polyculture scenarios requires all species to be considered. The objective is to exclude situations in which one species takes advantage of the rearing conditions to the detriment of another. Polyculture only makes sense if all species take advantage of these rearing conditions. A multi-trait assessment is useful for a balanced assessment between production and welfare objectives, with possible adjustment according to the varying and changing expectations of stakeholders.

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PRODUCTION DATA, BIOMASS ESTIMATION AND AI – A COCKTAIL FOR IMPROVED SUSTAINABILITY AND PROFITABILITY IN AQUACULTURE

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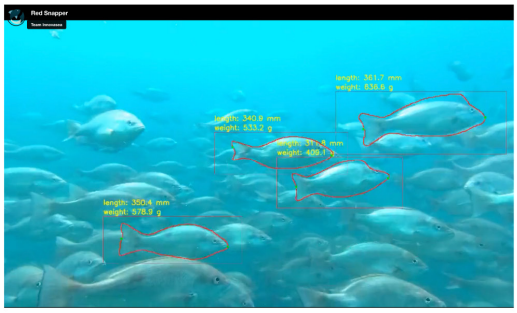
Aquaculture is an increasingly important sector in global food production. However, its growth is threatened by the high cost of feed, which accounts for up to 70% of the production costs. Feed conversion ratio (FCR) is a key indicator of the efficiency of aquaculture production and refers to the amount of feed required to produce a unit of fish biomass. Improving FCR is crucial to increasing profitability, sustainability, and environmental performance. Artificial intelligence (AI) and production data can be leveraged to optimize feed management, while biomass estimation cameras can improve feeding accuracy and reduce wastage.

AI can be used to analyze production data and identify patterns that can inform feed management decisions. Feeding is a complex activity in that feed ingestion by fish depends on many factors, environmental, genetic, husbandry, nutritional, behavioral, etc. By analyzing these variables, AI systems can optimize feeding regimes to improve FCR and fish growth. Advanced farm management software will help feed these AI models and deliver continuous improvement of feeding and growth models specific for each farm.

But a feeding regime can only work when farmers have a good notion of the biomass in each pen. Biomass estimation cameras can improve the accuracy of feeding by estimating fish size and quantity in real time. This data is then used to adjust feed delivery rates and reduce overfeeding, which can lead to poor water quality, disease, and mortality. Innovasea has developed its BiomassPro solution to reliably estimate fish weight distribution in several farmed species, with more than 97% accuracy for most species and up to 99% accuracy in others. In addition to enabling more precise feeding, these cameras also help farmers forecast production yields, optimize harvest schedules, and reduce fish waste.



DATA MANAGEMENT FOR BROODSTOCK FACILITIES AND ORGANIZATIONS DOING RESEARCH IN FISH REPRODUCTION, GENETICS AND OTHER FIELDS

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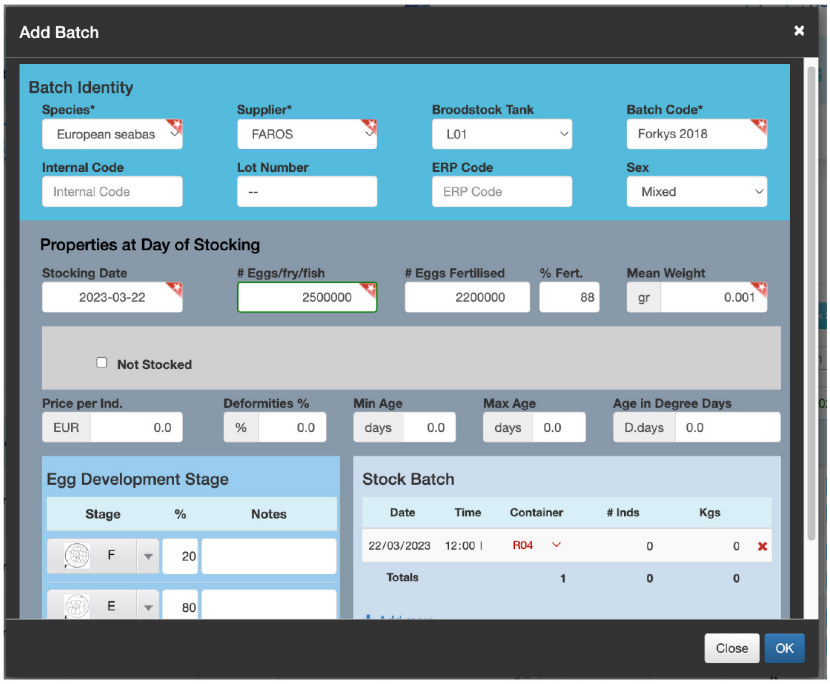
In many marine and freshwater broodstcok facilities, fish are tagged so they can be individually handled according to the plans of facility managers. Similarly, in many public or private research organizations working fish reproduction, genetics, pathology, or nutrition, tagged fish are used and tracked individually. Managing data from these activities using non-standard applications such as Excel or Google spreadsheets is far from ideal. Data is complex, multi-dimensional and difficult to track as fish are moved between tanks or pens.

The Cloud-based software service Aquanetix Broodstock, developed with support from the NewTechAqua project, allows users to record all types of events linked to individual fish, such as samplings for weight or length, treatments, transfers between tanks, and more, as well as events linked to the group of fish in a tank, such as water parameters and feedings. The software keeps full traceability of both, groups of fish and individual fish, allowing researchers and broodstcok and hatchery managers to find the clear cause-effect relationship between treatments and performance. Much of the flexibility in the application is due to the possibility for users to define custom sampling types, that can be complex variables with many dimensions (for example the shape of fish, or others such as microsatellite haplotypes or specific treatments). The user can then record these samplings over time and link them of individual fish.

Data can be recorded using automated systems such as water parameter monitoring sensors, input by hand using the Aquanetix mobile app or uploaded easily from standard applications used by the main PIT tagging hardware companies. Analysis can be done using the reporting and BI platform linked to Aquanetix or easily exported into a number of formats for analysis in specialized applications.

Aquanetix Broodstock not only allows users to collect and track data from individual fish but stores it in a standard format that can be retrieved and analyzed against future research trials. The data belongs to the organization and is not lost when researchers or broodstcok managers move on to new positions.

NewTechAqua is a project funded by the European Commission. This project has received funding from the European Union's Horizon 2020 Programme under grant agreement No 862658.



INNOVATION FIRST! MANAGING OPERATIONAL RISK IN AQUACULTURE TECHNOLOGY DEVELOPMENT

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Introduction

Norway is a leading producer and exporter of Atlantic salmon, and open sea-based cages in the coastal zone has been the leading technology since the 1970s. In recent years, new production systems have been emerging, for both coastal and offshore locations. This may require adjustments in the regulatory framework, which has been described as fragmented.

In daily operations, employees at the fish farm and vessels have a responsibility to handle a range of operational risks to ensure personal safety, fish health and welfare and the environment. Studies find that workers are exposed to several hazards in their work environment, and may experience dilemmas related to for instance personnel safety and production or fish welfare and profits (1,2)health complaints and concerns, sickness absence, subjective health status and job satisfaction. Results The survey data show that physical and ergonomic exposures are the most common, and several workers report psychosocial exposures such as stress and lack of control in their workday. The most frequently reported health complaints were musculoskeletal (neck/shoulder/arm pain, back pain, hand/wrist pain, knee/hip pain.

Reducing risks, as well as utilization of new areas, were prevalent in the call for "development licenses", a temporary license aimed to spark innovation introduced in 2015 (3). A total of 104 applications were submitted by the deadline in 2017. Criteria for being awarded development licenses targeted some risks (e.g., salmon lice, escapes, waste) and not others (e.g., occupational health and safety). Furthermore, concepts had to be unique, which may also introduce unique risk scenarios that must be handled.

It was the Directorate of Fisheries (DF) who were responsible for the call and assessment of applications. Even though it is required to assess all risks in the operation of the farms, the Food Safety authority (FSA) (responsible for fish welfare), Norwegian Labour Inspection authority (NLI) and Norwegian Maritime authority (NMA) (responsible for occupational health and safety) had no formal role in the assessment processes.

While the criteria guided applicants towards tackling certain risks through technological innovation, the fish farmers who operate the farms must consider all operational risks in their risk management to ensure safe operations. The question guiding this study is: How were operational risks managed in selected innovation processes?

Material and methods

The research question is addressed through material from 44 semi-structured interviews with fish farmers and suppliers who have applied for development licenses, as well as interviews with authorities, organizations, and other key stakeholders. Interviews were conducted between January 2021 and January 2022.

Additional materials included publicly available documents such as consultation response, response letters to applicants, media coverage and other documentation from the projects.

Results

Interviews provided knowledge regarding applicants' approach to risk management in the innovation processes, including what type of competence they saw as important at different stages. In addition to the fish farming companies and suppliers, universities, research institutes and consultant agencies also provided advice for several of the projects.

Interviews showed that risk assessments were used to identify operational risks. The call asked for descriptions of how the project would affect fish welfare, and how this could be measured. Several projects were concerned with the welfare of the fish, fish mortality, how the fish would handle the new technology and making sure the personnel could perform operations and maintenance. Here, previous research was seen as an important source. The licenses' purpose was to test the technology, and one informant underlined that the licenses did not give them any chance to do a large-scale scientific study regarding the welfare of the fish. The Food Safety Authority also addressed issues regarding fish welfare stating that consideration of fish welfare and fish health has not been adequately addressed when licenses for production have been developed and awarded, pointing out the animal welfare law requirements of ensuring animal welfare in all technology.

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Occupational health and safety were not mentioned as a criterion in the call for licenses. However, and especially for offshore concepts with harsher weather conditions and increased distance from shore and potentially having to use helicopter transport, personnel safety in operations was addressed in the innovation processes. Concepts with more stable work platforms as less use of vessels in operations, compared to traditional net cages with plastic collars, were seen to reduce risk for workers. While the interaction with regulators varied among applicants, some contacted the NLI and the NMA to discuss concerns regarding risk management related to regulations for work hours. Following the innovations aimed for offshore locations, the government also started working on new regulatory requirements for occupational health and safety applicable for offshore aquaculture, aiming to finalise regulations in 2023.

Interviews show that the applicants applied different strategies to manage operational risks throughout the development licenses innovation process. New technologies can reduce risk, but also introduce new risks. To ensure safe operations and compliance with regulations, all risks must be handled when the farms are in operation. When technological innovation is first on the agenda, it is therefore crucial that the operational risks are identified and managed throughout the innovation processes.

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EMBRYOPHENOMICS AS AN APPROACH TO ASSESSING SENSITIVITY IN EARLY LIFE STAGES

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Phenomics is a 21st century approach to quantifying an organisms' observable characteristics with high dimensionality, however while they are increasingly applied in agriculture and medicine, they are not widely adopted in aquaculture. Here, we present new approaches to the study of developing aquatic embryos, incorporating both open-source bioimaging hardware and analytical software. A key strength of phenomics when applied to developing organisms is the ability to acquire high-dimensional longitudinal data at an individual level. We will present the automated analysis of both commonly used phenotypic measures such as heart rate, but also the ability of 'machine proxy traits' to measure complex organismal physiology as the integration of energy across a series of frequency spectra. These approaches have broad potential applicability to aquaculture, including breeding, sensitivity assessment and the use of embryos as biosensors within culture facilities.

GENOME EDITING FOR GENETIC IMPROVEMENT IN FINFISH AQUACULTURE

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Increasing ability to harness the power of genomics is forcing a rethinking of aquaculture genetic improvement strategies. Successful breeding programs will always be built on the careful selection of the next generation of broodstock, detailed record keeping, and accurate collection of phenotypic data. Genomics allows this base of phenotypic selection to be enhanced, and ultimately accelerated to increase genetic gain per generation. This is currently done in finfish at the most sophisticated level through the use of Genomic Selection. However, another exciting technology is on the horizon that will fundamentally change how we deliver genetic improvement. This technology is Genome Editing.

Genome Editing is a technology that can thought of as "precision breeding". It will be an important tool in the future toolbox for genetic improvement in aquaculture. The current state of the art in Genome Editing in aquaculture is impressive and on the cusp of significant commercial application. The basic concept is that enzymatic tools (such as CRISPR technologies) can be used to create variants in specific DNA sequences that create a desired phenotype (such as sterility, monosex, rapid growth, or disease resistance). The technique does not involve adding new DNA, so is not transgenic and does not create a GMO. It simply involves understanding the genomics and underlying genetic variant that is needed for a trait to be expressed, and harnessing natural processes to create that variant rather than sorting through many thousands of broodstock and many generations to achieve the same effect.

The power of genomic research is that we are beginning to understand the exact genes involved in performance traits, and how variation in those genes leads to improved performance. Harnessing the power of Genome Editing allows us to transfer this knowledge to application in commercial breeding programs for heritable, quantum advances in genetic improvement. Importantly, sterility will be a requirement in most applications of GE in aquaculture as a method of biocontainment to prevent escape to the environment, or the inadvertent application of genetically improved animals.

This presentation will provide background on how genome editing works, an update on regulation, and how this tool may be used to improve aquaculture genetics in the very near future.

THE EFFECT OF COPPER ON APPETITE, GROWTH AND HEALTH IN FARMED ATLANTIC SALMON: THE IMPORTANCE OF NUTRIENT SENSORS AND THE MICROBIOTA-GUT-BRAIN AXIS

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A major challenge in fish farming in cages is the formation of biofouling, i.e organisms and algae growing on the pens. This may compromise water circulation, undermine disease management and cause bacterial diseases to spread in the pens. To prevent biofouling, nets are frequently coated with antimicrobial copper alloys, which results in the release of copper into the surrounding water. Copper released from net coating is considered the largest source of environmental toxicants caused by the fish farming industry and it creates an environment in which the farmed fish within the net pen as well as species in the surrounding waters are subjected to chronic copper exposure. Given that copper exposure has been shown to cause reduced growth and feed conversion rate in numerous fish species, it is of high industrial interest to gain knowledge about copper toxicity as it relates to farmed Atlantic salmon.

Based on previous studies in both mammals and fish, we hypothesize that the reduced growth observed in fish exposed to copper in part is owed to an accumulation of copper in the gastrointestinal tract, which in turn affects the composition of the gut microbiota. The gut microbiota plays an important role in the host organism, partially due to its ability to convert feed ingredients like, starch, chitin and fibers into various forms of short chained fatty acids (SCFAs). The variants of SCFAs generated is determined by the composition of both the food and the gut microbiota. Following their formation, SCFAs bind to nutrient receptors in the intestines, the best characterized of which are «free fatty acid receptor 2» (FFAR») and «free fatty acid receptor 3» (FFAR3). Activation of these receptors contribute to the regulation of peristalsis, immune response, appetite, feed intake and more. The wide repertoire of functions is owed to the many possible combinations of fatty acids, receptors and cell types linked to different signaling pathways, and a shift in any of these variables can potentially have a profound effect on the host organism.

Most of the available knowledge surrounding the symbiotic relationship between the gut microbiota and host organism is derived from mammalian studies. While this relationship has also been demonstrated to be present in fish, available information regarding the processes involved is still limited. To investigate the underlying biological mechanisms in Atlantic salmon, we have analyzed its genome using *in silico* tools and identified several variants (paralog genes) of FFAR2/FFAR3. Early analysis shows substantial similarity between the receptors in salmon and in humans, with the characteristic seven transmembrane alpha helix structure preserved. We are currently working to characterize these receptors with regards to sequence, structure and expression pattern. We will investigate their role in nutrient sensing, how they are affected by copper exposure and how they correlate with growth, feed intake, the composition of the gut microbiota and SCFA, and expression of appetite regulating neuropeptides in the brain.

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ULTRASONOGRAPHY AS A NON-INVASIVE TECHNIQUE FOR MONITORING THE GONADS AND LIVER DEVELOPMENT IN INDIVIDUALS OF EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

Accurate comprehension of reproductive processes is essential for the design of aquaculture strategies aimed at optimizing yield and ensuring sustainability. Despite its pertinence, the estimation of fecundity and the dynamics of energy allocation to reproduction remains challenging, both at the farm and individual levels (Chary et al., 2022).

Bioenergetic models provide a mechanistic framework for elucidating many biological processes. Dynamic Energy Budget theory (DEB), for instance, proposes that an organism assimilates energy, storing it as reserves and subsequently expending it to fuel growth and reproduction. Under this paradigm, a fish's reproductive capacity at any given time depends upon the available energy reserves for this purpose (Kooijman, 2010).

This work is framed in the long-term objective of developing a DEB model capable of mechanistically elucidating principal reproductive processes, thereby facilitating estimations of energy investment in reproduction. The specific objectives of this study encompass 1) developing an ultrasound-based technique for quantifying gonadal and hepatic dimensions, and 2) establishing a statistical model capable of generating precise and accurate estimates of organ weights from ultrasound measurements.

Materials and Methods

European sea bass (*Dicentrarchus labrax*) serves as model species. A sample of 47 mature female individuals was selected for experimentation. These fish were housed at the IRFAP-LIMIA facilities within marine floating mesh cages. At the start of the study, the individuals weighted on average 2,383g ± 524 g. Over the span from November 2021 to May 2022, aligning with the species' spawning season (Pawson et al., 2007), biweekly samplings were conducted on each individual, involving ultrasound measurements of gonadal and hepatic structures. Echographic assessments employed an ultrasonic probe equipped with a linear array, operating at frequencies of 7.5-10 MHz, a focal depth of 25-35 mm, and a signal amplification (gain) of 85%. For gonads, 11 measurements of the left lobe were undertaken, capturing total length, and height and width at five equidistant points along the longitudinal axis. For the liver, three measurements (length, height, and width) were recorded. Before echographic procedures, individuals were anesthetized using phenoxyethanol in a dose of 1,000 ppm.
During the course of the experiment, 35 out of the 47 individuals were sequentially sacrificed to calibrate the method. Ultrasound measurements were consistently taken the day before sacrifice. Post-mortem examinations involved dissection, organ weighing, and identical measurements as those obtained through echography, but done using a calliper.
Organ volume was estimated after approximating organ shape to a simple 3D shape. For gonadal lobules, the volume was approximated using a combination of truncated cones with ellipsoid bases, while for the liver, a triangular prism shape was utilized. The estimated volumes for each organ were subsequently compared with their respective weights (obtained during necropsy) using linear regression.

Results

The regression models for gonadal lobes and liver yielded a p-value below 0.001 for the slopes of their respective regression lines. The correlation coefficients were $R^2 = 0.95$ and $R^2 = 0.48$ for gonadal lobes and liver, respectively.

The proposed model for estimating the weight of left gonadal lobes from ultrasound measurements is formulated as $W = 1.028V - 5.263$, where W (g) is the estimated weight of the gonadal lobe, and V (cm^3) denotes its volume. The proposed model for estimating liver weight from ultrasound measurements is expressed as $W = 0.725V + 12.681$, with the same variables.

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These models were applied to describe the gonadal and hepatic size changes for the 12 fish monitored over the full experiment (nearly 6 months). For these fish we describe clear between-fish differences in the temporal dynamics of the gonad size along the reproductive season.

Discussion

The obtained findings underscore the reliability of ultrasonography for *in vivo* measurements of internal organ dimensions and weight, specifically gonads and liver, within adult *D. labrax* specimens. The handling protocol has been validated as safe for fish welfare, thus allowing long-term, non-invasive monitoring.

Both volumetric reconstruction methods for gonadal lobules and liver demonstrate suitability in predicting organ weights. However, while gonadal lobe volume estimates displayed a robust correlation with the volumetric measurements derived from ultrasonography, liver weight estimates appear comparatively less precise. This discrepancy could be attributed to the more irregular shape of the liver, and its deeper intra-abdominal location.

The established association between ultrasonography-derived volume estimates and actual measurements of organ weight offers a means of precisely estimating organ weights exclusively through ultrasonography, facilitating repetitive measurements throughout the fish's lifespan. The potential of repeatedly estimate organ weights on an individual basis holds promise for characterizing species' reproductive dynamics. The resultant dataset, generated through this methodology, holds potential for improving bioenergetic models, particularly those derived from DEB theory. The overarching aim is to incorporate data of gonadal and liver weights alongside other variables, culminating in a comprehensive DEB model that mechanistically explicates core reproductive processes, which, ultimately, may contribute to improve the sustainability of aquaculture practices.

Acknowledgements

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METABOLIC CHANGES IN IN VITRO MODEL OF MARAENA WHITEFISH *Coregonus maraena* DUE TO TEMPERATURE INCREASE

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Introduction

Global climate change causes increasing water temperatures and decreasing oxygen levels, which leads to changing or destroying aquatic habitats. Additionally, the direct contact of the fish with the changed environment leads to physiological changes in the species, which can be studied using cell culture systems. In vitro models represent an essential tool in aquaculture-related research and a flexible system for investigating the effects of altered biotic and abiotic factors at the cellular level.

The maraena whitefish (*Coregonus maraena*) is an ecologically important species in the region of the Baltic Sea. Due to extensive fishing, habitat fragmentation, and eutrophication, these populations have declined sharply. Today, this vulnerable salmonid species is on the IUCN Red List. The effects of rising water temperatures on the already vulnerable species are not yet been well studied. In studies conducted by our working group, we have succeeded in establishing a cell line (CMA-fin1) derived from maraena whitefish (Grunow et al. 2021, Kaya et al. 2022). The aim of the present work was to use this in vitro model to examine the impact of increasing temperatures on growth and energetic parameters of CMA-fin1 cell line. Additionally, the use of cell lines helps to reduce the number of animals used in research in accordance with the 3Rs principle.

Materials and Methods

The proliferation of CMA-fin1 cells was analysed and compared at two different temperatures, at 20°C (control) and 25°C (high temperature). For this, all cells were cultivated in Leibovitz-15 Medium (L-15, Gibco) with 10% fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin. Growth and vitality were measured after 6 days by trypan blue staining and image-based automated cell counting (EVE™ Plus, NanoEnTek).

To conduct a comparative analysis (20°C vs. 25°C) of morphological cell changes, we visualized actin by Phalloidin-iFluor 488 Reagent staining. Additionally, mRNA abundance of selected temperature and stress-related genes was measured by quantitative reverse transcription PCR (RT-qPCR). To measure parameters related to mitochondrial function and glycolysis, we used the Seahorse XF Cell Mito Stress Test Kit and Glycolysis Stress Test Kit (Seahorse, Agilent Technologies). The tests were performed after 24 h for cell attachment. The detection of lactate dehydrogenase (LDH) is a tool for determining the status of necrosis of cells. The enzyme activity was measured by Cytotoxicity Detection Kit (Roche Applied Science) in the supernatant of the CMA-fin1 cells

USE OF GLYCERIDES OF SHORT CHAIN FATTY ACIDS (BALANGUT®) IN GILTHEAD SEA BREAM (SPARUS AURATA) JUVENILE CULTURE: EFFECTS ON MUCOSAL HEALTH AND DISEASE RESISTANCE

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Introduction

Modifications in diet composition, especially those addressing the reduction of traditional ingredients, may induce side effects on fish performance, feed utilization and health. Particularly, fish gut integrity and functionality has been demonstrated to be directly affected by those replacements, especially in relation to alterations in gut morphology, gut microbiota and impaired gut associated lymphoid tissue (GALT) immunological response, altogether facilitating the appearance of inflammatory processes and disease outbreaks. In this sense, functional ingredients, such as glycerides of short and medium chain fatty acids seem to be an accredited solution to reduce the above-mentioned health-related effects.

Materials and methods

Feeding trial: Four experimental diets were formulated to meet the nutritional requirements of gilthead sea bream. One of the experimental diets was devoid of the functional ingredient (Control diet) while the remaining diets were supplemented with glycerides of short and medium chain fatty acids (BalanGut® AQ P; BASF, Germany) at 0.3% (BG0.3), 0.5% (BG0.5) and 1% (BG1), replacing standard carbohydrates (wheat meal). Diets were formulated in a basis of a 15% fish meal, 5% poultry meal and a 6% fish (46% CP, 16% CL, 21.4 MJ/kg feed). The experimental trial was carried out at the facilities of the Parque Científico-Tecnológico Marino (PCTM) at University of Las Palmas de Gran Canaria (Telde, Canary Island, Spain). Gilthead sea bream juveniles of own production were randomly distributed into fifteen 500 L open flow-through water system tanks at an initial density of 3.7 kg·m^3 (30 fish/tank). Fish average initial weight and length were 62.41 ± 1.39 g and 14.23 ± 0.07 cm, respectively (mean ± SD). Diets were assayed in triplicate and fish were fed to apparent satiation three times a day, six days a week for 8 weeks. Two sets of three tanks were fed with the control diet and used as negative and positive controls for the challenge test. Fish growth performance was estimated after 4 and 8 weeks of feeding, and at the end of the feeding trial, fish were sampled for immune parameters activities determination and gut morphological and mucus production studies.

Challenge test: After 8 weeks of feeding, 25 fish/tank were transported to the Marine Biosecurity (MBS; PCTM-ULPGC) and challenged by intraperitoneal injection against *Vibrio anguillarum* (10^7 cfu/ fish, strain 507) along 10 days. Fish survival was recorded daily and described by Kaplan-Meier curves for each dietary treatment. *V. anguillarum* was confirmed as the causative agent of all the naturally dead fish.

Results

Fish accepted well the experimental diets, which had no effect on fish survival neither condition factor along the feeding trial. After 8 weeks of feeding, dietary supplementation did not significantly affect ($p>0.05$) fish weight gain, SGR and FCR (Fig. 1). However, although not significant ($p>0.05$), it increased SGR in an 8-12% after 4 weeks of supplementation in relation to fish fed control diet. Similarly, functional diets optimized in a 13-17% and in a 5-8% the FCR after 4 and 8 weeks of supplementation (Fig. 1). Graded functional additive supplementation did not affect ($p>0.05$) ACH50, lysozyme, bacteriolytic and bacteriostatic activities compared to fish fed control diet. However, fish fed BG0.3 diet presented a relative increase in ACH50, serum bacteriolytic and serum bacteriostatic activities of a 12%, 27% and 8%, respectively, compared to the control group. At the end of the challenge against *V. anguillarum*, fish fed BG0.5 and BG1 diets for 8 weeks presented higher survival ($p<0.1$) than fish fed the BG0.3 and control diets. (Fig.2). In terms of submucosa width, for anterior gut fish fed BG0.5 presented thinner ($p<0.05$) anterior gut submucosa compared to fish fed the control diet, whereas for posterior gut and rectum intestinal regions all the dietary levels fed reduced ($p<0.05$) gilthead sea bream submucosa thickness. In terms of gut mucus production, fish fed BG0.3 and BG0.5 diets for 8 weeks presented smaller ($p<0.05$) goblet cells than fish fed control diet along the whole intestine.

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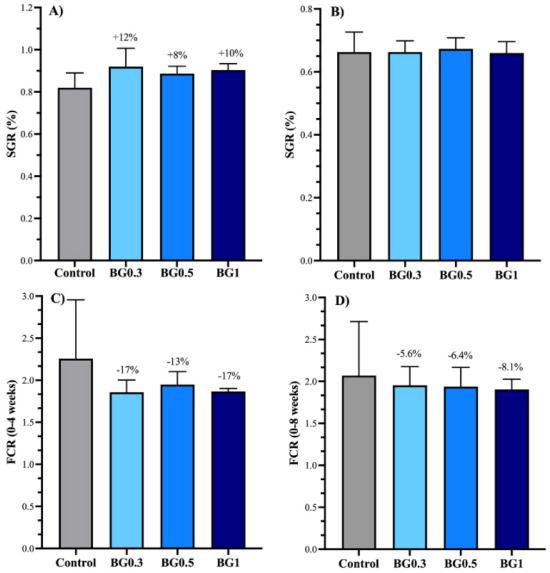

Figure 1. Gilthead sea bream (*Sparus aurata*) growth performance and feed utilization parameters. (A) SGR after 4 weeks of feeding, (B) SGR after 8 weeks of feeding, (C) FCR after 4 weeks of feeding and (D) FCR at the end of the feeding trial. BG0.3: 0.3% BalanGut® AQ P; BG0.5: 0.5% BalanGut® AQ P; BG1: 1% BalanGut® AQ P).

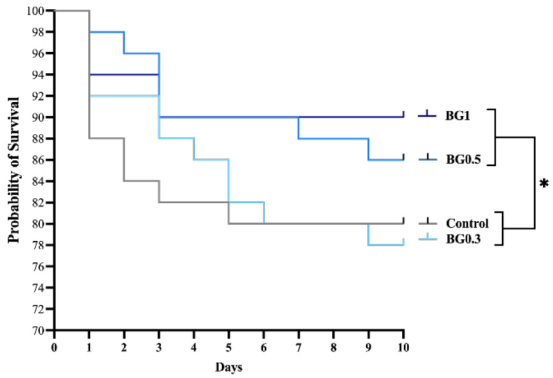

Figure 2. Cumulative survival (%) of gilthead sea bream (*Sparus aurata*) along the challenge test against *V. anguillarum* fed graded levels of glycerides of short and medium chain fatty acids. Symbols denote statistical differences ($p < 0.1$; Kaplan-Meier survival). BG0.3: 0.3% BalanGut® AQ P; BG0.5: 0.5% BalanGut® AQ P; BG1: 1% BalanGut™ AQ P).

Conclusions

The overall results obtained in the present study, profile the use of glycerides of short and medium chain fatty acids (BalanGut® AQ P; BASF, Germany) at 0.5% as a potential functional ingredient to promote gilthead sea bream gut health and disease resistance against *V. anguillarum,* when included in practical diets for this fish species.

COMPARATIVE GENOMICS OF THE OLFACTORY GENE REPERTOIRE IN SENEGALESE SOLE (*Solea senegalensis*) AND OTHER FLATFISH

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Introduction

The evolution of flatfish (order Pleuronectiformes) towards a sea-bottom lifestyle has involved sharp morphological changes, such as the acquisition of a flattened shape ("flatfish") and the characteristic bilateral asymmetry. Pleuronectiformes is a highly diverse polyphyletic group resulting from a quick adaptive radiation (around 700 species), dated back to the Pliocene (~70 MYA), and which has adapted to a wide variety of benthonic environments (Lü et al., 2022). Flatfish have evolved into compact genomes (500-700 Mb) with a low proportion of repetitive DNA elements (< 8%), which has facilitated assembly and annotation, so currently, Pleuronectiformes are among the fish with most confident and wide genomic resources (Robledo et al., 2017).

The particular environmental characteristics of the sea-bottom, like the low light radiation and low temperature, needs from specific adaptations to enhance vision (green light), as well as enhancement of alternative sensory systems, such as the olfactory system, as it has been hypothesized (Figueras et al., 2016). The early development of olfactory organs in fish underscores the essential role of the sense of smell in their interactions with conspecifics and the environment. In order to investigate the evolutionary history of the genes related to olfaction and better understand the evolution of the olfactory system in this group, we performed a comparative genomics study on some relevant flatfish species with chromosome-level assembly and well-annotated genomes pertaining to 5 different families: *Solea senegalensis* (Soleidae), *Hippoglossus hippoglossus*, *Reinhardtius hippoglossoides*, *Verasper variegatus* (Pleuronectidae), *Scophthalmus maximus* (Scophthalmidae), and *Cynoglossus semilaevis* (Cynoglossidae) using two bilateral and pelagic related teleosts as outgroups: *Oryzias latipes* and *Danio rerio*. We aim at identifying contractions or expansions in olfaction-related genes that could be eventually associated to specific evolutionary pressures, thus providing new insights into the role of olfaction in the adaptation these unique fish in their habitats. Additionally, our analyses have focused specifically on the Senegalese sole, an emerging aquaculture species in Europe that still faces challenges in captive reproduction, where chemical communication mediated by the olfactory system might be involved.

Material and Methods

The transcriptomes were downloaded from Ensembl. The longest protein for each gene was selected using a custom Python script. Orthology relationships were determined using OrthoFinder (Emms & Kelly, 2019). Phylogenetic relationships were represented using FigTree v1.4.4.

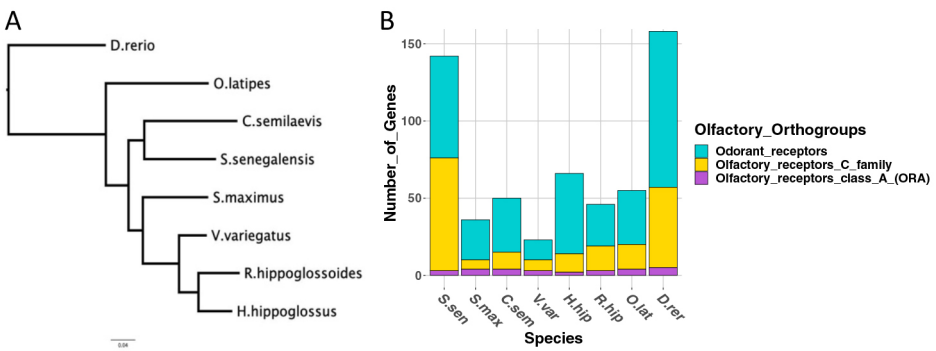

Figure 1: (A)Species tree obtained using Orthofinder, which recapitulates the known teleost phylogeny. (B) Barplot of the number of genes per species of odorant receptors, olfactory receptors class A (ORA) and class C.

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Results

A total of 184,534 genes from the 8 species were grouped into 20,151 orthogroups, of which 7,074 are single-copy orthogroups, and therefore represent single-copy genes across the whole teleost phylogeny. The species tree placed zebrafish and medaka as sister branches out of the Pleuronectiformes cluster, consistent with the teleost phylogeny (Figure 1A). Further, grouping of families in the order was also consistent with previous reports. We did not observe a common evolutionary trend in olfaction-related genes in flatfish, suggesting species-specific adaptations of the olfactory system. Nevertheless, some expansions in genes related with sensory perception were detected. Particularly, the Senegalese sole showed a high number of genes in some orthogroups related to sensory perception through olfaction unlike the remaining species of the order (Figure 1B). Additional analyses are required to further investigate the evolutionary patterns of olfactory genes in flatfish and to better understand the species-specific adaptations of the olfactory system. Furthermore, the role of the olfactory system in reproduction, as an essential aquaculture trait, should be explored.

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CAN DIETARY INCLUSION OF PHOSPHOLIPIDS HELP TO GILTHEAD SEABREAM (*Sparus aurata*) CULTURE DURING LOW TEMPERATURE PERIODS?

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Introduction

Among other abiotic factors, water temperature is key for fish culture. Temperature changes during culture have a notable impact on multiple physiological processes in fish and other animals. Fish reared in ponds may have a more direct effect from water temperature change than fish reared in cages. Nevertheless, the cold temperatures during winter period affect fish performance in both production systems. Total production of gilthead seabream (*Sparus aurata*) in Europe and other Mediterranean countries in 2021 was estimated at 321,912 tonnes (APROMAR 2022 report), a 12.6% higher that reported in 2020. The main production method in Europe is cage culture. There are numerous reports and articles documenting multiple pathological effects of the low culture temperatures on the fish health during winter, i.e. malfunctions of digestive system, metabolism depression and plasma biochemistry changes (Ibarz et al., 2010). In order to counteract these effects, different nutritional strategies could be applied to help maintaining fish health, growth and survival, including the use of certain nutrients such as phospholipids (PLs) (Teodosio et al., 2021). PLs have been successfully used to improve the growth, survival, reduction of skeletal deformities and increase stress resistance in farmed fish (Kokou et al, 2021). Being a structural component of cell membranes, PLs (and cholesterol) are directly related to membrane fluidity. From the different PL molecules, phosphatidylcholine (PC) is the most abundant phospholipid class in the soy and legume products. PC is the type of PL mostly included in fish feed formulations. However, PL from marine origin (algal oils) are nowadays being evaluated as marine dietary PL source. The aim of this study was to investigate the inclusion of marine origin PL and terrestrial origin PL by the incorporation of i) an algae oil (Marine diet), replacing 91% of the fish oil and ii) soy lecithin (Terrestrial diet), without substitution of fish oil, against a Control diet without PL supplementation, in grow out diets for gilthead seabream cultured at low temperatures and close to the commercial size.

Material and Methods

A total of 162 fish with an initial weight of 277.85 ± 3.03 g were cultured at a stocking density of 12,5 km/m^3 (18 fish per 400-liter tanks) and a temperature of 16.0 ± 1.3ºC, simulating winter conditions, in a recirculation aquatic system (RAS) at the facilities of Testing Blue S.L. (Puerto Real, Cádiz). Three formulated diets (Table 1) were evaluated by triplicate tanks. Diets were isoproteic (45% protein) and isolipidic (18% lipid). The fish were hand-fed twice a day, six days per week, until apparent satiation, during a 60 days feeding trial. Growth, daily feed intake, feed conversion ratio and condition index were evaluated. At the end of assay period, plasma and bile samples were taken for biochemical analysis (nutritional metabolites, total bile acids, osmolality). In addition, tissue samples were obtained for the calculation of somatic indices and histological study of the intestinal epithelium (foregut, midgut and hindgut). Transepithelial electrical resistance of the epithelium in the foregut was evaluated by *ex vivo* electrophysiological techniques (Ussing chamber), as well as the possible improvement in the electrogenic transport of amino acids in the midgut of fish fed with the different diets.

Table 1. Lipid fraction ingredients of the experimental diets. Symbol (–) denote the absence of the ingredient.

	Diets		
Ingredients (g/100 g)	**Control**	**Marine**	**Terrestrial**
Fish oil	5.7	0.5	5.7
Colza oil	9	8.9	7.1
Algae oil	-	5.2	-
Soya lecithin	-	-	1.8
Cholesterol	-	0.1	0.1
Phospholipids	0.9	2.0	2.1

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Results and Discussion

No significant differences were found among fish groups for biometric, somatic indices and plasmatic cholesterol, triglycerides and lactate. However, differences were observed in total bile acids (TBA) values in plasma of fish fed with the Marine and Terrestrial diet, but not with respect to the fish fed the Control diet. Regarding bile analysis, no significant differences were found among fish groups for production (µl/g fish), osmolality and TBA values. Bile cholesterol values of fish fed with Marine diet were statistically higher ($p=0.006$) than those showed for fish fed with Terrestrial diet, but not differences were observed with respect to Control group. The histopathological study of the liver did not indicate any alteration or difference between groups. Likewise, no differences were detected in the histological evaluation of the foregut, midgut and hindgut of the fish fed with the different diets. Regarding the electrophysiological results, significant differences were observed in the epithelium intestinal resistance values between Control and Terrestrial group ($p=0.004$). Only fish fed with Marine and Control diets showed electrical resistance values higher (200 and 160 $\Omega.cm^2$, respectively) than usual for healthy gilthead seabream (120 $\Omega.cm^2$) (Fuentes et al., 2006). Therefore, these results suggest that the replacement of fish oil with algae oil could have positive effects on gilthead bream cultured at low temperatures.

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CAN SUPPLEMENTED DIETS REDUCE STRESS IN CULTURED FISH? EFFECTS OF INCLUSION OF A NATURAL ADDITIVE WITH "RELAXING EFFECTS" IN *Seriola dumerili* UNDER RAS CONDITIONS

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Introduction

Aquaculture is a productive area in continuous growth, where the constant search of new potential cultured species is a key factor for its industry development. Thus, inside the genus *Seriola* that include 12 species, the greater amberjack (*Seriola dumerili*) is considered the one of the species with the highest potential for aquaculture (Corriero et al., 2021). However, actually, there are many biological and zootechnical aspect to optimize for its industry expansion. Among this factor, the study of stress in fish has significantly increased in the last years, mainly due to its close connection to animal welfare. Based in the growing researches aimed to the use of dietary additives in fish stress mitigation (Herrera et al., 2019), the purpose of this study was to evaluate the effects of the inclusion of a relaxing additive (RELAQUAX) provided by BEDSON S.A. (Málaga, Spain) in the daily aquafeeds about the growth performance, metabolism and welfare of *S. dumerili* cultured to a medium stocking density, established in a previous trial, and later subjected to a thermal challenge under RAS conditions.

Material and Methods

Greater amberjacks were obtained from natural spawning at the ECOAQUA Institute from University of Las Palmas de Gran Canaria (Canary Islands, Spain) and transferred to CTAQUA facilities (Cádiz, Spain). Then, a total of 540 individuals (~144 g) were distributed in a RAS system with 9 tanks of 400 L (60 fish per tank), which constituted the 3 experimental groups (in triplicate). Fish were fed three daily times, until visual apparent satiety, with i) standard aquafeed without experimental additive (Control diet), ii) standard diet with 1 g of RELAQUAX/kg aquafeed (D1), iii) standard diet with 2 g of RELAQUAX/kg aquafeed (D2) during 69 days under control cultured conditions (22 ºC, O_2 saturation, 12L:12D). After this period, fish were maintained with the same diets for an additional period of 21 days more at 14 ºC water temperature, simulating winter Mediterranean conditions. After both feeding/thermal trials, a biometric sampling was done, and samples from plasma, liver, muscle and water from each experimental tank were taken. Somatic and zootechnical indices were also calculated.

Results and Discussion

For both assays periods, 69d at 22ºC (pre-challenge) and 21d at 14ºC (challenge), no significant differences were observed in the growth parameters, somatic and zootechnical indices of fish regarding the experimental diets ingested. However, similar to results showed in Fernández-Montero et al. (2018), significant differences were observed in Specific Growth Rate (SGR) and Feed Intake between the fish fed with the same diets during pre- and thermal challenge periods (Table 1), with worse SGR values in fish cultured at low water temperature. Even so, an improved Feed Conversion Ratio (FCR) at lower temperatures was more evident in fish fed with the D1 diet during thermal challenge compared with those fish fed D1 during pre-challenge period. These results would be indicative that a dietary inclusion of the experimental additive to 1 g/kg dosses could help to maintain, or even improve, the FCR in fish cultured under low temperature for short time periods.

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Table 1. Effect of supplemented diets and rearing temperature on growth performance and feed utilization of *S. dumerili*. Asterisk (*) within a row denote significant differences ($p < .05$) between fish fed same diets in pre-challenge (69 d to 22 ºC) and challenge (21 d to 14 ºC) periods.

	CTRL		***D1=1g/kg***		***D2=2g/kg***	
	CTRL_22	***CTRL_14***	***D1_22***	***D1_14***	***D2_22***	***D2_14***
Total weight (g/fish)	*265.25±5.15**	*304.9±1.20*	*265.12±10.86**	*303.36±3.63*	*265.53±5.93**	*301.3±7.89*
Δ weight(g/fish/day)	*1.75±0.07*	*1.52±0.44*	*1.75±0.16*	*1.82±0.34*	*1.76±0.08*	*1.70±0.33*
SGR (%/day)	*0.90±0.03**	*0.48±0.09*	*0.89±0.06**	*0.5±0.10*	*0.90±0.03**	*0.47±0.09*
Feed intake (g/fish/day)	*2.55±0.03**	*1.66±0.05*	*2.67±0.12**	*1.64±0.08*	*2.57±0.02**	*1.67±0.08*
FCR	*1.43±0.07*	*1.25±0.19*	*1.51±0.07**	*1.18±0.19*	*1.44±0.06*	*1.29±0.23*
K-Factor	*2.14±0.01*	*2.14±0.01*	*2.12±0.02*	*2.12±0.02*	*2.13±0.09*	*2.13±0.09*

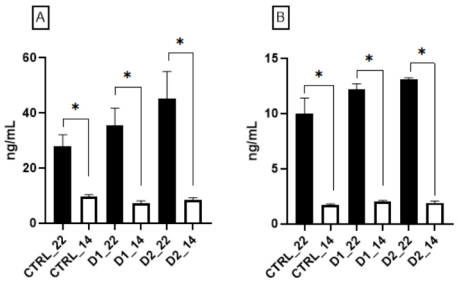

Figure 1. Cortisol levels in plasma (A) and water (B) of *S. dumerili* fed with supplemented diets and submitted to different water temperatures. Asterisk (*) over the box denote significant differences ($p < .05$) between fish fed same diets in pre-challenge (69 d to 22 ºC) and challenge (21 d to 14 ºC) periods.

In terms of somatic indices, the Hepatosomatic Index (HSI) showed significant differences attending only to cultured temperature, with an increase in liver weight in those fish groups subjected to thermal challenge, in line with a liver lipidic accumulation from muscle and perivisceral fat for attending an increased energy demand caused by the adaptation to thermal changes, in agreement with Ibarz et al. (2007). Regarding to intermediary metabolism, the results denoted the absence of negative effects of the dietary inclusion of the experimental relaxing additive in the energy mobilisation which can be invested in growth performance. Attending to temperature effects, a thermal drop caused a metabolism orchestration characterized for a decline in plasmatic levels of triglycerides and glucose, while lactate levels increased in plasma and liver. In contrast, muscle lactate levels decreased concomitantly with increased glycogen levels. This could suggest an increased metabolic recirculation, through the Cori´s cycle, in response to thermal stress produced by low culture temperature. Curiously, cortisol analysis did not show significant differences attending to diet supplementation, existing a direct correlation between circulant cortisol levels in plasma and those detected in the water collected from experimental tanks (Figure 1).

The analysis of the combination of results obtained in the present study seem to indicate that a dietary inclusion of the experimental additive tested have not detrimental effects in growth, health and welfare of fish species with high growth potential, as *S. dumerili*. However, it is considered important to carry out further assays that complement the results presented herein with this and others cultured species.

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EVALUATION OF STRESS MITIGATION USING A CARVACROL AND THYMOL EXTRACT AFTER INTRAPERITONEAL VACCINATION, IN THE SEA BASS (*Dicentrarchus labrax*)

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Introduction

Vaccination is the most powerful and safe prophylactic method to avoid pathologies and outbreaks caused by microorganisms, but the administration of vaccines can generate an episode of stress and a transient lack of welfare (Khansari et al., 2018; Vargas et al., 2018). The aim of this work was, first to determine the welfare conditions of the fish during one of the welfare critical points in the production process, i.e., vaccination, and secondly, to determine the attenuation capacity of di stress, discomfort, or deleterious health effects, through two specific products based on carvacrol and thymol plant extracts, applied in the moments or phases just prior to the vaccine administration (Montero et al., 2003).

Material and methods

Sea bass, *Dicentrarchus labrax* of 9-11g body weight were stocked for 1 month in a RAS facility of CTAQUA (Cadiz) under stable conditions of 22ºC, 3.5% salinity, 12:12 photoperiod and 9 kg/m3 density. After that time fish were treated with 30 ppm of either two products obtained from carvacrol and thymol extracts, or not treated for 10 minutes and then a subgroup of either treatment groups were vaccinated with a commercial vaccine ICHTHYOVAC VR/PD from Hipra (Spain). After vaccination fish were sampled at 1 hour, 24 hours and 21 days and the following samples were obtained: Plasma, mucus, brain, head kidney, liver, skin, gills, intestine, and spleen. The physiological response was measured at plasma (cortisol, glucose, and lactate) and the levels of cortisol were also measured in the skin mucus. The genomic stress response was measured by gene expression of selected stress genes (glucocorticoid receptor, corticotropin releasing hormone, heat shock protein 70) in different tissues, and particularly in the mucosal ones (skin, intestine and gills).

Results and discussion

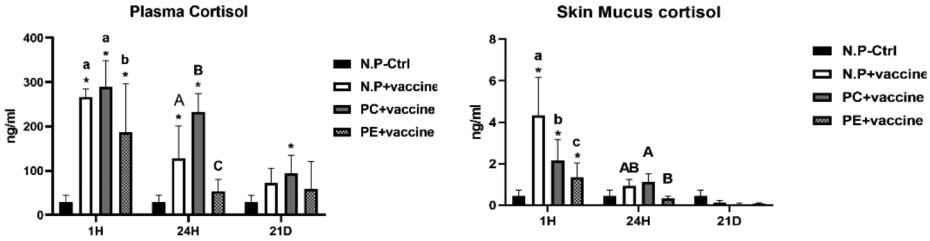

Figure 1: Effect of plant extracts on the expression levels of the stress associated gene gr in two tissues: Skin and intestine compared to the basal expression of controls (horitzontal line)

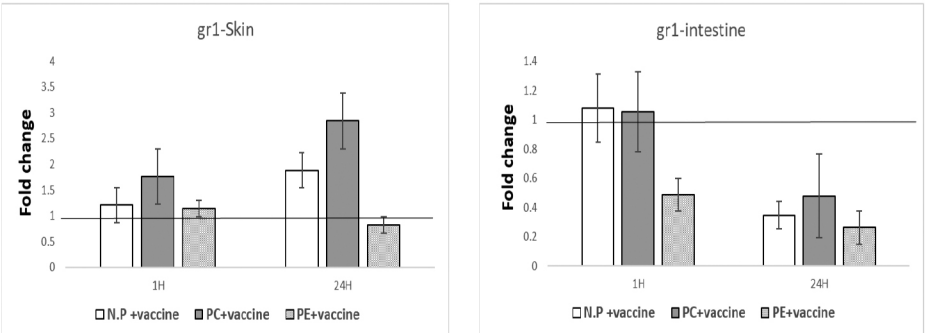

Figure 2: Effect of plant extracts on the expression levels of the stress associated gene gr in two tissues: Skin and intestine compared to the basal expression of controls (horitzontal line)

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One of the products used in this work showed the most effective performance in terms of mitigating the stress effects of the vaccine. Thus, plasma cortisol showed less responsiveness after the E carvacrol-thymol plant extract treatment product. When cortisol was measured peripherally in the skin mucus the results showed the same trend, i.e., low cortisol levels with the same product (Figure 1), and the same for plasma glucose and lactate (results not shown).

In terms of gene expression, the E plant extract product also showed mitigating effects as not only levels were similar to the vaccinated controls, but the levels were even lower than the non-vaccinated controls (see figure 2).

Overall, the results show that carvacrol-thymol plant extracts are able to reduce the stress responsiveness of sea bass to the vaccination response, both at the gene and the hormonal and metabolic level. Further analysis looking at inflammatory cytokines such as Interleukin-1-beta also showed a lower degree of induction of such response (data not shown). As vaccination is one of the critical points of the production process in fish farms, the reduction of such responsiveness by these extracts involves positive effects, as less stress mediators such as stress hormones or proinflammatory cytokines will diminish the risk of imbalances and will help to overcome the critical but unavoidable stress episodes such as vaccination.

Acknowledgements

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BIOCHEMICAL RESPONSE AND GENE EXPRESSION PROFILING OF GILTHEAD SEA BREAM (*Sparus Aurata*) FED WITH *Thymus vulgaris* ESSENTIAL OIL AND ITS EFFICIENCY AGAINST *Sparicotyle chrysophrii* NATURAL INFECTION

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Introduction

Gilthead sea bream (*Sparus aurata*) is a teleost fish found in the Mediterranean Sea. It is economically important and natural stocks are subject to intensive exploitation. However, intensification of its production has been accompanied by an increased occurrence of parasitic diseases (Sitjà-Bobadilla, 2004). *Sparicotyle* (syn. *Microcotyle*) *chrysophrii* is one of the most pathogenic ectoparasites for the seabream aquaculture and research efforts are focused on identification of therapeutic and preventive agents. The use of medicinal plants as aquafeed supplements provide a sustainable way of fish protection in a cost effective way. Several studies have proved that medicinal plants have extensive antimicrobial, immunostimulant, antioxidant, anti-stress, and growth-promoting properties including thymus essential oil extracts. Aim of the present study was the assessment of *Thymus vulgaris* feed supplementation effects on sea bream (*Sparus aurata*) and the fish immune responses in natural *Sparicotyle chrysophrii* infection.

Materials and methods

Two experimental diets containing different amounts of *Thymus vulgaris* (T) essential oil extract (0.25% T and 0.50% T) and a positive control diet were used. The feeding trial was continued over a period of two months. At the end of the feeding trial macroscopic examination of fish gills revealed a natural infection with *Sparicotyle chrysophrii* parasite in 0.50% T tanks. Fish were collected from each tank, their mucus was isolated, weight and length were measured, and blood was drawn by the caudal vein. All gills were examined by microsopy. Head-kidney, spleen, liver and gill tissues were removed aseptically and stored at – 80 °C. All animal handling and sampling procedures were conducted in accordance with Greek and EU laws and regulations.

To assess the *S. chrysophrii* infection effects on sea bream fed with 0.50% T experimental diet, mucus and plasma samples were used for biochemistry and immunological parameters assessment, according to well established protocols. Non-specific immune parameters (i.e. nitric oxide, lysozyme, myeloperoxidase, complement C3, proteases and anti-proteases), antibody responses (total antibodies, immunoglobulin M, anti-microcotyle and anti-*T.maritimum* antibodies), oxidative stress (CYP1A1, metallothionine (MTT)) and metabolism markers (glucose, alkaline phosphatase (ALP) were determined. Total RNA was extracted from fish spleen, head-kidney, liver and gills, and real-time PCR assays were carried out to analyze the expression levels of genes related to antioxidants (SOD1, GPx1), cytokines (Il-10, TGFb1, Il-1b, TNFa), anti-bacterial peptide (Hepcidin) and heat shock protein (GRP75).

Results and Discussion

The 0.50% T diet had no effects on fish weight, length and splenosomatic indexes compared to the control group. The total protein amount, the nitric oxide levels, and C3 in serum was stable, however serum lysozyme was significantly lower as well as mucus total protein and nitric oxide in fish fed with 0.50% T diet. Cytochrome 1A1 was significantly lower in experimental diet but metallothionine, glucose, and alkaline phosphatase were stable. All tested genes expression was unaffected by the experimental diets.

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The *S. chrysophrii* infection had no effects on fish weight, length and splenosomatic indexes on 0.50% T diet group. The total protein amount in serum was stable, however the mucus total protein was significantly lower in the infected fish. Glucose and alkaline phosphatase levels weren't significantly altered by the infection both in serum and mucus. Non-specific immune responses were assessed in fish serum and plasma by measuring the levels of nitrite ions, lysozyme, complement C3, myeloperoxidase, as well as protease and anti-protease activities. No significant differences were found in serum nitric oxide, lysozyme and C3 levels; however, mucus nitric oxide levels were slightly lower in infected group. The myeloperoxidase levels were also not altered by the experimental diets. The remaining parameters (proteases and anti-proteases activities) were not affected by any of the experimental diets. The total antibody levels remained stably low, however the IgM levels appeared to be lower in the infected group. Both cytochrome 1A1 and metallothionine had a decreasing trend without significant differences. Those results indicate a mild systemic immune response of sea bream to *S. chrysophrii* infection.

Gene expression profile was analyzed in four different organs to evaluate the modulation of immune-, oxidative stress- and metabolism-related genes in sea bream infected with *S. chrysophrii*. The studied genes in sea bream spleen were related to cytokines (Il-1b, Il10, TGFb1, and TNFa), oxidative stress (SOD-1 and GPx1) and metabolism (hepcidin and GRP-75). All analyzed genes were differentially expressed more intensively in head-kidney as expected at least for immune genes. Infection seems to suppress expression of all tested genes in all other organs, except IL-10 which remains stable at all tested organs.
The suitability of *T. vulgaris* as efficient food supplement for immune status improvement was investigated and the results indicated that it could be used as dietary additive since it appears to have some potential as a natural immunostimulant. Serum and mucus tested parameters revealed that *S. chrysophrii* didn't influence 0.50% T fed *S. aurata* non-specific immune and oxidative stress profile. Moreover, gene expression levels in spleen, liver and gills seem to be down-regulated by the infection, but slightly up-regulated in head-kidney. Overall, even though the experimental diets considered to be beneficial for fish immune system, natural infection with *S. chrysophrii* revealed poor immune responses in *T. vulgaris* fed sea bream.

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GILTHEAD SEA BREAM SKIN MICROBIOTA SHAPES PROACTIVE AND REACTIVE BEHAVIOUR IN FISH UNDER HIGH STOCKING DENSITIES

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Introduction

The intensification of aquaculture production must deal with inadequate stocking densities that increase the risk of health issues and welfare status due to a variety of stressful conditions such as feed competition, aggressive interactions and reduced O_2 availability. In gilthead sea bream facing changes in water O_2 concentrations, mitochondrial bioenergetics of blood cells are finely adjusted at the transcriptional level (Martos-Sitcha et al., 2017). Likewise, different O_2 status and rearing densities changes induce different tissue-specific expression patterns of genes related to energy metabolism and endocrine growth (liver > muscle > blood) (Martos-Sitcha et al., 2019). All this evidences the fish plasticity to cope changes in the environment, and several components of the *Gh/Igf* system have emerged as hypoxic imprinting genes during critical early life stages (Naya-Català et al., 2021a). There is now a large body of evidence linking changes in mucosal microbiota communities with fish health and welfare, affecting both local and systemic physiological functions (Naya-Català et al., 2021b). However, it remains to be unravelled how changes in mucosal microbiota composition are linked with changes in physiological traits and welfare indicators in a broad sense. Thus, this study aimed to assess how high-stocking densities in concurrence with reduced O_2 availability affect host physiological traits, with focus on skin microbiota and associated shifts in other behavioural and welfare indicators, using a gathered biomarker approach.

Methods

Two-year-old gilthead seabream (450-500 g) were pit-tagged and distributed in 3,000 L tanks to achieve three different initial rearing densities (low, LD: 6 kg/m^3; medium, MD: 12 kg/m^3; high, HD: 22 kg/m^3). Fish were fed close to satiety with a commercial diet from May to June (8 weeks) under natural photoperiod and temperature conditions. The concentration of dissolved O_2 varied from 6-5 ppm in LD fish to 5-4 ppm and 4-3 ppm in MD and HD fish, respectively. At the end of the trial, 10 fish per group were randomly selected for the continuous and simultaneous recording of swimming activity and respiration rates over 48h, using implanted AEFishBit devices (Calduch-Giner et al., 2023). The same fish were used for assessing external damage using a scoring system from 1 to 5. Also, from the same fish, samples of skin mucus, blood, liver and white skeletal muscle were taken for microbiota, biochemical and transcriptional analyses. For skin microbiota profiling, the 16S rRNA v1-v9 regions were sequenced with the ONT MinION device and processed with an in-house pipeline. Muscle and liver gene expression was assessed by qPCR array layouts designed for the simultaneously gene expression profiling of two tissue-specific panels of 44 genes each.

Results

At the end of the trial, welfare scores of epidermal status varied among the three groups ($P < 0.05$), showing the worst status fish held at HD and gradually improving with the decrease in density. However, correlation analysis indicated that external damage appears associated with an active feeding behaviour. Concerning skin microbiota, discriminant analysis showed that the skin microbiome of the three different groups differed in bacterial abundance, and LEfSe analysis revealed six strong microbial markers for these experimental conditions, being the abundance of *Alteromonas* and *Massilia* largely increased in the HD group, though a closer look evidenced an opposite trend for these two discriminant taxa. At the transcriptional levels, five genes related to growth (*igf1, igf2*), lipid metabolism (*cyp7a1*), and oxidative metabolism (*cs, cox1*) were positively correlated with a higher abundance of *Alteromonas* within the HD group. Otherwise, seven differentially regulated muscle genes related to growth (*ghr1, ghr2, igf2*), antioxidant defence (*grp170, grp75*), and energy metabolism (*sirt1, hif1α*) were positively correlated with a higher abundance of *Massilia*. This integrative behavioural, metagenomic and transcriptional approach supported stressful mediated responses that serve to alert and adapt the system in different ways. Hence, a higher abundance of *Massilia* was connected to a proactive behaviour with increased skin damage and locally regulated growth, while *Alteromonas* abundance and low cortisol levels appeared related to a reactive behaviour and systemic growth regulation via the liver *Gh/Igf* system.

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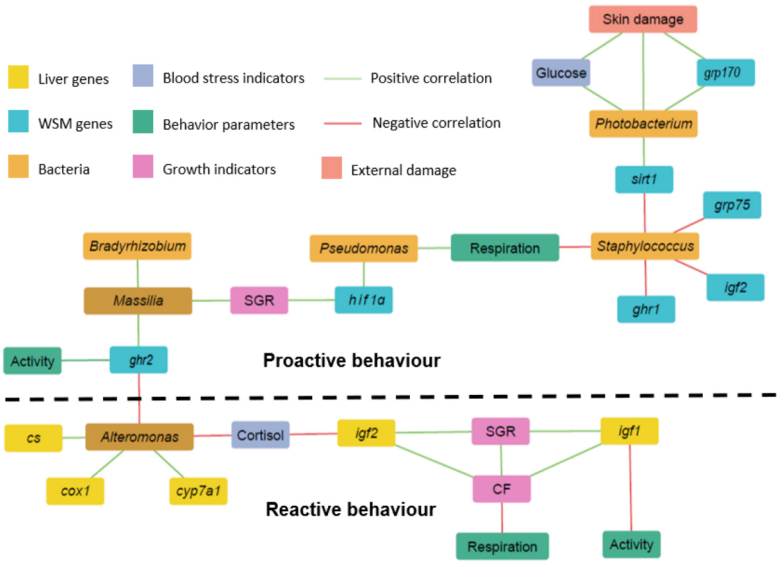

Figure 1: HD correlation network showing significant positive (green lines) and negative (red lines) correlations (Spearman, $P < 0.05$) of discriminant skin microbiota (light orange, reduced abundance in HD; dark orange, increased abundance in HD) with biomarkers of external damage (red), growth (pink), behaviour (green), blood stress indicators (purple), and liver (yellow) and muscle (blue) differentially regulated genes.

Concluding remarks

High-stocking densities had a significant impact on behavioural and physiological traits, that correlated with significant changes in the associated skin microbial population. This gathered biomarker approach served to infer and regulate new operational biomarkers for increased stress resilience in a context of global warming and intensive rearing conditions to cover the increased demand of sea food products.

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NOVEL TREATMENT FROM NATURAL HERBAL SOURCES FOR FISH LICE (*Caligus* spp.) PARASITIC ON RABBITFISH (SIGANUS GUTTATUS) CULTURED IN VIETNAM

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The research was conducted to identify the infection level of fish-lice (*Caligus* spp) on Rabbitfish (*Siganus guttatus*) which cultures in Thua Thien Hue province, Vietnam, and to clarify the effect of extraction from Saudau (*Azadirachta indica*) plant on the fish lice. The total of rabbit fish that were used for experiments is 120 fish, and fish were collected in 6 months at two different sites. The extraction of the Saudau plant was collected from leaf, and seed and then concentrated for experiments at ppm (0; 10; 50; 100; 500; and 1000) to evaluate the effect of extraction on fish lice. The results showed that the infection rate of fish lice on rabbit fish is 62,5% and infection intensity averaged at 19,2 lice/fish. Based on the collection data, it was clearly shown that in January, February, and March, fish lice were found much more than in other months ($p<00.05$). After fish lice were treated with the extraction from leaf and seed for 30 hours, we found that the half effective concentration (EC_{50}), and 90% effective concentration (EC_{90}) of leaf extraction are 148ppm and 928ppm, respectively. The EC_{50} and EC_{90} of seed extraction are 62ppm and 397ppm, respectively. The results from our research frankly show the potential of using extraction from Saudau leaf and seed to treat the parasite disease caused by fish lice on Rabbitfish.

UNRAVELLING THE ROLE OF MITOCHONDRIAL DNA METHYLATION IN TEMPERATURE-DEPENDANT GROWTH OF NILE TILAPIA (*Oreochromis niloticus*) THROUGH NANOPORE SEQUENCING

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Introduction

Thermal plasticity allows organisms to adapt to temperature changes through physiological, developmental, and behavioral responses. This adaptability is crucial for species survival, especially in the face of global warming. In fish, temperature fluctuations in early developmental stages can alter metabolic rate, induce stress, and potentially cause long-lasting developmental anomalies, all of which impact their overall growth, feeding performance, and energy allocation. Mitochondria as cell organelles play a crucial role in generating ATP, which is vital for cellular growth, and its structure and function are affected by temperature (Chung and Schulte, 2020). DNA methylation, is an epigenetic mechanism that modifies gene expression and aids organisms in adapting to temperature fluctuations, impacting growth (Campos et al., 2013). Hence, we hypothesize that fluctuations in temperature may influence the mitoepigenome, subsequently impacting growth. Our prior studies on Nile tilapia (*Oreochromis niloticus*) mtDNA based on bisulfite sequencing identified cytosine methylation (5mC) in a non-CpG context (Nedoluzhko et al., 2021). However, methods like Illumina short reads and bisulfite sequencing are limited to analyzing methylation in 5mC context. Nanopore sequencing offers the advantage of directly detecting additional DNA modifications such as 5hmC and 6mA without the need of chemical or enzymatic treatments. Hence, the aim of the present study was to investigate how thermal plasticity is linked to mitoepigenome changes and growth using Nile tilapia as model, and utilizing nanopore sequencing as the methodological approach.

Materials and methods

Nile tilapia embryos were incubated at three temperatures (24 °C, 28 °C, 32 °C) in triplicate groups. At the opercular stage they were transferred to a common temperature of 28 °C and fed with a commercial diet. After 45 days post fertilization, nine males from each temperature group were randomly selected and fast muscle was carefully dissected. Genomic DNA was isolated and used for construction of PCR-free DNA libraries using SQK-NBD114.24 library preparation kit (ONT, UK). The libraries were sequenced on the Nanopore MinION Mk1C with adaptive sampling. The resultant pod5 files for each sample were used for basecalling and mapping in dorado (v0.3.4, ONT) for 5mC, 5hmC and 6mA. The bam files were used for methylation calling and analysis with modkit (v0.1.13, ONT) and methylKit (v1.26.0; Akalin et al., 2012), respectively. The experimental set-up and workflow are shown in Fig. 1.

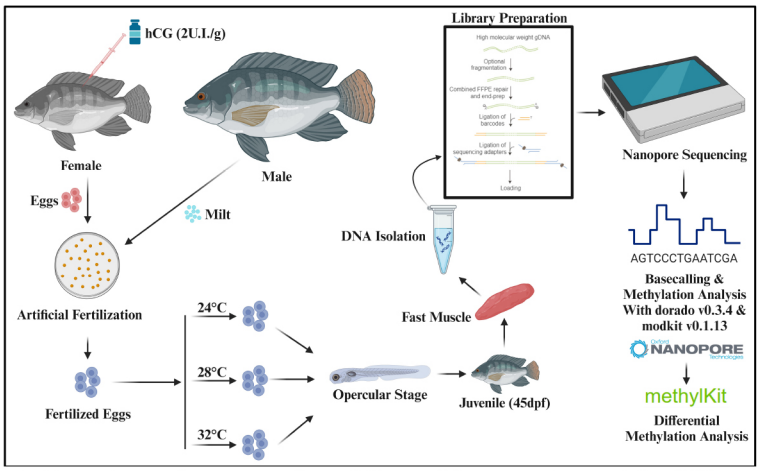

Fig. 1. Experimental set-up and workflow of the experiment. The differential methylation ($p<0.05$) was analyzed with methylKit v1.26.0.

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Results

The average reads per sample, percent of reads mapped to mtDNA and coverage were 349715, 83% and 419×, respectively. The mitogenome exhibited minimal methylation, and notably, the methylation occurred in non-CpG context. In 5mC context, methylation levels in the mitochondrial H-strand increased from 24 °C (1.2%) to 28 °C (1.9%), then slightly decreased at 32 °C (1.8%). The L-strand showed a rise in 5mC levels from 24 °C (1.1%), 28 °C (1.5%) to 32 °C (1.9%). Regarding the 5hmC context, methylation on the H-strand peaked at 28 °C (5.3%), while the L-strand had its highest at 24 °C (2.6%). In the 6mA context, the H-strand's methylation decreased from 24 °C (6.8%) to 32 °C (4.9%), whereas the L-strand steadily decreased from 24 °C (1.2%) to 32 °C (0.9%). In both 5mC and 5hmC contexts, the L-strand exhibited higher methylation than the H-strand across all groups. However, in 6mA context, the H-strand had more methylation than the L-strand. Fish mitogenomes at 24 °C were hypermethylated in all contexts (5mC, 5hmC, 6mA) compared to those at 28 °C and 32 °C.

Conclusion

In conclusion, the results indicate that the mtDNA methylation patterns in Nile tilapia was influenced by embryonic incubation temperature. While the L-strand consistently showed higher methylation levels for both 5mC and 5hmC, the H-strand dominated for 6mA. Notably, Nile tilapia mitogenomes were hypermethylated at 24 °C across all examined DNA modifications as compared to the ones at 28 °C and 32 °C. This highlights the intricate relationship between temperature and mtDNA methylation in fast muscle of Nile tilapia. Methylation data generated from nanopore long-read sequencing will constitute a valuable resource for future research on the mitoepigenome related to muscle growth, metabolism and thermal plasticity in farmed fishes.

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ASSESSING BAY-SCALE EFFECTS OF AQUACULTURE OPERATIONS ON THE DISTRIBUTION OF PELAGIC FISHES AND PREDATORS

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The aquaculture industry has grown rapidly in Canada during the last three decades, and represents a significant source of employment and revenue for coastal communities. However, this development has altered both the physical and biological attributes of aquatic ecosystems at multiple scales and could displace vulnerable species that utilize these areas as critical habitats. As the aquaculture industry is expected to expand further, there is an increasing need to better understand bay-scale impacts of aquaculture on ecosystem functions and services before aquaculture sites expand into new areas. The objectives of this project are to assess long-term and bay-scale impacts of aquaculture operations on the distribution and abundance of pelagic fishes and other large predators in coastal marine ecosystems. Four complementary approaches are used to determine their distribution and abundance of pelagic fishes and large predators in three separate Bay Management Areas (BMAs) in Southwest New Brunswick and a reference area: 1) stationary hydroacoustic devices, 2) acoustic receivers, 3) water environmental DNA, and 4) high resolution thermal imaging drones. A summary of the key results of the project will be presented and discussed.

MODELLING THE DISPERSION OF THE INFECTIOUS SALMON ANEMIA VIRUS (ISAV) FROM ATLANTIC SALMON FARMS IN THE QUODDY REGIONS OF NEW BRUNSWICK, CANADA, AND MAINE, USA

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Outbreaks of disease caused by the infectious salmon anemia virus (ISAV) are an important threat to Atlantic salmon aquaculture production. Pathogen dispersal from an infected farm into the surrounding ocean poses further risks of infection to wild fish and other farms but is difficult to predict. This study aimed to build a framework using ocean circulation and particle tracking models in conjunction with a dynamic infection model and virus inactivation model to simulate the waterborne dispersal of ISAV from Atlantic salmon farms in the Quoddy Region (QR, New Brunswick, Canada and Maine, USA). Using a particle tracking method , simulated particles were released from hypothetically infected farms at rates estimated by a dynamic infection model, and were then advected by modelled currents. Inactivation of viral cohorts by ambient ultraviolet (UV) radiation and natural microbial communities was simulated during advection. Simulations were conducted on thirteen farms in the QR area to demonstrate this modelling system. Maps showing hypothetical spatiotemporal changes of viral concentrations in ambient water were produced for farms under simulated worst-case scenario outbreaks. The advection distances of infectious particles were calculated. A farm connectivity matrix in terms of viral infection was produced, with mutual and asymmetrical connectivity patterns identified between farms. Factors that impact the simulation of viral shedding and inactivation, hydrodynamic effects on dispersal, model application to aquaculture management, and future development were discussed. This framework provides an approach and opportunities to simulate waterborne viral transmission by considering the biology and epidemic features of significance for pathogens and dynamic ocean conditions.

GAIN VALUE BY LOSING DATA: ENHANCING DATA QUALITY THROUGH DATA REDUCTION - AN ILLUSTRATION USING A BLURRINESS FACTOR FOR DEPTH ESTIMATION IN A FISH BIOMASS MODEL

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Introduction

Accurate biomass estimation serves as a cornerstone for effective fish farm management, enabling optimized feeding strategies, health assessment, disease control, and resource allocation. By precisely quantifying the total mass and average weight of fish within a farming system, farmers can make informed decisions that promote efficient production and minimize environmental impacts. Given the labour intensiveness of counting, weighing, and sorting fish manually computer vision solutions are on the rise. By applying artificial intelligence algorithms, this technology empowers fish farmers with real-time, data-driven insights into their fish batches.

While hardware costs and computing power have been reduced in many applications, they are still the limiting factor in biomass estimation due to the three-dimensional information necessary. Elaborate methods such as 3D-image synthesis, stereo-vision cameras or special sonar systems are technologically high-end solutions best suited for intensive industrial farms. For a wide-spread application of AI-based biomass estimation in the aquaculture industry a more technically and financially feasible solution is needed.

Animal Welfare Assessment and Control System

Urban Blue offers a software solution which enables land-based aquaculture farms to monitor key farm aspects and manage, analyse, and visualize this data to enable a better system operation. The Urban Blue system is a combination of a computer-based platform, hardware sensors and a mobile phone app allowing to assess the system (pumps, tanks, valves) and manage the operational workflow (task, routines, lists).

Together with the Zurich University of Applied Science Urban Blue has launched the Innosuisse innovation project AWACS (Animal Welfare Assessment and Control System for fish farms) in order to develop an automated assessment of fish-based parameters. The goal is to provide the aquaculture industry with a comprehensive solution allowing fish-farms to constantly monitor, automatically assess and visually analyse fish health and welfare. As part of this project a biomass model was develop using computer-vision and regression models to calculate the weight of a fish based on a lateral picture (Fig. 1) (Aftab *et al.* 2023).

Figure 1: Biomass model using computer-vision algorithms to define the fish species and calculate the body weight from a lateral picture.

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Biomass modelling in a 3D-environment

This model was further developed to calculate the body weights from single frames extracted from videos from underwater cameras. In order to maintain the simplicity of the model, which later ensures applicability, the information about the third dimension was calculated from the camera's focal length. By increasing the aperture of the camera, the depth of field decreases, leaving any object in front or behind a given distance blurry (Fig. 2). Object detection enables the computer to find fish in the frame and define their blur value excluding fish that are not within the focus area, leaving only fish with a known horizontal distance from the camera lens (Alphonse & Sriharsha 2021)with increase in concern about public safety and security, human movements or action sequences are highly valued when dealing with suspicious and criminal activities. In order to estimate the position and orientation related to human movements, depth information is needed. This is obtained by fusing data obtained from multiple cameras at different viewpoints. In practice, whenever occlusion occurs in a surveillance environment, there may be a pixel-to-pixel correspondence between two images captured from two cameras and, as a result, depth information may not be accurate. Moreover use of more than one camera exclusively adds burden to the surveillance infrastructure. In this study, we present a mathematical model for acquiring object depth information using single camera by capturing the in focused portion of an object from a single image. When camera is in-focus, with the reference to camera lens center, for a fixed focal length for each aperture setting, the object distance is varied. For each aperture reading, for the corresponding distance, the object distance (or depth.

Figure 2: The camera aperture settings define the blurriness of objects in given distances and allow to select for sharp image areas for features extractions with a known horizontal distance from the camera lens.

Figure 3: AI-based selection of single frames in underwater videos provides images of sharp objects in a known horizontal distance from the camera.

Data reduction increase data quality and ensures simplicity

The concept of detecting and excluding objects with high blur values allows to screen the videos and to select only those frames where a fully visible fish with a low blur value is detected (Fig. 3). By consequence these fish are in a given known distance from the camera, allowing to apply the previously develop biomass model where length and height of the fish are used to estimate their body weight.

The reduction of data from a fully day long video with 30 f/sec to a subsample of several hundred high-quality single frames with fish suitable for the biomass model considerably increases the quality of the biomass estimation and drastically reduces the computing power and digital storage necessary. Further, the concept reduces the costs and handling of the hardware.

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EFFECTS OF PLANT-BASED DIETS SUPPLEMENTED WITH DIFFERENT LEVELS OF ZINC ON GROWTH, BONE HEALTH AND ANTIOXIDANT STATUS OF GILTHEAD SEABREAM JUVENILES (*Sparus aurata*)

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Introduction

Zinc (Zn) is a vital micromineral crucial for various biological functions and metabolic processes in fish (Lall and Kaushik, 2021). Zn is an integral component of copper-zinc superoxide dismutase (CuZnSOD), an antioxidant enzyme that defends against free radicals. Zn has a significant role in the bone metabolism and maintaining the normal bone homeostasis in fish (Nguyen et al., 2008). Additionally, Zn is a cofactor for alkaline phosphatase, an enzyme involved in this process for promoting mineralization (Yamaguchi 2010). The use of plant-based ingredients in fish feed to reduce fish meal has become common in aquaculture, but it can lead to Zn deficiencies due to lower Zn content in plant-based ingredients (NRC, 2011). Additionally, the presence of anti-nutritional factors in plant-based ingredients can reduced the absorption of minerals in fish (Kumar et al., 2012). This may cause Zn deficiency to fish, leading to reduced growth, short body dwarfism, cataracts, oxidative stress, poor mineralization, an increased incidence of skeletal anomalies, and increased mortality in fish (Izquierdo et al., 2017; Lall and Kaushik, 2021). On the other hand, excessive Zn can cause toxicity to fish, reducing the capacity of antioxidant defenses, increasing oxidative damage, and competing with other minerals such as Fe, Mg, Mn, Ca, P (Huang et al., 2018). The present study aims to assess the impact of different Zn levels in plant-based diets on growth, bone health, and antioxidant status in gilthead seabream juveniles.

Materials and methods

Six plant-based experimental diets containing low levels of fish meal (FM, 10%), and fish oil (FO, 6%), were formulated with six different levels of Zn. A diet without supplied Zn was used as control (contained: 46 mg Zn/kg). Other five diets were supplemented with Zn sulfate heptahydrate ($ZnSO_4.7H_2O$), containing 50, 56, 63, 71, and 89 mg Zn/kg. A total of 378 initial fish (body weight: 72.67 ± 0.08 g, total length: 16.37 ± 0.02 cm) virtually selected without anomalies, were randomly distributed into 18 tanks (170 L/tank/21 fish). Fish were manually fed with approximately 2% of body weight in three times per day until apparent satiation, the experiment period was 101 days. Fish body weight and total length were measured every 2 weeks. At the end of trial, fish were collected for determining growth performance, liver TBARS values, vertebral column mineral analysis, skeletal anomalies and gene expression.

Results and Discussion

After 101 days of feeding, gilthead seabream juveniles fed diets with different levels of Zn (46, 50, 56, 63, 71, 89 mg/kg) presented no statistically significant ($P>0.05$) differences for growth performances and no mortalities were recorded (Table 1). While, dietary Zn level for increase growth defer vary with fish species and developmental stages, such as early stage of gilthead seabream require higher dietary Zn levels for optimal growth (Tseng et al., submitted) Liver TBARS value showed significantly lowest ($P<0.05$) in fish fed with non-supplemented diet (46 mg Zn/kg) and increased in fish fed with the elevation of dietary Zn levels (Figure 1), indicating an increased risk of lipid peroxidation. Whereas vertebral mineral composition (Zn, Se, Mn, Cu, Fe, Ca, P and Ca/P ratio) was not significantly ($P>0.05$) affected in all treatments. In fact, vertebral Zn content was maintained at plateau levels between 44.33 to 47.67 mg Zn/kg. Except the cobalt content was significantly highest in fish fed with dietary Zn level at 46 mg/kg, and reduced as the dietary Zn levels increased. In term of bone health, fish fed with non-supplemented diet (46 mg Zn/kg) showed the lowest incidence of total skeletal anomalies although no significantly differences was determined among the groups. Interestingly, fish fed with non-supplemented diet (46 mg Zn/kg) showed a significantly ($P<0.05$) highest *opn* expression of vertebrae which was reduced with increasing dietary Zn levels. A similar tendency was also found in other bone-related gene including *bmp2*, *on*, *alp* expression. Similar findings were observed in gilthead seabream larvae fed with optimum dietary Zn levels (Tseng et al., Submitted)

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Table 1. Growth performance of gilthead seabream juveniles fed with different dietary Zn levels

	Diets						QR	
	Zn 46	Zn 50	Zn 56	Zn 63	Zn 71	Zn 89	R2	P value
FBW (g)	143.66 ± 4.75	143.90 ± 3.56	141.36 ± 6.06	140.01 ± 5.22	139.75 ± 5.16	148.68 ± 6.68	0.32	0.07
WG (%)	97.34 ± 6.90	98.05 ± 4.54	94.35 ± 7.84	93.20 ± 7.24	92.38 ± 6.92	104.82 ± 10.12	0.31	0.08
FTL (cm)	20.27 ± 0.21	20.35 ± 0.15	20.18 ± 0.20	20.05 ± 0.15	20.19 ± 0.24	20.48 ± 0.24	0.31	0.07
SGR (%)	0.67 ± 0.03	0.68 ± 0.02	0.66 ± 0.04	0.65 ± 0.04	0.65 ± 0.04	0.71 ± 0.05	0.28	0.10

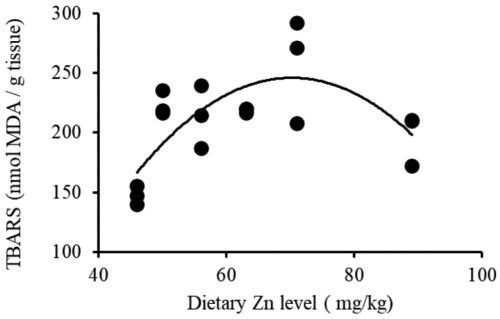

Figure 1. Liver TBARS value of gilthead seabream juveniles fed with different dietary Zn levels for 101 days. The lines represent quadratic regression analysis of the TBARS on different dietary Zn level (x-axis), the statistical significance was set as $P < 0.05$. Quadratic regression (QR): $y = -0.13x2 + 17.67x - 374.08$, $R2 = 0.46$, $P=0.01$.

Conclusion

Gilthead seabream juveniles growth and survival was not affected by dietary Zn levels from 46 to 89 mg/kg. Fish fed with a non-supplemented diet (46 mg Zn/kg) showed reduced lipid peroxidation, increased vertebral cobalt content, up-regulated bone biomarker and antioxidant gene expression, alongside fewer skeletal anomalies. Conversely, those fed with Zn-supplemented diets led to increased lipid peroxidation, reduced vertebral cobalt content, lower bone biomarker and antioxidant gene expression, and more skeletal anomalies. Vertebral Zn content maintained at plateau level (44.33 to 47.67 mg Zn/kg) and along with the results, suggesting a Zn dietary level at 46 mg/kg would be sufficient to cover the requirements in seabream juveniles when fed with practical plant-based diets.

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DETECTION OF GENETIC LOCI ASSOCIATED WITH BODY WEIGHT AND PHENOTYPIC TRAITS IN MEAGRE *Argyrosomus regius*

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Introduction

Selective breeding is commonly accepted to be the foremost approach to drastically amplify the aquaculture production and the quality of aquaculture products. Meagre (*Argyrosomus regius*) is a fast-growing sciaenid species which recently presents a rising interest to the Mediterranean aquaculture diversification and industry sustainability. However, the study of genetic parameters on economically important traits in meagre is nowadays limited and the aim of the current work is to identify genetic markers associated with body weight and phenotypic traits at different growth timepoints.

Materials and Methods

We used juveniles originating from a hormone-induced spawning event of thirteen broodstock, reared together to 297 Days Post Hatching (DPH) until grading was performed and small- and big-sized fish were transferred to different cages. At 394 DPH, 600 fish from each cage were randomly selected, individually tagged with a Passive Integrated Transporter, fin-clipped and transferred into a single cage. Weight was measured on all the surviving offspring at 394 DPH (BW1), 770 DPH (BW2) and 978 DPH (BW3). Additionally, length was measured on all surviving offspring at 770 and 978 DPH (Len2 and Len3, respectively), and last deformities were recorded.

Fin clips sampled from all broodstock and offspring fish were used for DNA extraction and library preparation for double-digest random amplified DNA sequencing (ddRAD- seq) using enzymes *Sbf*I and *Nla*III (Nousias *et al*. 2022). Four libraries were prepared and sequenced on Illumina NovaSeq PE150 aiming at 400-500 G raw data per library. Genomic information was filtered using plink (SNP call rate>80% MAF>1%, HWE<10^{-6}). After quality control, 4650 SNPs remained and used to perform the parentage assignment using the Apparent software (Melo & Hale, 2019) in R. Then, heritability of each body weight using the restricted estimation of maximum likelihood method (REML) was performed and a univariate animal model was used in AIREMLF90, which is illustrated by the formula,

$$Y = \mu + Zu + e$$

where Y corresponds to the vector of the phenotype, μ is the mean, Z is the incidence matrix, e is the residual, u is the additive genetic effect using the Genomic Relationship Matrix (GRM) and it is described as $\sim N(0, G\sigma_a^2)$(G is the GRM and σ_a^2 is the additive variance).

Furthermore, a Genome Wide Association analysis was performed for the body weight using GEMMA, which is illustrated by the following linear model,

$$Y = W\gamma + Z\upsilon + e$$

Where additionally, γ is the vector of SNPs, W is the regression coefficient of the SNP effect, and Z is the corresponding design matrix. The thresholds of significance of the p-values after Bonferroni correction were 4.69 for the 0.05 and 4.66 for the 0.1. The proportion of phenotypic variance (PVE) explained by the statistically significant SNP was calculated as described in Shim et al. (2015).

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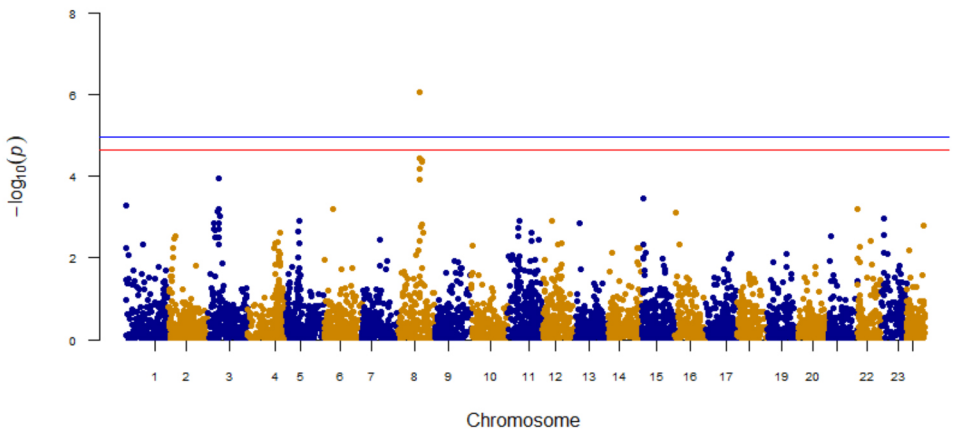

Figure 1. Manhattan plot for the BW1. The blue and red lines illustrate the threshold for 0.05 and 0.1 after bonferroni correction, respectively.

Results & Discussion

Based on a strict parentage assignment, 93% of the fish were identified belonging to 23 families. The number of offspring ranged from 1 to 251 per family while per parent was 2 to 376. The average number of markers per chromosome was 194 SNPs (range from 121 to 242).

A high genomic heritability (0.53 (0.06)) of the body weight was estimated only for the early stage (BW1). Additionally, a QTL was detected in chromosome 8 (Papadogiannis et al., 2022) which explains approximately 2.01% of the phenotypic variation. Even though the QTL has a minor effect, it is notable that there is an increasing trailing of the p-value of SNPs in this chromosome when only 214 SNPs appeared.

Interestingly, GWAS for body-weight and length at later stages did not show significant association with any marker. Last, GWAS for the pughead deformity, a rare skeletal anomaly, detected a QTL in chromosome 13.

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ASSESSING THE IMPACT OF PLANT BIOACTIVE COMPOUNDS ON THE GROWTH OF SEABREAM AND SEABASS JUVENILES

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Introduction

Marine fish farming holds great significance in Mediterranean aquaculture, and it is primarily carried out in open seas, subject to varying environmental conditions and the risks of infection by parasites and pathogens. To thrive under these challenging circumstances, the ultimate objectives are to ensure robustness and expedited growth. In the pursuit of these objectives, the use of bioactive compounds with immunostimulant and growth-enhancing properties is gaining recognition (1,2). However, identifying such compounds necessitates novel screening approaches to evaluate their effectiveness in achieving robust and rapidly growing marine fish populations, in the face of environmental oscillations and disease risks. In this study, the impact of bioactive compounds obtained from olive (*Olea europea*) and *Spirulina platensis* (*Arthrospira)* processing by-products on the growth hormone (GH) signaling pathway and myogenesis in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) juveniles was studied.

Materials and Methods

Fifteen experimental feeds were formulated with the addition of varying concentration of each bioactive compound in a standard feed formulation and were screened *in vitro* for the presence of antinutritional factors and the levels of dietary protein autohydrolysis and digestibility in each fish species. Based on the results *in vitro*, two feed per species were selected for a feeding trial. For each species three groups of fish in duplicate were fed with control feed (commercial) and two experimental feeds, Diet 1 (D1) and Diet 2 (D2). At the beginning and at the end of the trial, tissues (white muscle, liver) were collected from eight individuals per condition per species and immediately stored in RNAlater. Following that, RNA was extracted from tissues and cDNA synthesis was carried out. For the growth hormone signaling pathway, the expression levels of three genes were investigated in liver. Two of those genes were growth hormone receptors (*ghrI* and *ghrII*) which bind GH, and their levels are analogues to the ones of GH, while the third gene was the insulin like growth factor 1 (*igf1*), which is one of the outcomes of GH signaling to the liver and is involved in mediating growth and development. For myogenesis the expression levels of two genes *mylpfa* and *mylpfb* were determined in white muscle. The genes *mylpfa* and *mylpfb* are coding for white muscle myosin light chain 2 and are markers for different types of muscle growth (3). The gene *mylpfb* is a marker for hyperplasia, which is the recruitment of new muscle fibers, and it is the dominant isoform in early development of the fish, whereas *mylpfa* gene is a marker for hypertrophy, i.e the growth of already existing muscle fibers, the dominant form of muscle growth after fish development. Primers were designed for the genes of interest as well as two reference genes (per tissue - per species) and gene expression was measured using real-time PCR. A Wilcoxon signed-rank test was used to determine whether there were significant changes in the expression levels between the condition. At the p0.05 level, differences were considered significant.

Results and Discussion

No significant differences were observed in fish growth of D1 and D2 groups compared with the control group as indicated by fish weight and length in both species. A trend for heavier and more elongated fish was observed in seabream that were fed on the experimental feeds. On the contrary, a drop in weight and length of seabass fed with D2 was observed. Experimental feeds did not impact the GH signaling pathway in seabass. In seabream, *ghrII* expression was up regulated in both experimental diets compared with the control, whereas *igf1* and *ghrI* expression was not affected by diet. Myogenesis molecular markers, *mylpfa* and *mylpfb* genes were downregulated in both D1 and D2 groups in seabass. In seabream, while no differences were identified in the expression levels of *mylphb,* up regulation of *mylpha* gene was observed in both experimental feeds compared with the control, with the differences being statistically significant.

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In conclusion, the bioactive compounds assessed in this study had no impact on seabass growth, yet they had a negative effect in myogenesis regulation. Then again in seabream both experimental diets appeared to elicit a response of the GH growth signaling pathway and they clearly affected myogenesis by promoting hypertrophic growth of white muscle. Furthermore, it is possible that clear differences in seabream growth (weight, length) were to be observed in the fish with the experimental diets, if the experiment had been prolonged. The study provided evidence of the possible beneficial properties of bioactive compounds of olive and spirulina in seabream growth, and underlined the differences in nutrient utilization and metabolism between seabream and seabass.

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THE EFFECT OF DIGESTED OLIGOSACCHARIDES AS FOOD ADDITIVE ON THE GROWTH OF SEABREAM AND SEABASS JUVENILES

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Introduction

The sustainability of marine fish farming in open seas is challenged by varying environmental conditions and disease risks. Fish robustness and accelerated growth are paramount and bioactive compounds with growth-enhancing properties are gaining increasing attention (1). This study investigates the effects of digested oligosaccharides derived from Spirulina's processing on the growth hormone (GH) signaling pathway and myogenesis in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) juveniles. Origin and properties of oligosaccharides are known for their prebiotic like properties promoting growth, by improving intestinal morphology, increasing gut absorptive area, microvilli density and height, and villi structure complexity in sea bream (2). An effect was also seen in the antioxidant capacity of sea bass, indicating a positive effect on the reduction of hepatic reactive oxygen species production (3).

Materials and Methods

An experimental diet was formulated with the addition of 0.05% of digested oligosaccharides in the formulation of the control diet. The two diets were compared based on their *in vitro* properties; their digestibility of dietary protein was determined *in vitro* by pH-stat titration according to the method developed by Dimes & Haard (4). Protein digestibility of each feed was measured in the presence of a standard quantity of crude extract of digestive enzymes prepared by the pyloric caeca of either gilthead sea bream or European sea bass. Autohydrolysis rates of each feed were measured with crude enzyme extracts being replaced by distilled water. Enzymatic hydrolysis was calculated by subtracting autohydrolysis from protein digestibility. The presence of antinutrient factors was assessed by measuring Kunitz Trypsin Inhibitor (KTI) against trypsin activity and Bowman-Birk Inhibitor (BBI) against chymotrypsin activity (5). Two groups of European seabass and gilthead seabream were fed either control or experimental feed containing digested oligosaccharides. Tissues (white muscle and liver) were collected from eight individuals per condition per species at the beginning and end of the trial, stored in RNAlater and used for RNA extraction and cDNA synthesis. The expression levels of three genes (*ghrI*, *ghrII*, and *igf1*) in the liver, related to the growth hormone signaling pathway, were investigated. Additionally, the expression levels of *mylpfa* (hypertrophy) and *mylpfb* (hyperplasia) genes, associated with myogenesis in white muscle, were analyzed. Real-time PCR was used with two reference genes per tissue per species. The Wilcoxon signed-rank test was used to identify significant changes in gene expression levels between conditions, with significance set at $p < 0.05$.

Results and Discussion

The *in vitro* digestibility assay revealed that, autohydrolysis, enzymatic hydrolysis and total dietary protein digestibility were higher in the experimental diet compared with the control. In seabream, KTI content did not differ between the two diets, whereas BBI content was nearly double in the experimental compared with the control diet. In seabass both antinutritional factors were identified higher in the experimental feed. No significant differences were observed in fish growth between the experimental and control feed, as indicated by fish weight and length in both species. A higher hepatosomatic index (HSI) was observed in the fish fed on the experimental feed in the case of seabass. No response of the GH signaling pathway to the experimental diet was observed in seabream. On the contrary, all examined markers (*igf1*, *ghrI*, *ghrII*) were significantly upregulated in seabass fed on the experimental diet compared with the control. No effect on myogenesis molecular markers was observed in seabream. In seabass, the expression levels of *mylpha* gene were significantly lower in the experimental diet compared with the control. In conclusion, the bioactive compound assessed in this study, had no impact on seabream at any level checked. Then again it seems that the active compound had a negative effect on the regulation of myogenesis in seabass. The combination of the GH pathway up regulation with the downregulation of myogenesis and a higher liver mass in seabass fed on the experimental feed, suggests that while the feed was ingested by the fish, there was a need for higher levels of digestive enzymes, probably caused by the elevated antinutritional factors of the active compound. The fact that even higher levels of BBI were tolerated by seabream, underlines the differences in nutrient uptake and metabolism between the two species.

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Acknowledgement
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SUSTAINABILITY ASSESSMENT OF ALTERNATIVE PACKAGING MATERIALS OF FISH

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Introduction

Fish and seafood waste ranges 30-50% and is attributed mainly to spoilage and quality deterioration during post-harvest handling, packaging, storage and transportation. Novel and efficient packaging solutions to improve food quality and shelf life and reduce food waste while not raising the amount of packaging waste is a significant challenge for improving the sustainability of food supply chains (Tsironi and Taoukis, 2018). At the same time, environmental sustainability plays a crucial role for the selection of appropriate packaging materials for food products (Ladakis et al., 2020). According to the EU Directive 2019/904 on the reduction of the impact of certain plastic products on the environment, food packaging materials will contain at least 25% and 30% recycled plastic from 2025 and 2030, respectively. Biodegradable polymers have been developed with the aim to increase the sustainability in the food packaging sector. Biopolymers have been considered promising materials for this purpose. The properties and overall performance of the developed materials depend on formulation and processing parameters. In active packaging, the traditional role of packaging is accompanied by the conservation role of antimicrobials, antioxidants, and other components (Oreopoulou and Tsironi, 2021). Novel processes, such as cold atmospheric plasma (CAP) can modify the barriers and enhance the protective effect of the packaging materials (Pankaj et al., 2014).

The objective of the study was to evaluate the applicability and environmental sustainability of conventional and alternative packaging materials for fish and seafood, considering their recyclability, biodegradability and preservative effect on packed products.

Materials and methods

The tested packaging materials were conventional or recycled polyethylene (PE) and polypropylene (PP), polylactic acid (PLA), cellulose, gelatine, chitosan and alternative combinations of biopolymers. CAP treatment was applied using an atmospheric pressure cold plasma jet (kINPen® IND, neoplas GmbH, Germany) supplied with argon at a flow rate of 4 L/min and a conical probe of 16 cm diameter for uniform plasma application onto the film surface. Gas (O_2) permeability and water vapor transmission rate (WVTR) were evaluated using standardized methods (ASTM F2622, ASTM E96/E96M). Wettability of the packaging materials was characterized based on the determination of the contact angle (Theta Flow Optical Tensiometer, Biolin Scientific). Fresh farmed fish fillets were packed using the different packaging films and stored isothermally at 2°C. Quality evaluation was based on microbial spoilage (enumeration of total viable count and *Pseudomonas* spp.) and sensory evaluation. Computational estimation of environmental impact, according to the Life Cycle Analysis (LCA) methodology using LCA GaBi software, was made.

Results

CAP modified OTR, WVTR and wettability of conventional and alternative polymers with the increase of treatment time. Appropriate processing conditions, resulting in better barriers and higher hydrophobicity, were selected for the materials used for fish packaging. Considering the limit of acceptability of 10^7 cfu/g for total viable count (ICMSF, 1986) the shelf life of fish fillets at 2°C ranged 8-10 days. No significant differences were observed in the shelf-life of fish using the different packaging materials. Based on the results of the study, the maximum environmental benefit would be achieved by replacing conventional polymers with recycled materials and at the same time increasing their recycling rates. The partial or overall replacement of conventional polymers with edible or biodegradable materials proved as an effective to decrease environmental impact of fish packaging.

Discussion and conclusion

The application of novel processing methods and packaging materials offers a great potential for more environmentally friendly packaging systems resulting in lower emissions and sustainable fish and seafood products.

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QUALITY AND SAFETY EVALUATION OF ALTERNATIVE PACKAGING MATERIALS FOR FISH AND SEAFOOD: BIOACTIVITY, TOXICITY AND VIRUS TRANSMISSION POTENTIAL

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Introduction

During the last decade, joint efforts by the packaging and the food industries aimed to reduce the amount of petroleum-based polymers and food packaging waste. Biopolymers have been considered promising materials for this purpose. The properties of the developed materials depend mainly on formulation and processing parameters. Edible packaging systems may also be used as thin layers of materials to coat or wrap food products, aiming to extend their shelf life and reduce the required polymers for food preservation (Tsironi and Taoukis, 2018). The performance and preservative effect of several alternative food packaging materials is being investigated for a wide range of food applications. Novel processes, such as cold atmospheric plasma (CAP) can be used for the surface modification of polymers, with the aim to modify the polymer properties and create active materials with potential antimicrobial and/or antioxidant activity (Pankaj et al., 2014).

The aim of the present study was to evaluate the quality and safety aspects of alternative edible and non-edible packaging systems, considering their bioactivity, toxicity, and antiviral activity potential.

Materials and methods

We report the development and testing of alternative biopolymers, including edible materials, such as carboxymethyl cellulose (CMC), pectin, chitosan, and non-edible films, such as polylactic acid (PLA), polyhydroxybutyrate (PHB), polyethylene (PE). Standard methodologies and model systems, determined by the current legislation, regarding putative effects on the cell viability by the migration of substances from the packaging materials to the packaged food was used (Commission Regulation (EU) No 10/2011). In brief, carboxymethyl cellulose-based membrane (possible edible) or the classical plastic membrane of low-density polyethylene (LDPE), were used to assay putative cytotoxicity by the migration of substances to solvents simulating food contact. The approved for food packaging LDPE membrane served as control. Specific dimensions of the above membranes were weighted and incubated in pre-weighted glass vials using different solvents. Based on the current legislation, the solvents used simulated the contact to aqueous (dH_2O), alcoholic (10% ethanol in dH_2O), milky (50% ethanol in dH_2O), fatty (95% ethanol in dH_2O) or acidic (3% acetic acid in dH_2O) foods. The incubation was carried out for 10 days at 20 ^oC or 4 ^oC to resemble the room temperature or the refrigerator conditions of food storage, respectively. At the end of the incubation period, the remaining undissolved membrane-related material was withdrawn, the organic part of the solvents was evaporated under a stable nitrogen stream while the water part was completely dried in fridge-dryer. The remaining was weighted, rediluted in dimethyl sulfoxide (DMSO) and used to perform cell-based viability assays (resazurin-metabolism assays). A piece of carboxymethyl cellulose-based membrane was completely dissolved in dH_2O with the use of vortex and used also in cell-based viability assays. Bioactivity was assessed in terms of antimicrobial and antioxidant activity. The evaluation of the antiviral activity on the surfaces of the tested packaging materials was performed using different viruses (Vaccinia virus, Norovirus, Adenovirus, Poliovirus, and SARS CoV-2) according to standard test methods (ISO 21702:2019).

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Results

To check for putative effects on cell viability due to substances which are possibly released by the CMC- or LDPE-membranes in the different solvents, serial dilutions of the DMSO-dissolved remaining of each sample were applied on Caco2 and Huh7 cells. Similarly, Caco2 and Huh7 cells were treated with different concentrations of the dH_2O-dissolved CMC-based membrane. Treated cells were incubated for 72 h and then the cell viability was determined by the resazurin-based assay. Our results showed that the CMC-membrane had no negative effects on the cell viability with any of the solvents used and in any conditions of incubation. Similarly, with the used concentrations of the dH_2O-dissolved CMC-based membrane no negative effects on the cell viability were observed. The results of the study showed the potential of the tested biopolymers as alternative packaging materials, appropriate for fresh and perishable food products, such as fish and seafood, in terms of migration and toxicity, according to the EU regulation for food contact materials, and their bioactivity for the development of active packaging materials. In addition, Vaccinia virus, SARS CoV-2, and Adenovirus exhibited low reduction rates in their titers (5%, 4.5%, and 3%, respectively) when tested using the selected non-edible packaging surfaces. In contrast, no antiviral effect was observed either for Norovirus and Poliovirus on the edible surfaces or for all the tested viruses on the non-edible surfaces.

Discussion and conclusion

The application of processing methods, such as cold plasma, and the incorporation of bioactive components into (bio) polymers offers the potential for effective active packaging for improving quality and safety of fish and seafood products. Moreover, the lack of negative effects on cell viability of CMC-based membrane with all solvents used and with any conditions of incubation (room temperature or refrigerator) suggests that this membrane can be used to wrap food of any composition to extend its shelf life and to reduce the required plastic material for its preservation.

Acknowledgment

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THERMAL STRESS CHANGES TELOMERE DYNAMICS IN THE MANILA CLAM *Ruditapes philippinarum*

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Introduction

The effects of climate change (CC) are a contemporary reality that will have increasingly negative and severe consequences in the near future. One of major component of CC is constituted by heat waves (HWs), extreme climate events that can cause massive mortality of organisms, especially in costal habitats. HWs are increasing in frequency, intensity and severity and represent a serious threat, not only for species living in coastal habitats, but also for all human activities connected to those habitats, such as clams aquaculture. To counteract the negative effects of these stressors on clams it is necessary to identify stress markers and to investigate the physiological and molecular mechanisms triggered by them. One of the consequences of thermal and oxidative stress is faster ageing caused by an impairment on telomeres, which are non-coding DNA sequences that protect chromosomes. The length of these protective structures is generally associated with lifespan and some evidence has shown a significant decrease in their length following thermal stress. In this work, we evaluated the telomere length of clams subjected to a simulated HW. We demonstrated that HW had a tangible effect on the dynamics of telomeres, which appear to shorten following HW. However, much remains to be understood about how this occurs.

Materials and Methods

To evaluate the impact of HWs on the Manila clam (*Ruditapes philippinarum*) at physiological and molecular level, we purchased, from the hatchery SATMAR, a batch of clams that were produced from the same mass spawning event, to ensure that all animals had the same biological age (thus preventing bias in telomeres due to differing ages). After 14 days of acclimatization at 20°C, clams were divided into two groups: treated and control. The treatment consisted in mimicking for 15 days an HW which naturally occured in the Venice lagoon in the past years, with water temperatures oscillating daily from 31°C to 35°C, while controls were kept at 25 °C. After 15 days, the HW was terminated and all animals underwent a resting phase (25°C for 18 days) at the end of which the gills from 40 control and 40 treated clams were sampled to analyze the impact of HW on telomere length. After the extraction of high molecular weight DNA from the gills, a protocol was applied for the amplification, via qPCR of the telomeric DNA and of a reference gene (GAPDH) and for the subsequent calculation of the relative telomere length of the telomeres. (T/S ratio)

Results

Exposure to HW significantly reduced the treatment population by 60%, with a mortality peak on the 15th day of HW and a more reduced but constant mortality over the following days.

We then tested whether there was a link between clam length and survival rate to understand if larger or smaller clams were more resilient. However, no correlation was found among these parameters. Finally, we looked at telomere dynamics and found that while in the control population a positive correlation existed between shell length and telomere length, an opposite trend was observed between HW-exposed clams: in fact, HW-surviving clams that were longer in length had shorter relative telomeres lenght. These results suggest an important change in telomere dynamics following HW stress, that should cause the erosion of telomeres (i.e. shortening). However future experiments are needed to properly quantify the the extent of this shortening.

HEAT PRIMING TRIGGERS SURVIVAL MECHANISMS IN MANILA CLAMS EXPOSED TO HEAT WAVE

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Introduction

Aquaculture is one of the growing sectors in the world and 60% of world aquaculture production is made up of bivalve, especially clams, oysters, mussels and scallops. However, climate change and associated extreme weather events such as heat waves (HWs) are seriously threatening coastal environments, the species that inhabit them and all human activities connected to those habitats. Since the vast majority of global clam aquaculture is produced in coastal areas it is essential to find solutions to counteract the negative effects of these stressors on clams. Possible mitigation strategies lie in heat-priming. Heat priming consists in the exposure of animals to a sub-lethal stressful condition which renders them less vulnerable to a subsequent lethal exposure with the same stressor. In this study, we tested the effect of priming on Manila clam, *Ruditapes philippinarum*, at physiological and transcriptomic level, highlighting some mechanisms that could be triggered by this innovative treatment.

Material and methods

To investigate the effect of priming, we used 500 individuals generated from SATMAR, one of the biggest hatcheries in Europe, and, after an acclimatization period of 10 days at 18 °C, we divided them into two groups: primed and controls. The first group was left for 7 days at a temperature of 30°C, while the control group was kept at 25°C. After a resting period of 15 days (both groups at 25 °C), both groups were exposed to a simulated HW for 7 days where the temperature of the aquariums went daily and gradually from 31 to 35 °C. Survival of animals was monitored for the following 15 days (where temperatures were back at 25 °C) and, finally we measured the condition index -CI (a parameter which gives a general indication of the well-being of the clam), and the hepatosomatic index -HSI (to evaluate the energy reserves stored in the body). In addition, digestive glands were collected and stored in RNAlater to study the transcriptomic response.

Results

At the end of the experiment, the primed clams had a near 100% chance of survival, while the clams that did not receive treatment before the HW had 70% chance of survival. This confirmed the fact that priming improves the survival of clams after HW. However, it emerged that, after the HW, primed clams have ad a lower CI and HSI. Transcriptomic analysis revealed that primed clams had 32 differentially expressed genes (24 down regulated and 8 up regulated) compared to the group that was only exposed to HW. Despite the low number of differentially expressed genes, specific functional groups emerged from the functional analysis via Gene set Enrichment Analysis. Specifically, entries related to the development of endothelial and epithelial cells and tissue organization are up regulated. Furthermore, entries related to the synthesis of molecules involved in carbohydrate and lipid metabolism such as pentose-phosphate shunt, regulation of ketone biosynthetic process, NADPH regeneration, regulation of lipid metabolic process were up-regulated in primed clams. These results highlights a reorganization of the organ responsible for the stock of energy reserves and the metabolic processes that are triggered following priming. Other up-regulated pathways via GSEA were related to the response to viruses and bacteria (response to cytokine, defense response, type I interferon signaling pathway). Taken together, these results indicate that priming is able to trigger defence responses to stress, directing the clams energies towards processes that guarantee a better response in terms of survival to HW.

REARING OF EUROPEAN SEABASS (*Dicentrarchus labrax*) IN AQUAPONIC SYSTEMS: EFFECTS OF ENRICHED BLACK SOLDIER FLY (*Hermetia illucens*) PREPUPAE MEAL ON FISH LIVER

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Introduction

As the global human population is projected to reach 9.7 billion by 2050, concerns about the future food supply have intensified. Aquaculture is expected to play a crucial role, with an estimated 62% of seafood production coming from aquaculture by 2030. However, to meet sustainability criteria and European Union requirements, aquaculture must limit organic pollution and water consumption, maintaining high production standards. Insects, particularly black soldier fly larvae, have gained attention for their environmentally friendly characteristics. They can be reared on organic waste with low water input, aiding waste biodegradation and aligning with the circular economy concept [1]. Black soldier fly prepupae meal (*Hermetia illucens*; HI) is rich in protein, essential amino acids, vitamins, and minerals, making it a potential ingredient for aquafeed. However, the fatty acid profile lacks certain essential long-chain polyunsaturated fatty acids (PUFA) for fish, raising concerns for marine species. Despite this nutritional drawback, several studies aimed to use the full-fat HI, exploiting the property of the black soldier fly larvae to modulate their fatty acid profile based on their feeding substrate. In this regard, it has been demonstrated that the inclusion of microbial dried biomass from marine protists or cyanobacteria, such as spirulina (*Arthrospira platensis*), in the insects' growth substrate represents a valid procedure to improve HI's nutritional value in terms of both long-chain PUFA and antioxidant molecules content. In addition, the inclusion of spirulina-enriched HI in diets intended for both fish and crustaceans resulted in positive responses in terms of fish growth performance and welfare. In this context, this study examined the effects on the biochemical profile of fish liver of the partial substitution of fish meal with spirulina-enriched HI in the diet of European seabass (*Dicentrarchus labrax*) juveniles, reared in aquaponic systems to further promote sustainability in aquaculture.

Materials and Methods

Three different diets for European seabass were designed, aiming to meet their nutritional requirements. The control diet (HI0) contained conventional marine-derived ingredients (40% fish meal and 12.5% fish oil) along with vegetable ones. The other two diets, named HI3 and HI20, were based on the control diet by substituting 3 or 20 % of fish meal with spirulina-enriched HI, respectively. Two hundred and seventy E. seabass juveniles were acclimated for two weeks in a 1000 L tank with controlled water parameters. After acclimation, the fish (initial weight: 19.3±0.1 g) were divided into nine media-based aquaponic systems (600 L fish tank and 120 L hydroponic unit) with 30 specimens per tank. The trial lasted 90 days during which fish nearly tripled their weight (final weight: 60.5±0.6 g). Fish were fed the experimental diets at 2% body weight, with diet adjustments every two weeks. At the end of the trial, all fish were euthanized, individually weighed, and livers were collected for the following analysis: (i) histological analysis was performed to determine hepatic lipid and glycogen deposition; (ii) spectroscopic (FTIR) analysis aimed to evaluate the biochemical composition of the liver tissue; (iii) chemical analyses in which livers were singularly homogenized then analyzed for the total lipid content, oxidative status (as conjugated dienes and thiobarbituric acid reactive substances) and antioxidant capacity (2,2-diphenyl-1-picrylhydrazyl). Data were subjected to one-way ANOVA followed by Tukey's multiple comparison test. Significance was set at $p < 0.05$.

Results and Discussion

Liver serves as a significant lipid storage site in various marine fish species, including E. seabass. However, the use of full-fat HI in aquafeeds has a notable drawback due to its unbalanced fatty acid profile. This imbalance often results in increased saturated fatty acid (SFA) content and a high n-6/n-3 ratio, which have been linked to the promotion of hepatic lipid accumulation. As expected, in the present study, despite the absence of pathological alterations observed in the liver parenchyma, the histological analysis of the HI20 group showed a significantly higher fat accumulation compared to the other dietary treatments. This outcome was further corroborated by the FTIR analyses, which revealed an increase in both the total lipids and fatty acids content in livers from the HI20 group. Additionally, both the histological and spectroscopic

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analyses indicated that liver energy storage in this group could primarily rely on fatty acids rather than glycogen. However, chemical analyses did not show significant differences among the experimental groups regarding total lipid (all around 11%) and SFA (ranging from 251.89 to 327.38 mg of FA/100 g fresh tissue) contents. The contradiction found between the results obtained using different analytical methods can probably rely on their different sensitivity. Indeed, in the case of the total lipid analysis, the liver was homogenized by blending before analysis while for histological/spectroscopic analyses, whole liver samples were fixed without homogenization. Thus, the heterogeneous spatial distribution of lipids in the liver may have affected the results [2].

Finally, lipid oxidation results revealed that conjugated dienes (CD) (mean value: 0.54 mmol kg^{-1} fresh tissue) and thiobarbituric acid reactive substances (TBARS) (mean value: 0.11 mg MDA-eq. kg^{-1} fresh tissue) contents in the liver were not significantly influenced by diets. These aspects reflected a good health status of the liver since, when it is inflamed, TBARS usually tend to increase. The same positive results have been found for the antioxidant capacity which did not differ among groups. In conclusion, the marginal impact on the liver underscored that the enriched full-fat HI is a viable and eco-friendly option as an aquafeed ingredient for rearing E. seabass juveniles. Indeed, diets did not adversely affect the biochemical composition and health status of the liver. Thus, further studies could test higher inclusions of enriched HI. Further studies could also help to shed light on the discrepancy in the results for lipid deposition liver found with the analytical methods used. In addition, this feeding trial represents an example of how the rearing of a commercially significant fish species can be carried out using more sustainable aquafeeds and farming techniques, like aquaponics. This approach promotes the adoption of circular economy principles and zero-waste concepts in aquaculture, contributing to a more environmentally conscious and efficient industry.

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A COLLABORATIVE RESEARCH APPROACH FOR THE SUSTAINABLE DEVELOPMENT OF SHELLFISH FARMING IN ITALY

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Introduction
The Italian shellfish industry is a significant contributor to both the volume and value of the national fisheries and aquaculture production.

The sector is mainly composed by three branches: Clams, Mussels and Oyster.

The clam (*Tapes philippinarum* and *Tapes decussatus*) industry continues to present a satisfying production level, as Italy is stably the leading European producer. Oyster (*Crassostea gigas* and *Ostrea edulis*) production is relatively recent to Italy, yet it presents great potential for development as the country has the capacity to breed high-quality products, and the demand for internal consumption is primarily met through imports.

On the contrary, the Mussel (*Mytilus galloprovincialis*) industry is currently experiencing a challenging period, with a diffused difficulty to get enough revenues to remunerate the productive factors and make structural investments.

Considering that shellfish production plays a central role in the current European food strategies, the sector's primary challenge is thereby to identify a sustainable development pattern, able to ensure a conservative use of resources and the food safety, while meeting the growing demand for seafood.

Framework
This paper is part of the dissemination process of the VALUE-SHELL project, financed by the Italian Ministry of Agriculture, Food Sovereignty and Forests. The project has been financed as part of the institutional and technical-scientific support activities for the implementation of the National Strategic Plan of Aquaculture.

Purpose/scope
Moving from the findings gathered during the VALUE-SHELL project, the purposes of this paper are to present the main problems and needs of the Italian shellfish sector, to highlight the current state of the industry and to suggest sustainable intervention actions. Policy recommendations should be addressed to solve or mitigate current weaknesses and support the development of the opportunities and the potentials of the sector.

Materials and methodology
The study was conducted during 2022 through a desk research on molluscs' industries structures and the different typologies of public interventions and support.

To assess the needs of the sector it was used a collaborative approach, consisting of interviews to stakeholders, such as local administrators and research centers, and focus group meetings with the fundamental participation of small/medium producers, often organized in cooperatives.

Stakeholders have been singularly interviewed about the main sectorial problems from their professional point of view.

Producers were consulted by organizing five focus group meetings on a territorial basis (Liguria, Emilia-Romagna, Sardegna, Puglia and Campania). The realization of the focus group meetings has been made possible by the collaboration of AMA (Mediterranean Aquaculture Association), the most representative molluscs producers association in Italy.

Moreover, an additional focus group meeting has been conducted within the EATIP experts to share the main results of the project and acquire more knowledge at European level.

Problems and needs emerged during the project's activities has been categorized according to their prevalent nature within the four sustainability pillars (environmental, economic, social, and institutional).

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Results

The collaborative process allowed to confront the problems and needs experienced by producers from different areas and overall situations.

From a sustainability point of view, the main reported critical aspect regards the Institutional side, due to the lack of a specific aquaculture law governing the sector and the current fragmentation of competences among different entities. In this field, the most relevant issues concern the bureaucratic concessions of marine areas for aquaculture use and the ongoing process of delimitation of the AZA (Allocated Zones for Aquaculture), an instrument that could solve the conflicts between different spatial uses and grant to shellfish producers the ability to plan mid and long-term investments.

With regards to the economic sustainability, some structural characteristics do create issues in the overall economic performance. Between those should be named the increased production costs and the difficulty to introduce income integration by aquaculture tourism and offer diversification.

Environmental sustainability does involve the relationship between shellfish production and the environment on which it is carried on. Main problems from this aspect comprehend global warming, the need for ecosystem equilibrium in the sea populations (predatory species) and measures to reduce both sea pollution and cementing of coastal freshwater springs.

For the social sustainability, from the focus group meetings emerged a diffused fear on the generational turnover, also connected to the scarcity of adequate professional training and scholastic sectorial education.

The project, both in its policy review and in the collaborative meetings components, found out the main bottlenecks in the sectorial policies that do not allow to completely fulfill the producers' needs.

THE IMPACT OF FERTILIZER SAVINGS ON QUALITY OF LETTUCE (LACTUCA SATIVA) IN AQUAPONICS. COMPARISON OF AQUAPONIC, FERTILIZED AQUAPONIC AND HYDROPONIC NUTRITION

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Introduction

Aquaponics is a promising technology due to its efficient transfer of nutrients from fish waste to plant biomass, resulting in reduced fertilizer and water usage. However, the nutrient content in aquaculture water varies greatly and may not be optimal for plant growth, which can affect plant yield and quality. Scientific literature on this topic is inconclusive and exhibits a high level of disparity. The objective of this study was to compare plant growth and quality aspects in aquaponic, fertilized aquaponic, and hydroponic nutrition systems.

Materials and methods

The experiment was done in three runs. After the first run, the light photoperiod was increased from 11 to 18 hours of daylight to achieve more realistic lettuce weight for commercial production. Experimental set-up was placed indoors in a climate chamber allowing environmental control. Each experimental treatment was implemented in three technical repetitions placed in individual growing chambers. Lettuce, green butterhead variety of Salanova Aquino (Rijk Zwaan) was grown. The total number from all the runs was 229 lettuces. Aquaculture water (aquaponic treatment) was sourced from professional recirculating aquaculture system (RAS) at the Leibnitz Institute of Freshwater Ecology and Inland Fisheries. RAS was placed in the nearby greenhouse, with total water volume of 16 m^3, 4 fish tanks stocked with *Oreochromis niloticus* (tilapia) and *Colossoma macroponum* (red bellied pacu), kept separately with total weight of 182 kg. Used feed was Aller Aqua Claria Float 2mm (45 % crude protein, 12 % crude fat, 25,1 % NFE, 6,4 % ash, 3,5 % fiber, 0,9 % phosphorus). Aquaponic fertilized treatment was prepared using Hydrobuddy program (programmed and designed by Dr. Daniel Fernandez, available at www.scienceinhydroponics.com). Water from RAS was analyzed on ICP and Ion chromatography. Aquaponic water was supplemented with nutrients to obtain similar concentrations as for hydroponic 70% or 80% Howard-Resh solution, depending on nutrient content of aquaculture water, primary targeting for comparable NPK (nitrogen, phosphorus, potassium) content. Hydroponic treatment was prepared with 1:1 tap and distilled water. After mixing, pH was adjusted to the same level in all treatments, either lowered by nitric acid (6,5% HNO_3) or raised by sodium hydroxide (2M NaOH). After 30 days, lettuces were cut at the basis and weighted. Bottom defective leaves were removed before sampling. For nitrate content analysis samples were oven dried at 85°C for 24 hours, grinded to powder, extracted by boiled water (1/100, w/v; 0.1 g of samples and 10 ml of deionized water (conductivity < 0.055 μS cm^{-1}; Adrona, Latvia) and samples were placed to 1 min in ultrasonic bath. Suspension were filtered through a 0.45 μm nylon membrane filter. Nitrates were determined by means of capillary ion-exchange chromatography with suppressed conductivity (capillary high-pressure ion chromatography - HPIC). Dionex ICS 4000 and ICS 6000 (Thermo Scientific, USA) system equipped with Dionex IonPac AS11-HC 4 μm (Thermo Scientific, USA) guard and analytical columns were used. For sensory analyses whole lettuces were stored fresh at 4°C for 27 hours, until assessed by experienced sensory panel. The sensory profile was assessed using intensity scales (0-100 mm) which were transformed into numerical scale for statistical analysis. Each evaluator assessed 9 samples for 9 descriptors. To determine microbiological parameters: total counts of bacteria, Aeromonas, coliform bacteria, Salmonella and Listeria, standard media and methods were used in accordance with relevant ISO standard.

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Results and conclusions

Our study found that lettuce grown in aquaponic treatment exhibited a 30 % decrease in growth (112,2 ± 27,2 g) compared to fertilized aquaponics (164,4 g ± 47,1 g) and hydroponics (160,7 g ± 44,1 g). Surprisingly, the suboptimal nutrient composition in aquaculture water did not exert a significant influence on the quality traits, sensory profile or microbiological status of the lettuce. The fertilized aquaponic treatment resulted in significant savings in fertilizer usage, with nitrogen savings of 93 %, phosphorus by 7 %, and potassium by up to 12 %, compared to hydroponics. The limit of nitrate content in the lettuce was not exceeded in either treatment. For sensory analyses statistically significant differences were not observed in the descriptors examined. Aquaponic lettuce exhibited the highest rank in overall acceptability (74.3), intensity of taste, crispiness and intensity of aftertaste, however the differences were not significant. Aeromonas bacteria were detected in all treatments, with an average concentration of 1.52 log CFU/g on the leaves and 3.11 log CFU/g on the roots. The presence of Aeromonas was not specific to treatments utilizing aquaculture water. Notably, the lettuce roots exhibited a higher bacterial load. Coliform bacteria were found at relatively high levels, with average values of 4.6 log CFU/g on the leaves and 6.69 log CFU/g on the roots, reaching up to 5.6 log CFU/g on the leaves and 7.7 log CFU/g on the roots.

This study provides a comprehensive evaluation of the quality of aquaponic lettuce. The results indicate that aquaponics offers a viable solution for sustainable agriculture by reducing reliance on synthetic fertilizers, producing high-value end products, and environmental sustainability. Aquaponics is a sustainable agricultural system that eliminates the need for synthetic fertilizers by utilizing organic fertilizer derived from fish feed. The method of fertilized aquaponics does not compromise the quantity, quality, sensory or microbiological properties of the resulting product when compared to hydroponics. When no fertilization is applied, aquaponics still maintains comparable quality and sensory traits, although the biomass yield is reduced by 30%. The absence of fertilizer usage in aquaponics can lead to a decrease in nitrate content in lettuce, which can be beneficial. However, it is important to note that aquaponic lettuce may have lower levels of certain nutrients, such as iron. Overall, the aquaponic method effectively saves the use of fertilizers, mainly nitrogen, phosphorus, potassium, and other essential nutrients, without presenting any risks to consumers.

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INTERACTIONS BETWEEN BIOPHYSICAL FACTORS, TECHNOLOGY, PRODUCTION STRATEGIES AND ECONOMIC OUTCOMES IN OFFSHORE SALMON AQUACULTURE

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Introduction

Offshore oceans provide biophysical environments and risks that are new to the salmon farming industry. New innovations are required in several stages of the value chain. This paper analyses biophysical challenges and risks, and implications for technological innovation, investments, production strategies and financial returns. We examine different smolt size and offshore production cycle choices, accounting for a range of biophysical and technological constraints and opportunities. Our analysis show that production strategies have significant effects on production costs and returns on investments, and implications for the competitiveness of offshore aquaculture and opportunities for sustainable growth. The design of government regulations that affect production strategy and capacity utilization will ultimately play a critical role for the economic sustainability of offshore aquaculture. Here, we examine the impacts of tax regimes.

Empirical analysis

Offshore aquaculture requires investments in an entire value chain, starting with onshore production of salmon fingerling, smolt. This value chain will have a different configuration than the present inshore value chain, with new production and logistics stages. Figure X depicts the conventional inshore value chain, which today represents almost all salmon production, together with alternative offshore value chains. In two of the value chain alternatives an additional production and sea transportation stage is added, postsmolt production in sea. Furthermore, the offshore value chains at several stages require new knowledge and innovation, and involve new risks. These risks are related to biosecurity, fish health and welfare, technological performance and economic performance.

It should be recognized that total investments and production costs in an efficient inshore value chain will be lower than in an offshore value chain. An offshore farm producing 20000 tonnes of salmon may require value chain investments of approximately 5 billion NOK (500 USD, exchange rate 10 NOK/USD), while an inshore value chain producing the same quantity may require investments of only 1.5 billion NOK. The difference in investments is driven mainly by the offshore installation, but also higher share of post-smolt production and specialized vessels increase investment costs.

The economic analysis:
1) Assess suitable production cycles based on temperature, current and waves.
2) Use the TGC growth model for the different production cycles taking temperature for the different production cycles into account and find new salmon weights as a function of time.
3) Creates a model involving both the number of smolts and the weight of the growing fish as a function of time.
4) When the potential biomass minus the maximum allowable biomass (MAB) from government is positive, the surplus of the MAB is harvested. All the biomass needs to be harvested prior of the planned fallowing.
5) The production cost for each identified production cycle is found.
6) Net Present Value analyses are undertaken with and without resource tax and with sensitivity analyses.

Figure 1 shows one example of an NPV profile with different tax regimes, building on proposals from the Norwegian government.

Conclusions

Our analysis shows that the production strategies of offshore salmon aquaculture value chains have significant consequences for cost productivity and financial returns. Capacity utilization in terms of biomass is a critical factor. The size of the smolt or post-smolt released into the farms have significant effects on economic performance. Finally, the design of the tax regime is critical for the investment decision, involving investments typically in the order of 500 MEUR for a value chain serving one offshore farm.

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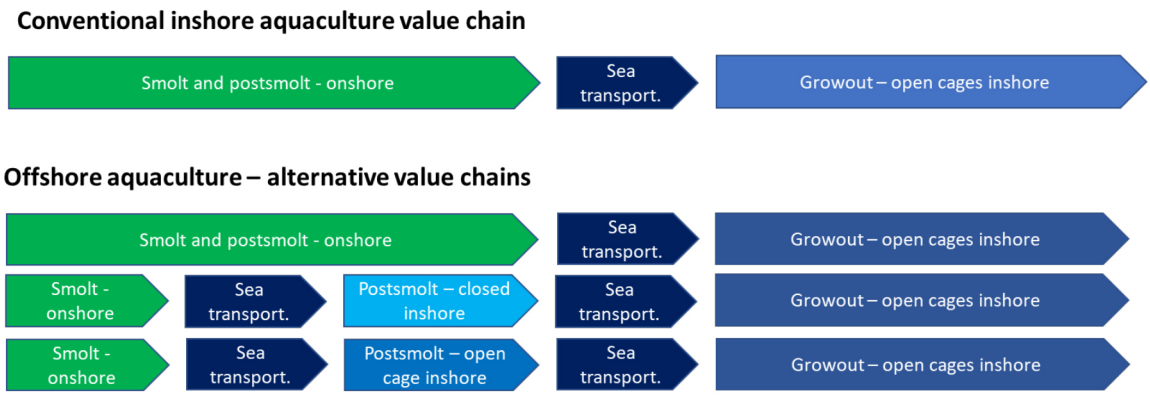

Figure X. Conventional inshore vs offshore aquaculture value chain

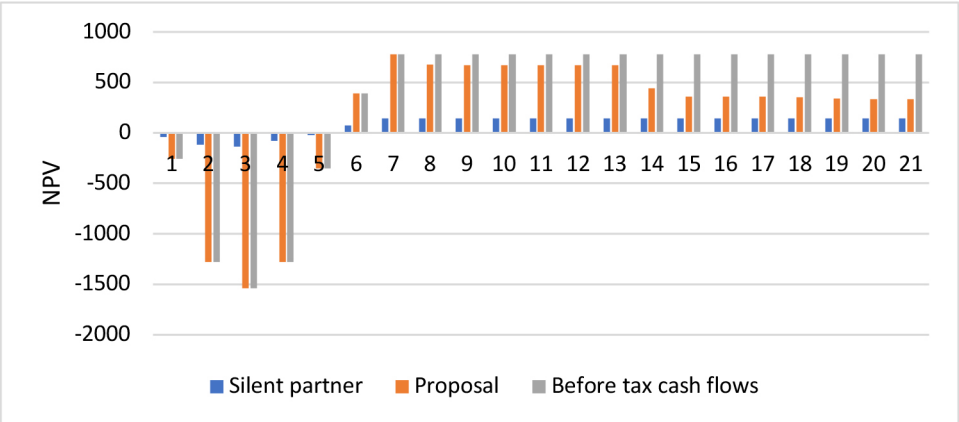

Figure 1.Net Present Value (NPV) with different tax structures

VITAMIN K_3: AN ANTI-MINERALIZING NUTRIENT IN AQUACULTURE AND BIOMEDICAL STUDIES

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Introduction

Minerals play a central role in the growth, development, and health of aquaculture species. Anti-mineralizing agents, such as bisphosphonates, and citrate, help prevent mineral accumulation in unwanted areas such as soft tissues or organs (Li and Uitto, 2013), in some case calcitonin, and vitamin K_2 also act as factor used to reduce vessel ectopic calcification. Excessive doses of anti-mineralization agents can hinder mineral deposition in fish bones and result in skeletal abnormalities, increased susceptibility to diseases, and higher mortality rates, particularly during the early stages of fish development in aquaculture species. In animal models, anti-mineralization agents are used to inhibit ectopic deposition of minerals that can contribute to pathological conditions. For example, anti-mineralization strategies can significantly prevent the formation or growth of mineral-based structures that could potentially obstruct or harm organs. The present study aims to assess the anti-mineralogenic effect of vitamin K_3, in its menadione form, in an aquaculture context using gilthead seabream (*Sparus aurata*) and in a biomedical context using zebrafish (*Danio rerio*).

Materials and methods

In vivo experiment #1: Gilthead seabream larvae (initial length 6.26 ± 0.42 mm, body dry weight 0.28 ± 0.41 mg) of 21 dph (days post hatch) fed increasing levels of vitamin K_3 (Table 1) were analysed for growth performance, skeletal anomalies, vertebral mineralization, and expression of bone markers.

In vitro experiment: Gilthead seabream mineralogenic cells VSa13 induced for mineralization (Pombinho et al., 2004) and exposed to increasing levels of vitamin D_3 (cholecalciferol) and vitamin K_3 (menadione) (Table 2), were analysed for cytotoxicity, extracellular matrix (ECM) mineralisation and expression of bone markers.

In vivo experiment #2: Embryos of the zebrafish mutant *grate* (model for Pseudoxanthoma elasticum (PXE) characterized by the abnormal calcification of soft connective tissues and the fragmentation of elastic fibers) were exposed to different levels of vitamin K_3 (Table 3) and analysed for the expression of mineralization markers.

Table 1. Dietary levels of vitamin K_3 fed to gilthead seabream larvae (mg/kg of diet)

Vitamin K_3 (mg/kg of diet)						
Analyzed vitamin K_3	1.32	1.62	4.98	12.26	22.90	58.51

Table 2. Levels of vitamin D_3 and vitamin K_3 (ppm; in ethanol) applied to mineralizing VSa13 cells

Vitamins D_3 and K_3 (ppm)									
	Ethanol	DM1	DM2	DM3	DM4	DM5	DM6	DM7	DM8
Vitamin D_3	0*	0.02	0.02	0.08	0.08	0.31	0.31	1.25	1.25
Vitamin K_3	0*	2.5	10	2.5	10	2.5	10	2.5	10

*Ethanol 0.1% was used as vehicle for vitamins D_3 or K_3

Table 3. Levels of vitamin K_3 (ppm; in ethanol) applied to zebrafish embryos

Vitamin K_3 (ppm)			
Vitamin K_3	0*	2.5	10

*Ethanol 0.1% was used as vehicle for vitamin K_3

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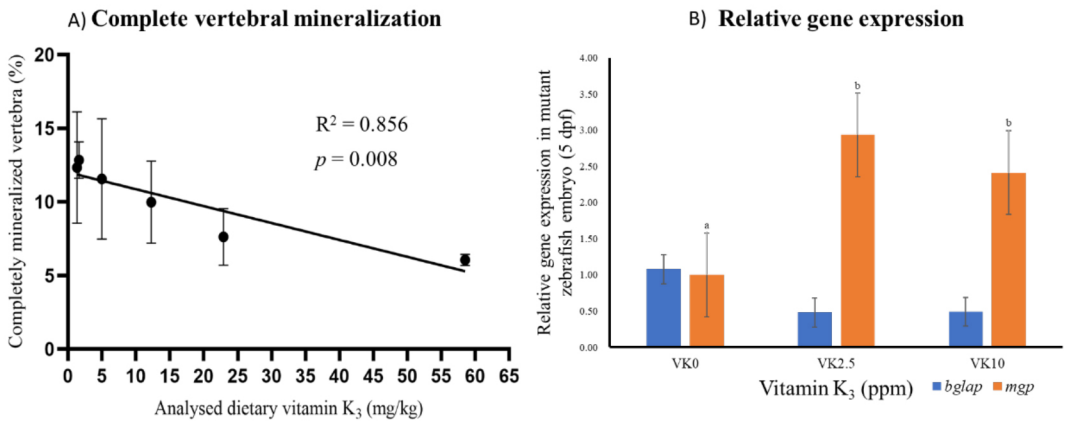

Figure 1: (**A**) Effect of dietary vitamin K_3 on vertebra mineralization in gilthead seabream larvae (linear regression, $R^2 = 0.856$, p = 0.008) and (**B**) relative expression of *bone gamma-carboxyglutamate protein* (*bglap*) and *matrix Gla protein* (*mgp*), two genes coding for vitamin K dependent proteins, in mutant zebrafish embryo.

Results and Discussion

Larvae fed increasing levels of vitamin K_3 exhibited a reduction in complete vertebral mineralization (Figure 1A), but also an increase in abdominal kyphosis and a reduced survival rate. A downregulation of *bone gamma-carboxyglutamate protein* (*bglap*), a gene central to bone mineralization, was observed with increased levels of dietary vitamin K_3. *In vitro* data showed an increase in ECM mineralization in cells exposed to higher levels of vitamin D_3 and lower levels of vitamin K_3. Conversely, ECM of cells were exposed to high concentrations of both vitamins was significantly less mineralized. However, ECM of cells exposed to high vitamin D_3 and low vitamin K_3 was significant more mineralized, indicating a possible antimineralizing effect of vitamin K_3. In this regard, mutant zebrafish embryos exposed to increasing levels of vitamin K_3 exhibited an increased expression of *matrix Gla protein* (*mgp)*, a gene coding for an inhibitor of tissue mineralization, and a reduced expression of *osteocalcin* (Figure 1B), as seen in gilthead seabream larvae. Our dataset agrees with observations reported by Elshaikh et al. (2020) and confirmed that excessive intake of vitamin K_3 can disrupt mineral deposition, leading to imbalanced bone mineralization. It also indicates that vitamin D / K ratio has to be optimized for achieving optimal matrix mineralization during bone development, as proposed by Ziemińska et al. (2021).

Conclusion

Increasing levels of vitamin K_3 reduced the mineralization of gilthead seabream vertebrae *in vivo* and bone matrix *in vitro*, and upregulated *mgp* expression in mutant zebrafish embryo providing strong evidence for an anti-mineralization effect of vitamin K_3. Optimizing levels of vitamin K_3 in fish diet may enhance skeletal status of farmed fish thus their health and welfare. In human, it could also contribute to the advancement of therapeutical approaches for ectopic calcification disorders.

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EXPLORING INFORMATION ENTROPY BASED APPROACH FOR PROCESSING AND ANALYSIS OF FISH TELEMETRY DATA TO ASSESS WELFARE

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Introduction

Analyzing telemetry data and translating it into meaningful indicators of fish welfare remains a challenge, as it is necessary to distinguish between typical and atypical behavior.

Entropy approaches can be used to analyze telemetry data and detect changes in fish behavior, providing valuable insights into fish welfare. Telemetry is an important tool for studying fish behavior and allows for the real-time monitoring of fish movements. However, analyzing telemetry data and translating it into meaningful indicators of fish welfare is a challenge. Entropy-based methods, which use information theory to quantify the complexity and unpredictability of animal behavior, provide a more comprehensive understanding of the animal state.

By analyzing data probability density function with entropy approaches, it is possible to identify atypical behavior that may indicate compromised welfare. These methods can detect irregularities in fish behavior and provide insight into the animal‘s state.

Results and Discusion

Typical behavior is not a single type of distribution, but rather a set of distributions. Entropy analysis is an effective method for identifying atypical behavior in fish welfare assessment, as it provides a more robust evaluation of telemetry datasets than classical statistical analysis. Entropy analysis allows for continuous monitoring of behavior and can identify when fish start behaving atypically. It can also determine which fish and which values are atypical or typical. By analyzing the variability of feeding behavior, social interactions, and behavior in response to different environmental conditions or stressors, entropy analysis can provide insights into the complexity and variability of fish behavior and promote more effective management practices. Entropy approaches can help to improve telemetry data analysis and provide objective indicators of fish welfare for management and regulatory purposes.

Acknowledgment

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THE CURRENT STATE OF HUNGARIAN AQUACULTURE EDUCATION, CHALLENGES, FUTURE OPPORTUNITIES AND SOLUTIONS

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Introduction

The European Aquaculture Society held its annual conference in the Italian coastal town of Rimini in September 2022. The motto of the conference was "Innovative solutions in a changing world", using the word "challenges" in a different context, but strongly indicating the need to analyse the socio-economic-environmental impacts and the importance of developing strategies for solutions. The increase in energy prices in the sector, the extreme rise in feed materials, and consequently in feed prices, created a dark atmosphere at the conference. This was compounded by the unpredictability of consumer markets, the unpredictability of the price sensitivity and tolerance of the buying public, and the cluelessness of the sector players in the face of a lack of skilled and trained labour. These problems affect all aquaculture subsectors, be it marine or freshwater, traditional or advanced (precision systems), production, or processing.

A special section of the conference focused on the state of education in the sector: a factual presentation of the present and a realistic view of the future. The consensus was that action is needed because the sector is threatened by a shortage of manpower, which cannot be offset by technical solutions and increased mechanisation, especially in the current economic situation. Although the aquaculture sector in Hungary is predominantly characterised by freshwater fish production, it can be said that it is facing the same major problems as the sector as a whole.

Background

The young generation has a very different experience of education today than even those who were in the classroom 10-15 years ago. This is partly due to the increasing demand for digital technologies in an accelerated and globalised world, and partly due to the impact of Covid, the online education at home. Young people are inundated with a constant stream of information from online platforms that they cannot and do not want to absorb: they decide whether to be interested or move on based on impulses acquired in seconds. Online education has reduced the social sphere and the opportunities for personal contact, the full impact of which we have yet to measure. It can therefore be said that reaching effectively the next generation, is a human and professional challenge.

The aquaculture sector needs to attract young people who are motivated, interested in the profession (who love it) and practice-oriented. On the other hand, young people who are not influenced by family or friends in their environment find themselves in serious competition with other, more attractive and well-communicated industries. It has become clear that a paradigm shift in aquaculture education and training marketing is needed as soon as possible. We need to attract the interest of young people to the sector with buzzwords that capture the imagination of a thinking teenager (they learn about it at school, they often see it on social media, they get news on their smartphones, etc.): environmental pressures, climate change, the importance of local food, etc.

In addition to the economic environment, the educational environment is constantly changing, to which some sectors (automotive, information technology, biotechnology) have reacted quickly. The traditional education system does not provide young people (primary and secondary school) with enough information on the importance, let alone the beauty, of agriculture, especially aquaculture. The young generation cannot be expected to turn to aquaculture of their own accord. Action must therefore be taken, involving all stakeholders, be they for-profit businesses, educational or research institutions, sectoral stakeholders, or regulatory bodies. There are already good examples of this, which should be adapted to the sector's environment and specificities, and the processes should be regularly monitored and restructured, and improved, incorporating feedback.

Conclusions

To summarize, the situation in aquaculture education is mixed, with plenty to do and plenty of work to be done. However, these tasks can and should be developed in a structured way, in agreement with and with the involvement of the relevant stakeholders.

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The first and most important step is to raise the profile of aquaculture as a profession and/or vocation among young people:

- introducing the basics of aquaculture in public education courses, e.g. in a biology or natural science subject (nature studies, environment);
- organising summer camps for primary and secondary school pupils, where young people can learn about the industry through a variety of practical activities;
- teacher training: to familiarise teachers with the aquaculture sector through study trips for teachers with a biology or science degree;
- summer student placements in aquaculture companies, where young people can gain knowledge and experience in the sector;
- raising wages in the sector to bring them on par with other agricultural sectors.

In order to increase the sector's popularity and attract interest, it is necessary to renew and update the training courses offered in the sector. Main tasks (not exhaustive):

- identify the labour market requirements of the practical (for-profit) partners for each level of training,
- develop the basis for funding outside the school system and, in some cases, within it (adult training), and a system for allocating partner contributions and, where possible, funding from grants, in addition to public funding,
- developing the interaction between theoretical and practical curricula, and preparing training places to carry out the tasks required,
- integrating digital learning opportunities and new teaching methods into traditional training systems,
- adopting good practices from abroad and adapting them to the national system,
- developing the marketing of training courses, using uniform campaigning and marketing tools (coordination),
- development and implementation of a comprehensive training strategy for fisheries (aquaculture) and angling, and wide communication.

Our primary interest is to find the answers to these questions as soon as it is possible through a common reflection that could provide the basis of a new educational concept which we believe is one of the key factors for the survival of the aquaculture sector.

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GENERAL INFORMATION OF THE SERBIAN POND FARMING SYSTEM BASED ON A PRACTICAL CASE STUDY

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Introduction

Like other carp-producing countries in Europe, Serbian pond farming has a long history and a strong tradition. To understand the background of the production and the functioning of the farming technology, we can get an idea of how a pond farm works. These ponds are known locally as the Óbecse fish pond system, and are located close to the Serbian-Hungarian border. The fish ponds were established in 1968 and are located between the cadastral municipalities of Óbecse and Bácsföldvár. The fish ponds cover an area of 650 ha and are supplied with water by the Ferenc-canal with gravity water control. The used water is discharged into the "Old Tisza" after the end of the production technology. The average annual production of the pond system over the last 20 years is 450 - 500 tonnes of market size carp, 150 tonnes of market size silver carp, 50 tonnes of grasscarp, 3 - 3 tonnes of European catfish and pikeperch, 120 - 150 tonnes of two-summer old carp juvenile and 30 - 50 tonnes of one-summer old carp fingerling.

Background

In Serbia, two types of production technology are used: two-year and three-year production systems (Table 1).

The two-year production system is used for intensive pond fish farming systems, using only extruded feed concentrates. The three-year production system is a traditional cereal-based feeding system, where concentrates are used only in the first year of rearing, and only cereals are fed to the stock in the second and third years.

Agrotechnological operations:

- It involves drying out the pond and keeping it dry due to oxidation processes that take place in the pond sludge. Drying out during the summer period involves resting the structures for 15 to 20 days. In winter, the duration of this process is doubled (30 to 40 days). The mineralisation of organic matter in the sludge is doubled at higher temperatures.
- Liming or disinfection: this is mostly carried out with burnt lime and, according to the original technology, there is an initial or zero liming (after the pond is fished, the remaining wild fish are destroyed). Each depression must be controlled, and disinfection is successful if the water is clear and transparent after treatment. The dose applied is 1,5 - 3 t/ha.
- Continuous liming or disinfection is carried out every 15 to 20 days throughout the production process at a dose of 50 to 100 kg/ha.

In small and intensive ponds, aerators are used to enrich the water with oxygen, both deep and surface aerators. In case the amount of feed exceeds 40 - 120 kg/ha, aerators should be operated during the night (mostly between 18 - 06 h).

Conclusions

European carp producers are divided on the future of feeding: the use of cereal-based feeds and complete feeds will be determined primarily by economic factors.

Table 1. Carp feeding systems in Serbia and average carp weight

Production technology	Duration of production (year)		
	1	*2*	*3*
two-year	0,25 – 0,5 kg	1,8 – 2,5 kg	-
three-year	0,10 -0,15 kg	0,6 - 1,2 kg	3,0 – 4,0 kg

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A good example of the use of complete feeds is what our Serbian fish farmers in Vojvodina territory are using in their pond farming systems. Due to the strong pressure from cormorant, they have been forced to rapidly increase the population of one-summer- and two-summer old carp, which they feed with complete feeds.

The results show that the grain-based feeding (2.5 - 7.0 kg FCR) resulted in an average of 5.0 kg, which is similar to the Hungarian results (based on literature data, 4.0 kg of grain can produce 1.0 kg of fish meat).

The results obtained with complete feeding are thought-provoking. The FCR of 1.78 kg of feed to produce 1.0 kg of fish meat is an excellent result, which supports the argument that there is a justification for feeding complete diets for certain carp age classes.

Serbian pond farmers are also struggling with water quality problems, which can only be alleviated by continuous monitoring and control. The KHV is also present in Serbia. Its management and prevention is a priority task and issue of everyday production organisation.

It is interesting to consider how the 2-year carp production system, the current input material prices and the drastic changes in the weather condition could be introduced into the production technology of other European countries.

OVARIAN DEVELOPMENT IN AFRICAN CATFISH (*Clarias gariepinus*) JUVENILES FED VARYING LEVELS OF COCOA BEAN MEAL

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Intoduction

African catfish (*Clarias gariepinus*) is a widely cultivated freshwater fish species in the world. In addition to poor nutrition and adverse environmental conditions, the high variability and unpredictability of fish reproductive performance pose significant challenges to the mass production of fingerlings and overall productivity (Uedeme-Naa and Nwafili, 2017). To address these challenges, the use of plant and organic feed additives to improve gonadal maturation and egg viability has gained attention in aquaculture (Al-Khalaifah et al., 2020). Cocoa (*Theobroma cocoa*) products have been shown to possess a wide range of health benefits and are rich in bioactive compounds, which have shown positive impacts on fish growth, health, and reproduction (Al-Khalaifah et al., 2020). There is a paucity of information on the effect of cocoa bean meal (CBM) on the reproductive development of catfish. Therefore, this study aimed to investigate the effect of varying dietary levels of CBM on the ovarian development of *C. gariepinus*.

Materials and methods

A total of 200 female *C. gariepinus* post-fingerlings, 9 weeks old and with similar body weights (23.12 ± 0.71 g), were procured from the Fish Multiplication Centre of the University of Nigeria, Nsukka, and used for the study. The fish were acclimatized in a concrete pond for 3 weeks and were then randomly divided into five treatment groups (T1, T2, T3, T4, and T5) in a completely randomized design with four replications. Each replicate was kept in a 100 L basin with 10 fish each. Five iso-nitrogenous (35% crude protein) and iso-caloric (2.51 Mcal/Kg) diets were formulated, with CBM at inclusion levels of 0, 10, 20, 40, and 50%, respectively, for T1, T2, T3, T4, and T5 diets. The fish received the diets for 9 weeks at the rate of 3.5% of the total biomass. The water was changed twice every week to ensure optimal quality. At the end of the study, fish samples were collected from each replicate, weighed, and taken to the laboratory for further processing. The fish were stunned, dissected, and the gonads were harvested, weighed, and fixed in Bouin's fluid for 24–48 hours before proceeding with histological analysis, as described by Cevaco et al. (1997). The Gonadosomatic index (GSI) was estimated as described by Çek et al. (2001). The fish ovarian developments were assessed on gross morphologically and histologically, as adopted and modified from earlier characterizations and classifications (Cek et al., 2001; Saka and Adeyemo, 2015). Body weights, ovarian weights, and GSI measurements were subjected to analysis of variance using IBM SPSS (Version 21), and mean differences were separated using Duncan's New Multiple Range Test procedure, accepted at the 0.05 probability level.

Table1. Ovarian development of C. gariepinus catfish fed varying dietary levels of CBM

Indices	T1	T2	T3	T4	T5	SEM
B. W (g)	33.24^{ab}	37.73^{a}	34.47^{ab}	28.24^{b}	29.33^{b}	1.652*
O. W (g)	0.358^{b}	2.356^{a}	0.579^{b}	1.269^{ab}	0.359^{b}	0.175*
GSI	1.124^{b}	6.22^{a}	1.646^{b}	3.951^{ab}	1.207^{b}	0.439*

^{abc} Row means with different superscripts are significantly (P<0.05) different; B. W.: Final body weight; O.W.: Ovary weight; GSI: Gonadosomatic index.

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Results

The results of the study showed that feeding fish with CBM at 10% dietary inclusion (T2) resulted in a significant improvement ($P<0.05$) in body weight (BW), ovarian weight (OW), and GSI values compared to the other groups. The OW and GSI values in the control, T3, and T5 groups were similar ($P\geq0.05$). The OW and GSI values in T4 were similar ($P\geq0.05$) to T2 and those of the other groups. Moreover, the inclusion of cocoa bean meal in the diet of *Clarias gariepinus* catfish improved ovarian growth and development, as reflected in the gross morphology of the ovary. At week 9, the ovaries of the fish in T2 and T4 were observed to be better developed (stage IV) than those in the other treatment groups. The ovaries of the fish in the control group were in stage II of ovarian development, while those of T3 (20% CBM) and T5 (50% CBM) were in stage III of ovarian development.

The study observed a group synchronous type of ovarian development in the fish ovary. The results of the histology studies revealed that CBM caused a general improvement in the follicular development of the catfish ovaries. While oocytes in all developmental stages were observed in all groups, T1 showed the least development, with the secondary growth phase being predominant. On the other hand, the ovaries of T2, T3, T4, and T5 fish predominantly had oocytes in the maturation phase (stage 6). Additionally, ovaries of T2 (10% CBM) showed the optimum maturation, with sparse levels of oocytes in the secondary growth phase.

Conclusion

From the observation of this study, it can be suggested that CBM inclusion in fish feed can facilitate ovarian maturation. However, optimum ovarian maturation can be achieved with 10% CBM inclusion in fish meals.

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COMPARATIVE ANALYSIS OF THE ENVIRONMENTAL SUSTAINABILITY OF MACROALGAE, OYSTERS, AND LOW TROPHIC LEVEL FISH NATIVES TO SOUTH AMERICA

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Introduction

According to the UN (2019), there are predictions that we can reach up to 10 billion people in 2050. Aquaculture can help overcome the protein deficit generated by the high population. For this, the production of aquatic organisms should increase, and thus, it is essential to use effective production systems to prevent adverse effects on the producer, the environment, or society (SAAD et al., 2018). In this way, farming low trophic species may be more sustainable than farming high trophic species. In this study, environmental sustainability indicators of producing organisms (macroalgae), filter feeders (oysters), and allochthonous food ingesters (low trophic level fish) were comparatively analyzed using a benchmark framework.

Materials and methods

Secondary data were compiled for 19 environmental sustainability indicators (Valenti et al., 2018) previously obtained for seven aquaculture systems. Five are monocultures. They include the farming of the macroalgae *Hypnea pseudomusciformes* (Pereira et al., 2021), the oyster *Crassostrea gasar* in tropical (Sampaio et al., 2023) and in subtropical regions (Miraldo, 2015), the fish tambatinga, a hybrid of *Colossoma macropomum* and *Piaractus brachypomus* (Gilson, 2019), and tambaqui, *Colossoma macropomum* (Dantas, 2017). Two are integrated systems: tambaqui in hapa inside Amazon river prawn (*Macrobrachium amazonicum*) ponds (Tambaqui IMTA-Hapa) and tambaqui and Amazon river prawn both free in ponds (Tambaqui IMTA-Free) (Dantas, 2017). These seven systems were used as models to assess the environmental sustainability in low trophic species aquaculture. The benchmark tool was applied, establishing reference values for comparing indicators between the systems.

Results

Macroagae showed the highest sustainable score for ten indicators, tropical and subtropical oysters for seven, and tambatinga for one. Tambaqui IMTA-Hapa received the lowest score for eight indicators, subtropical oyster for six, tambatinga and tambaqui monoculture for three, and Tambaqui IMTA-Free and tropical oyster for two.

Discussion

Environmental sustainability showed different patterns in the culture of organisms from different trophic levels. Considering the culture models used in the present study, environmental sustainability seems to decrease as the farmed species' trophic level increases. Integrated low-trophic fish farming systems did not show environmental sustainability superior to monocultures, as could be anticipated. Nevertheless, comparison between production systems is complex due to their large variations. The tremendous biological differences between cultivated species require very different culture systems. In addition, there are various levels of intensification, feeding, and management to maintain water quality. The sustainability indicators measure common points in different farm systems necessary for sustainability analysis. The difficulty in comparing them in an integrated way arises from using variables of various dimensions and unities. Therefore, a standardization and integration technique is needed. Generally, the benchmark analysis met this need. It effectively showed the differences between the indicators in the various analyzed systems in a more accessible and standardized way.

Conclusion

The benchmarking framework carried out with seven aquaculture systems indicated that macroalgae, tropical, and subtropical oyster cultures are more environmentally sustainable than low trophic level fish cultures. The cultures of these extractive organisms do not depend on the supply of an allochthonous diet and are performed at sea. Integrated low-trophic fish farming systems have not shown environmental sustainability much superior to monocultures as expected. Indeed, the culture designs and the species used play a preponderant role in the sustainability of the aquaculture systems.

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Using the benchmark framework proposed in the current study effectively ranks the production systems according to each indicator, allowing quick visualization. The magnitude of the differences in an indicator in the various systems is somewhat masked by standardization when there is a substantial asymmetry in the distribution of the numerical values. Modifications in the standardization formulas should be studied to make it easier to see the differences.

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(AquaVitae – Horizon 2020; Sustainable Aquaculture Network; CNPq; FAPESP)

SILVER NANOPARTICLES SYNTHESIZED IN PLANT EXTRACT AGAINST ACUTE HEPATOPANCREATIC NECROSIS OF WHITE SHRIMP, MINIMUM INHIBITORY CONCENTRATION ESTIMATED BY MULTIPLE MODELS

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Worldwide, Mexico is the sixth place in production of farmed white shrimp (*Penaeus vannamei*). This activity has suffered great economic losses due to the acute Hepatopancreatic Necrosis disease (AHPND) which is caused by a strain of *Vibrio parahaemolyticus*. For control, the first option is the application of antibiotics in food, causing changes in the environment and bacterial communities, this has caused greater virulence and resistance of pathogenic bacteria. An alternative treatment is silver nanoparticles (AgNPs) generated by green synthesis, which have been shown to have antibacterial capacity, by destroying the cell membrane or passing into the interior of the cell. However, the doses at which these are effective are still unknown. For this reason, the objective of this research is to calculate the minimum inhibitory concentration (MIC) using the Gompertz, Richard and Logistic model of AgNPs-Ep biosynthesizes against a strain of *V. parahaemolyticus*, and to test the effect of applying different doses and concentrations of those synthesized from *Euphorbia prostrata* (Ep) on *V. parahaemolyticus* causing (VpAHPND) in white shrimp. Aqueous and ethanolic extracts of Ep were obtained, after which an analysis of phenols and flavonoids was carried out. In the antibiograms, the AgNPs with 20% alcohol extract (Ep) and 30% alcohol extracts were the treatments with the highest inhibition 18±1.73 and 17.67±2.08 mm (well dilution) for *V. parahaemolyticus*. A microdilution in broth was carried out and the inhibitory agents (aqueous extracts, ethanolic and AgNPs-Ep) and 20 µL of Vibrio inoculum, the MIC was taken as the lowest concentration of antibacterial agents that inhibit the growth of *Vibrio*. Finally, extract concentration with inhibition was (OH at 10% was 57-73; 20% from 65-81 and 30% from 32-65 mg/mL) and nanoparticles (from AgNPs-EpOH of 10-30% was 6.2 ug/mL). The calculation of the inhibitory concentration of V. parahaemolyticus was determined within the previous intervals of the optical density (OD) tests. Emphasized the usefulness of modified templates: Gompertz, Richards and Logistic. The Akaike index (AIC) was used to choose the winning model to calculate the models and inhibition curves of *V.parahaemolyticus* with different concentrations of Ep and AgNPs-Ep.

DEVELOPMENT AND APPLICATION OF AN INDIRECT ELISA TEST FOR TILAPIA LAKE VIRUS (TILV) DETECTION IN *Oreochromis niloticus*

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Introduction

Tilapia lake virus (TiLV) is an emerging virus that causes large-scale mortalities in tilapia farming and considered a threat to the global aquaculture industry with devastating economic losses. A serological test for detecting Tilapia lake virus (TiLV) in *Oreochromis niloticus* might be helpful for epidemiological studies. For which an indirect enzyme-linked immune sorbent test (iELISA) was developed for the detection of TiLV antigen in tilapia tissue and mucus using polyclonal antisera against TiLV.

Materials and methods

The TiLV-positive tilapia samples were collected and the inoculum was prepared for the production of polyclonal antisera against TiLV in rabbit. The iELISA test was developed with the optimization of each step using the polyclonal antisera. The developed immunodetection test (iELISA) was validated against the existing reverse transcription-polymerase chain reaction (RT-PCR) based molecular detection method. The sensitivity and specificity of the developed iELISA were evaluated following the establishment of the cut-off value and optimisation of antigen and antibody concentrations. The iELISA test was applied for an epidemiological screening of TiLV in the field samples.

Results

The study discovered that the sample concentration of 50 µg/well was optimum and the dilution of the polyclonal antibody was appropriate at 1:4000 (Fig. 1) and the enzyme conjugated secondary antibody was appropriate at 1:65,000. The optimised iELISA showed high analytical sensitivity with average specificity. The Positive Likelihood Ratio (LR+, LR-) was 1.75, and the Negative Likelihood Ratio (LR-), 0.29. The test's estimated Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 76.19% and 65.62%, respectively. The developed iELISA's accuracy was calculated to be 73.28%. When samples from the field (India) were used for an immunological survey, using the developed iELISA, 155/195 fishes tested positive, suggesting a 79.48% positive rate for the TiLV antigen. The mucus had the highest positive rate of TiLV (92.3%) than all the tissue samples tested. By adopting a non-invasive approach to collect mucus as a sample for iELISA, the newly developed iELISA demonstrated sensitive detection and may be useful for thorough assessments of TiLV infections and monitoring disease status even from samples that appear healthy.

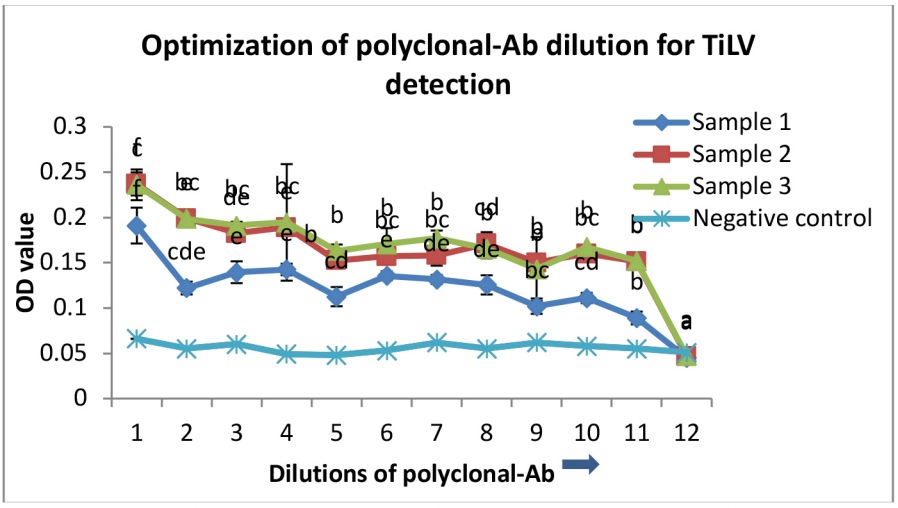

Fig 1. Detection limits of TiLV in fish tissue extracts (pool of kidney, liver, brain) by iELISA with various polyclonal-Ab dilutions. Mean±SD. Sig diff $p \leq 0.05$ for dilutions marked with different superscripts.

FIGHTING THREATS WITH MANY OTHERS: CHARACTERISATION OF THE LARGE NF-κB INHIBITORS FAMILY IN SALMONID FISH

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Introduction

The evolutionary ancient family of NF-κB (nuclear factor kappa-light chain-enhancer of activated B cells)/Rel transcription factors is activated by environmental and endogenous cues including viral and bacterial pathogen-associated molecular patterns (PAMPs) or cytokines. The activated NF-κB pathways play a major role in immune and stress responses. The inhibitors of NF-κB (IκB) regulate the activity of NF-κB through a dynamic interplay with their antagonists, IκB kinases (IKK). In mammals, the family of NF-κB inhibitors comprises nine members with different or mutual affinities for the various combinations of NF-κB/Rel dimers. In lower vertebrates, a detailed analysis of the family is still lacking. This study provides the first characterisation of the entire NF-κB-inhibitor family in a salmonid fish (rainbow trout, *Oncorhynchus mykiss*).

Material & methods

The different NF-κB inhibitors in rainbow trout (*Oncorhynchus mykiss*) were identified using the NCBI-database. We conducted structural analyses using phylogeny, synteny and 3D-modelling of proteins to characterize the canonical iκbα and iκbε proteins, the nuclear iκbδ and iκbζ proteins and bcl3. Additionally, comprehensive iκb overexpression studies in CHSE-214 cells were performed for functional analyses of iκbα and iκbε.

Results

In this study, six *nfkbia*, two *nfkbie*, two *nfkbid*, two *nfkbiz* and two *bcl3* genes in rainbow trout were identified. The sequence identity of ohnologous *nfkbi*-encoded iκb proteins from rainbow trout ranges from 82% to 100%. The two pairs of the iκbα ohnologs a1/a2 versus b1/b2 share about 60% identity. However, the comparison of the iκbα paralogs a1/a2 or b1/b2 versus c1/c2 revealed a sequence identity below 30%. A phylogenetic analysis across the amino-acid sequences of all IκB proteins from human and fishes revealed that this pair of ohnologous iκbα sequences (c1 and c2) cluster with the human IκBβ factor, while the other two pairs of iκbα ohnologs (a1 and a2 as well as b1 and b2) cluster with the human IκBα factor.

Functional analysis revealed significantly higher transcript levels of *nfkbia-a,* compared to the transcript levels of *nfkbia-b* and *nfkbia-c,* in immune tissues and immune-cell fractions. Also, the expression of *nfkbie-a1* was significantly higher than for *nfkbie-a2* in different immune tissues. With regard to tissue-specific expression patterns, the levels of *nfkbia-a* and *nfkbie* were significantly higher in immune-relevant tissues including head kidney, gill and spleen, but there was (almost) no differential expression of other *nfkbi* genes between tissues. Confocal imaging indicated a distinct localisation of iκbα and iκbε constructs in salmonid fish cell model. The concentration of iκbα was higher in the cytoplasm compared to the nucleus, whereas iκbε factor seem to be evenly distributed across cytoplasm and nucleus (Figure 1).

The overexpression of iκbα and iκbε robustly reduced the basal NF-κB activity down to a 0.09-fold and 0.06-fold, respectively, compared to the non-transfected controls (Figure 2). Stimulation of the non-transfected CHSE-214 cells with the fungal cell-wall component zymosan doubled the NF-κB activity (2.0-fold) compared to the basal state. Increasing amounts of the overexpressed iκbα and iκbε factors from rainbow trout lowered this stimulated nf-κb activity in a dose-dependent fashion compared to the non-transfected cells.

Furthermore, stimulation with zymosan increased the transcript levels of characteristic inflammatory markers such as *il1b* and *cxcl8*, but also *nfkbia*. Nevertheless, the transcript levels of the induced immune genes in non-transfected cells versus cells expressing iκbα or iκbε were not significantly different after stimulation with zymosan.

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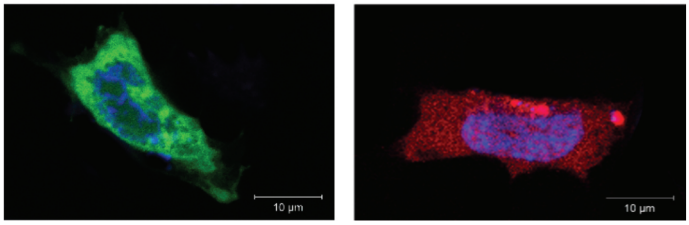

Figure 1. Confocal analysis of overexpression of (a) gfp-tagged iκbα (green fluorescence) or (b) plum-tagged iκbε constructs (red fluorescence) in salmonid model cells.

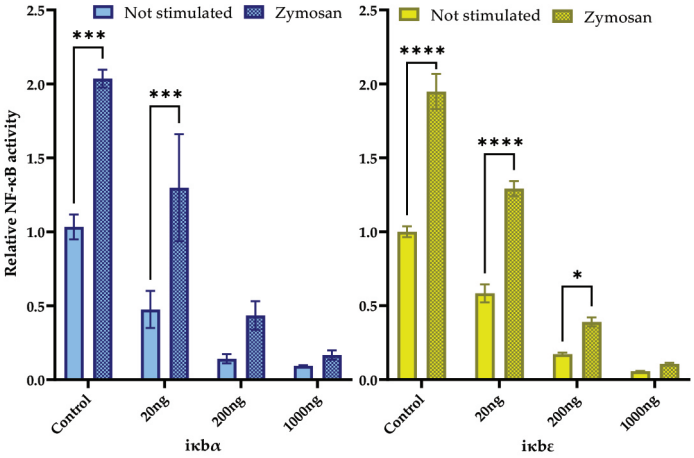

Figure 2. Overexpression of gfp-tagged iκbα (green fluorescence) or plum-tagged iκbε constructs (red fluorescence) in salmonid model cells.

Discussion and conclusion

In this study, we describe the iκb family in rainbow trout which comprises each multiple gene copies coding for iκbα, iκbε, iκbδ, iκbζ and bcl3. We compared canonical iκbα and iκbε proteins, the nuclear iκbδ and iκbζ proteins and bcl3 and identified various structural differences. Our comprehensive overexpression studies in fish cells confirmed a NF-κB-regulation potential of the iκb factors investigated and reveal the first functional results on iκbε in lower vertebrates.

THE ROLE OF AQUACULTURE IN CIRCULAR FOOD SYSTEMS IN EUROPE

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Introduction

Designing circular food systems is seen as a promising way to reduce the pressure of food systems on ecosystems. In circular food systems, biomass from arable land and water bodies is prioritised for human food and other basic needs, rather than animal feed (Boer & Ittersum, 2018; Muscat et al., 2021; van Zanten et al., 2019). Along this paradigm, farm animals, including aquaculture species, should not consume human food, but instead convert by-products from crops, livestock, and fisheries that are inedible for humans into edible biomass.

Previous research on food system modelling has primarily focused on livestock, while aquaculture can also play an important role in circular food systems. In aquaculture, species from a wide range of trophic levels can be cultivated, opening various opportunities for upcycling of food system by-products.

Our aim is to determine the role of aquaculture in circular food systems in Europe. We will use circular food system modelling to gain insights into which aquaculture species could be produced, how much aquatic food can be produced when animals are fed exclusively with food system by-products and what by-products can be recycled as fish feed.

Materials and Methods

We used a resource allocation model to allocate by-products (including by-products from fisheries), food-waste (derived from the EAT-Lancet diet) and grass resources to livestock and aquaculture species (fig 1.) to maximise animal source protein (objective). Livestock systems included in the model were based on van Hal et al., (2020) and van Selm et al., (2022), whereas we developed individual growth models for four commonly produced species in Europe: Atlantic Salmon (*Salmo salar*), European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata)* and blue mussel (*Mytilus edulis*). To this end, we used DEB modelling to estimate the growth and energy requirement of each species for different water temperature conditions representing marine-producing areas in Europe. Preliminary results of the model on what by-products can be recycled as fish feed and which aquaculture species could be produced will be presented.

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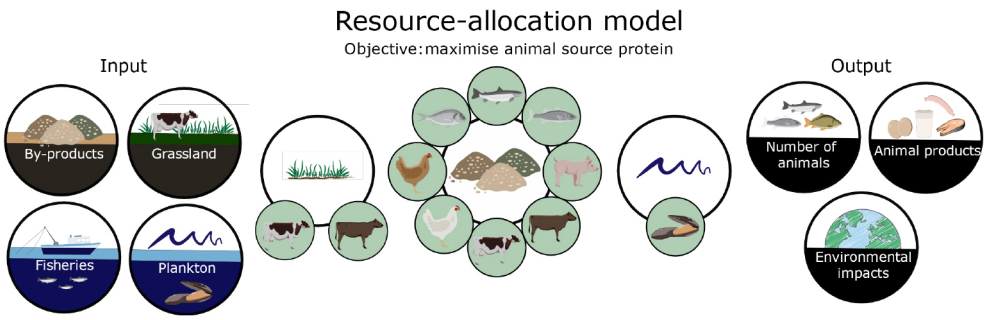

Figure 1 schematic overview of the resource-allocation model used in this study

ANTIMICROBIAL ACTIVITY OF OVOTRANSFERRIN AGAINST AHPND-CAUSING *Vibrio parahaemolyticus*

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Introduction

As the shrimp aquaculture industry is growing exponentially to meet increasing demands, it needs to overcome certain challenges. One of the challenges that come with intensification of aquaculture is the emergence of pathogens. Acute Hepatopancreatic Necrosis Disease (AHPND) is a bacterial infection caused predominantly by *Vibrio* (*V.*) *parahaemolyticus*. As the use of antimicrobials to fight infections is strongly discouraged due to the emergence of antimicrobial resistance bacteria, the search for alternatives is essential. Transferrins are a family of natural antimicrobial and immunomodulating glycoproteins, which have been shown to be effective against multiple pathogenic bacteria. Ovotransferrin (ovoTF) is an easily accessible transferrin, extracted from egg white. The aim of this study is to investigate the *in vitro* antimicrobial activity ovoTF towards AHPND-causing *V. parahaemolyticus*.

Methodology

Three AHPND-causing *V. parahaemolyticus* strains with different origins were used for these experiments. Different concentrations, ranging from 0.001 mg/ml to 10 mg/ml, of ovoTF were tested for their effects on growth curves, and concentrations ranging from 0.125 mg/ml to 1 mg/ml were tested for their effects on swimming and swarming motility, surface hydrophobicity and biofilm formation, and caseinase, lipase and phospholipase secretion. Live/dead flow cytometry was used to assess the ability of ovoTF to kill the bacterial cells. Furthermore, as ovoTF is able to act as a serine protease, its effect on the PirAB toxins produced by *V. parahaemolyticus* was investigated.

Results

Our results showed that ovoTF was able to delay growth of the bacteria significantly at a concentration of 0.1 mg/ml, and even inhibit growth at higher concentrations. Furthermore, biofilm formation by the bacteria is inhibited, which can be partly explained by the observed lower cell surface hydrophobicity after addition of the ovoTF. Swimming motility was inhibited, while swarming motility was induced. Secretion of lipase was inhibited by higher concentrations of ovoTF, while caseinase production was induced. On phospholipase secretion, the transferrin did not seem to have a significant effect. Results of live/dead flow cytometry and the degradation assay are to be included.

Clearly, ovotransferrin is putting the bacteria under pressure *in vitro* and from the growth assays, it can be concluded that it has a strong antibacterial effect on the AHPND-related *V. parahaemolyticus*. Together, these *in vitro* results indicate that ovoTF is a promising anti-microbial protein, and should be further investigated *in vivo* for its use as a natural compound for controlling AHPND in farmed shrimp.

SUCCESSFUL REPLACEMENT OF *artemia* BY CYOPRESERVED BARNACLE NAUPLII in EUROPEAN SEABASS LARVAL REARING

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Introduction

Live feeds have a key role in the larval rearing of marine fish. Their key advantage relative to dry feeds is that they can live together with the fish larvae in a tank and elicit their predatory behaviour, while providing high-quality bioavailable nutrients. The drawback of using them is the necessity to produce them simultaneously with the fish larvae, with risks of culture crashes that may jeopardize the production of fish larvae by lack of prey. Moreover, some of the most widely used live prey, *Artemia* and rotifers, often have a poor content in essential fatty acids (EPA and DHA), and have to undertake an additional step of enrichment to provide the desired nutrients to the fish larvae. Planktonic A.S is producing CryoPlankton, which are cryopreserved barnacle nauplii, stored in liquid nitrogen, and which can be revitalized after thawing followed by a short incubation in seawater. They have a good EPA+DHA content and provide an "off-the-shelf" solution for live feed provision to larval rearing, relieving the cost of live feed production and securing the daily provision of feed. They have shown good performance for the larval rearing of ballan wrasse *Labrus bergylta* (Malzahn et al., 2022). Thus, the present experiment aimed at testing their use against the standard "clearwater" protocol for European sea bass, which relies on a ten days dark phase followed by first feeding with newly hatched, non-enriched *Artemia* (Chatain, 1994).

Materials and methods

European sea bass fertilized eggs were produced on Nov 15, 2022 by artificial fertilization of 8 females and 15 males from a 3rd generation West Mediterranean seabass line selected for fast growth at Ifremer (Palavas-les-Flots, France). Nine experimental tanks of 0.5m^3 were each stocked with 33632 live fertilized eggs, leading to an estimated 30269 hatched larvae on Nov 18, 2022. These larvae were subjected to three rearing protocols, each in triplicate:

- Control: Clearwater rearing of sea bass with *Artemia* only. This protocol is the standard at Ifremer. It included a dark phase of 10 days post-hatching (dph) for vitellus resorption, before direct feeding with newly-hatched, non-enriched *Artemia* nauplii (AF, Inve) until 29 dph. Enriched *Artemia* (BF, Inve, 24 h post-hatching enrichment with Easy-DHA from Selco) were given from 25 to 56 days post-hatching (dph). Fish were weaned to dry feed (Biomar Larviva Pro-start) at 57 dph after co-feeding from 36 to 56 dph.
- CryoPlankton only (Cryo-Dry). In this protocol, there was no dark phase and fish were fed as soon as the mouth opens at 5 dph, and until 12 dph with CryoPlankton Small (cryopreserved *Balanus crenatus* nauplii from Planktonic A.S., Norway), then from 10 to 42 dph with CryoPlankton large (cryopreserved *Semibalanus balanoides* nauplii from Planktonic A.S., Norway). They were weaned to dry feed (Biomar Larviva Pro-Start) at 43 dph after co-feeding from 25 to 43 dph.
- CryoPlankton and enriched *Artemia* (Cryo-Art). In this protocol, there was no dark phase either, and fish were fed as soon as the mouth opens at 5 dph, and until 12 dph with CryoPlankton Small, from 10 to 31 dph with CryoPlankton large, and with enriched *Artemia* from 25 to 56 days post-hatching. They were weaned to dry feed at 57 dph after co-feeding from 36 to 56 dph.

The temperature was set to 16°C from 1 to 45 dph, was progressively increased to 22°C at 69 dph, then remained constant at 22°C. The photoperiod was set at 12L/12D, after a dark phase (0L/24D) until 9 dph for the Control, and immediately at hatching for the other two treatments. Salinity was decreased from 33 ppt at 0 dph to 25 ppt from 9 to 46 dph, when it was released to natural salinity (35-39 ppt). CryoPlankton was thawed for 5 minutes in natural seawater, rinsed for 10 minutes with seawater on a 100 µm sieve, then revived for 1h in 6°C, 25ppt seawater. All live feeds were distributed 12h/day with a peristaltic pump. Standard length (SL) was measured with ImageJ on pictures at 5, 10, 14, 24, 46 and 75 dph. Sample size was 30 fish/tank except at 14, 46 and 75 dph where it was 50 fish/tank. In addition, we estimated % swimbladder inflation on the pictures at 14 dph. At 75 dph, in addition, all fish were sorted for swimbladder inflation by flotation in 40ppt sea water, and the total number of fish per tank was estimated by weighing after a mean weight was estimated on a minimum number of 1500 fish per tank. The individual body weight (BW) of 50 fish per tank was measured to the nearest 0.01g. Data were analysed using mixed models with treatment as a fixed effect and tank as a random effect using lme4 in R. For swimbladder inflation and survival we used a logit transformation of the data.

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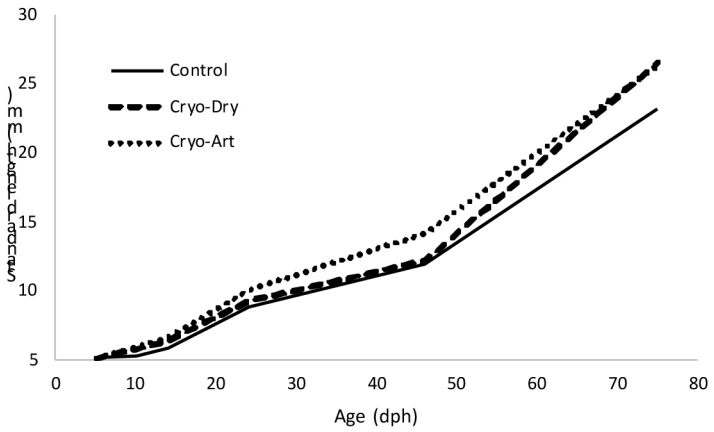

Figure 1: Growth in standard length over 75 days of European seabass with three larval rearing sequences using or not CryoPlankton.

Results

At 5 dph, before the first feeding of the Cryo treatments, the average SL of the larvae was 5.18 mm, and there was no difference between treatments (P>0.60). Treatments started to diverge at 10 dph, where both Cryo treatments were larger than the Control (P<0.01; Figure 1). The same difference was apparent at 14 dph (P<0.05) but became marginally significant (P=0.051) at 24 dph. At 46 dph, the Cryo-Dry treatment was similar to the Control, while the Cryo-Art treatment was larger (P<0.01). At 75 dph, both Cryo treatments had higher SL than the Control (P<0.001). This was also true for BW, which was 278±8 mg in Cryo-Dry, 269±8 mg in Cryo-Art vs. 178±8 mg in the Control.

Survival at 75 dph was higher in the Control (38.4%, 95%CI[37.0-39.9]) than in Cryo-Art (32.9%, 95%CI[31.6-34.2]) and in Cryo-Dry (28.9%, 95%CI[27.7-29.7]) but the biomass per tank was 20-28% higher (P<0.01) in the Cryo treatments (2761±72g in Cryo-Art, 2599±72g in Cryo-Dry) than in the Control (2163±72g)

Swimbladder inflation at 14 dph was lower in Cryo-Dry (75.0%, 95%CI[64.0-83.4]) and Cryo-Art (82.9%, 95%CI[73.3-89.4)] than in the Control (97.5%, 95%CI[93.1-99.1]). However, at 75 dph, the proportion of fish with uninflated swimbladders was negligible (lower than 0.3%) in all treatments.

Discussion

At the end of the larval rearing, both Cryo treatments clearly outperformed the Control in terms of growth (+56 % for Cryo-Dry, +51% for Cryo-Art). The difference started to appear at 10 dph, where the Cryo treatments had been fed for 5 days with CryoPlankton Small while the Control was still growing on its reserves, waiting for its mouth to be large enough to ingest AF *Artemia*. At 46 dph, the Cryo-Dry treatment had regressed to the level of the Control, however we must note that contrary to Cryo-Art and Control, which were at the beginning of co-feeding Cryo-Dry was at the end of its weaning period, which logically somehow restricts growth. It caught up with the Cryo-Art treatment at the end of the experiment. In terms of survival, the Control was better than the Cryo treatments. This can be related to the percentage of non-inflated swimbladders, which was more important in the Cryo treatments at 14 dph, while there were no uninflated swimbladders in any treatment at 75 dph. As swimbladder inflation is expected to be complete before 14 dph, this means that the uninflated fish in the Cryo treatments died before the end of the larval rearing. Surface skimmers were in place at 7 dph (as usual with the Control treatment), but at that time Cryo fish were already fed for two days, so probably the skimming of fat at the surface layer was not done early enough or with enough efficiency, which should be taken into consideration for practical implementation. Despite the lower survival, the biomass produced was higher with the Cryo treatments, showing the relevance of using CryoPlankton for sea bass larval rearing.

Acknowledgments

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USE OF EXOGENOUS ENZYMES TO IMPROVE THE NUTRITIONAL VALUE OF *Lupinus Albus* AS FEED INGREDIENT IN EUROPEAN SEA BASS (*Dicentrarchus Labrax*) NUTRITION

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Introduction

The expansion of the aquaculture production has been linked with the need for a rapid increase in the production of aqua feed products. Among alternative ingredients, the plant ones have been extensively studied. The European Union is highly depended on soybean imports for its domestic use and is highly vulnerable to risks linked to global trade. In this frame, the key of bridging the gap between consumption and demand is to identify alternative forage plant ingredients for sustainable production. For livestock production, forage legumes are promising candidates and highly valued feed components, however, the presence of several antinutritional factors (ANFs) still limits their use in feeds (Kaushik et al., 2018). The digestibility of many ingredients can be improved with inclusion of exogenous enzymes due to their ability of breaking down the complex cell wall structure that encapsulates nutrients present in plant based feed (Ojha et al., 2019). In addition, by increasing access to protein for digestive proteases, carbohydrases can act as enhancers to improve nitrogen and amino acid utilisation (Tahir et al., 2008). Aim of the present study was to evaluate the replacement of soybean meal by lupin meal treated with exogenous enzymes in European sea bass nutrition.

Materials and methods

Lupin seeds (*Lupinus albus* cv. Tennis) were grounded to produce lupin meal. A product fermented by *Aspergillus niger* containing several exogenous enzymes (Synergen™, Alltech Inc) was added at 0.05%. Moisture of lupin meal was adjusted to 45% and the lupin meal was treated for 4 hours at 50°C. Four commercial type diets, one control containing soybean meal as the sole plant protein at an inclusion level 15g/100g diet and three diets replacing soybean meal by treated lupin meal at three different levels (7.5 g/100g, 10 g/100g and 12.5 g/100g, Table 1) were formulated to be isonitrogenous (48%), isolipidic (17%) and isoenergetic. European sea bass juveniles (11.2g) were distributed in 12 tanks (50 fish/tank) in triplicate groups per diet. The experimental trial was conducted for 83 days in recirculation aquaculture system. Fish were fed *ad libitum* the experimental feeds three times per day. For a 2-week period prior to the end of the trial, faeces were collected, to evaluate nutrients digestibility.

Results and discussion

Cooking of legumes is a commonly used process that greatly improves the nutritional value of foods by reducing their ANFs (Patterson et al. 2017). In addition, the use of exogenous enzymes increases nutrient digestibility and bioavailability. In the current study inclusion of lupin meal, treated with exogenous enzymes, revealed positive results. Specifically, fish fed the Lupin1 diet showed significant higher final weight compared to the fish fed the control diet. Even though weight increase did not show significant differences, a trend (P=0.05) for higher weight was observed for fish treated with Lupin1 compare to those treated with control diet. Similar trend reflected also in feed conversion ratio (FCR) and specific growth rate (SGR) for the same groups. The growth parameters evaluated were not affected by the total replacement of soybean meal with treated lupin meal (Lupin3). Somatometric indices were fluctuated at similar levels, however, population fed the treated lupin meal presented lower liposomatic indices. Incorporation of exogenous enzymes treated lupin meal affected the digestibility of nutrients, the retention of protein and the activity of digestive enzymes (results are not shown). The present findings showed that lupin is a promising alternative to soyabean meal in the diet for *D. labrax* when treatment with exogenous enzymes is adopted.

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Table 1. Formulation of experimental feeds (g/100g).

	Control	Lupin 1	Lupin 2	Lupin 3
Fish meal	20.0	20.0	20.0	20.0
Krill meal	3.0	3.0	3.0	3.0
Blood meal	5.0	5.0	5.0	5.0
Wheat meal	10.3	12.1	12.8	12.6
Wheat Gluten	10.0	11.0	11.0	11.0
Corn gluten	15.0	15.0	14.8	15.0
Soybean meal	15.0	5.0	2.5	
Soy bean Concentrate	5.0	5.0	5.0	5.0
Lupin meal		**7.5**	**10.0**	**12.5**
Synergen™		**0.05**	**0.05**	**0.05**
Fish Oil	13.0	12.5	12.0	12.0
Nutrient composition (% wet weight)				
Protein	47.8	47.8	47.6	47.7
Fat	16.8	16.9	16.6	16.7
Starch	9.1	9.9	10.3	10.1
Fibre	1.0	1.2	1.3	1.4
DHA + EPA	3.3	3.2	3.1	3.1

Table 2. Growth parameters and experimental population indices.

	Control	Lupin 1	Lupin 2	Lupin 3
Final Weight (g)	59.96 ± 1.10^{a}	62.86 ± 1.65^{b}	60.56 ± 0.63^{ab}	60.11 ± 0.76^{ab}
Weight gain (g)	48.73 ± 1.09	51.64 ± 1.66	49.35± 0.63	48.89± 0.76
FCR	1.13 ± 0.02	1.05 ± 0.05	1.10 ± 0.02	1.12 ± 0.03
SGR	2.02 ± 0.02	2.08 ± 0.03	2.03 ± 0.01	2.03 ± 0.02
% consumption of BW	2.28 ± 0.04	2.15 ± 0.11	2.23 ± 0.05	2.25 ± 0.06
Viscerosomatic Index	13.46 ± 0.62	12.73 ± 2.62	14.65 ± 0.70	14.00 ± 0.99
Hepatosomatic Index	1.42 ± 0.12	1.55 ± 0.08	1.44 ± 0.15	1.52 ± 0.04
Liposomatic Index	5.56 ± 0.51	5.15 ± 0.73	4.89 ± 1.13	5.01 ± 0.57

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INVASIVE SPECIES FOR SUSTAINABLE AQUACULTURE – USE OF FISHMEAL PRODUCED FROM *Lagocephalus Sceleratus* IN DIETS FOR THE EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

Invasive species, also known as "Lessepsian species", have rapidly colonised the Eastern Mediterranean basin resulting in environmental and economic impacts on marine life, fisheries and human prosperity. Among them is *Lagocephalus sceleratus* (Gmelin, 1789), a highly opportunistic fish, which causes damages to fishing gear, catch and local biodiversity. It is a highly-toxic species with no commercial economic value for the Mediterranean market. The lack of predators in addition with the high growth and reproduction rate forecasts the future spread of these invasive species (Nader et al., 2012). This scenario leads to alternative management actions for these species. In addition, the expansion of aquaculture has been linked to the need for rapid growth in aquafeed production, however overexploitation of marine resources for fishmeal production is leading to alternative sources of protein. The objective of the current study was to evaluate the use of fishmeal produced from *Lagocephalus sceleratus* as a replacement for conventional fishmeal in nutrition of European sea bass.

Materials and methods

Individuals of *Lagocephalus sceleratus* were transferred to the laboratory of Applied Fish Nutrition of HCMR. LM was obtained after cooking, pressing drying and grinding. The produced LM was treated with two different temperatures to deactivate the tetrodotoxin (TTX) detected in the specific species. Low temperature LM (LT-LM) was treated at 160°C and high temperature LM (HT-LM) at 210°C. Five commercial type diets were formulated to replace fishmeal by unprocessed LM (UnP-LM) and by LM treated with low and high temperature. UnP-LM was used as a negative control to assess the tolerance of European sea bass to TTX. Formulation of the experimental diets are presented in Table 1. All the diets were formulated to be isonitrogenous (47%), isolipidic (17%) and isoenergetic. Micronutrients (essential amino acids, phosphorus, essential vitamins and minerals) were balanced among the experimental diets. The experimental diets were produced by extrusion at HCMR.

European sea bass individuals of 39g initial body weight were distributed in 15 tanks. The experimental diets were tested in triplicate groups of fish. The diets were fed in an open-flow system with controlled and monitored water parameters to the experimental groups over a period of 106 days. Temperature was held at 20°C and O_2 at >90% saturation. Experimental population fed *ad libitum* the produced feeds two times per day and feed consumed (g) was recorded daily. Fish were weighted individually at the beginning, intermediately and at the end of the experimental trial.

Results and discussion

At the end of the experimental trial fish were individually sampled and the Key production (KPIs) and somatometric indices were calculated (Table 2). Most of the production indices evaluated revealed significant differences between experimental population. Specific growth rate (SGR) was significant higher for group fed with unprocessed LM compare to the group fed low temperature LM. No significant differences observed for daily feed intake, although lower feed consumption was observed in fish fed unprocessed LM. Feed conversion ratio (FCR) showed significant lower FCR for fish fed diet UnP-LM. Viscerosomatic index (VSIndex) was similar for all treated populations. In contrast, hepatosomatic index (HSIndex) showed significant differences among experimental fish. Lower performance in KPIs for fish fed diets containing high and low temperature LM is probably associated with the degradation of the nutritional value of the produced LM since the high temperature used to deactivate TTX (160 & 210°C) leads to a higher oxidation status of the meals or feeds, degradation of highly unsaturated fatty acids and a reduction in the nutritional value of the produced LM (Phung et al., 2020). Concluding, further evaluation is required to assess the long-term dietary exposure to TTX in UnP LM.

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Table 1: Formulation of experimental diets (%).

	Control	UnP-LM	HT-LM	LT-LM	Combo
Fishmeal conventional	30	-	-	-	15
LM Unprocessed	-	30	-	-	-
LM High temp.	-	-	30	-	-
LM Low temp.	-	-	-	30	15
Wheat meal	10.9	10.9	10.9	10.9	10.9
Corn gluten	19	19	19	19	19
Soybean Concentrate	17	17	17	17	17
Soybean meal	9	9	9	9	9
Fish Oil	13	13	13	13	13
Monocalcium phosphate	0.5	0.5	0.5	0.5	0.5
Mineral & Vitamin	0.4	0.4	0.4	0.4	0.4
Lysine	0.1	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1	0.1

Table 2: Results of Key production indices and somatometric indices

	Control	UnP-LM	HT-LM	LT-LM	FM/LT-LM
Final Weight	103.3 ± 4.3^{ab}	111.8 ± 2.4^{a}	98.5 ± 4.4^{b}	98.9 ± 3.0^{b}	106.1 ± 5.9^{ab}
SGR (%/day)	0.94 ± 0.04^{ab}	1.00 ± 0.05^{a}	0.89 ± 0.04^{ab}	0.87 ± 0.05^{b}	0.92 ± 0.05^{ab}
Daily feed intake (% BW/day)	1.22±0.10	1.17±0.09	1.31±0.03	1.29±0.01	1.24±0.01
FCR	1.45 ± 0.16^{ab}	1.32 ± 0.11^{a}	1.64 ± 0.02^{b}	1.65 ± 0.10^{b}	1.53 ± 0.08^{ab}
VSIndex	10.98±0.44	10.86±0.25	11.77±0.60	10.80±0.35	10.82±0.45
HSIndex	1.67 ± 0.03^{a}	1.31 ± 0.10^{b}	1.77 ± 0.03^{a}	1.50 ± 0.01^{c}	1.48 ± 0.04^{c}

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DISSECTING THE ROLE OF CHAPERONE-MEDIATED AUTOPHAGY IN THE INTOLERANCE TO CARBOHYDRATES OF THE RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Sustainable aquaculture production requires a greater reduction in the use of fish-based ingredients, and one avenue investigated over the past years is the increase in the proportion of plant-derived digestible carbohydrates in aquafeeds. However, this strategy presents a number of drawbacks for high trophic level teleost fish such as rainbow trout (RT, *Oncorhynchus mykiss*), which show, for still unknown reasons, reduced growth associated with persistent postprandial hyperglycemia and hepatomegaly, when fed diets containing more than 20% of carbohydrates (1,2). Among the factors that may be involved in the apparent glucose intolerance of RTs, one of the major pathways of lysosomal catabolism known as Chaperone-Mediated Autophagy (CMA) attracted our attention. In mammals, CMA is described as a critical player in the turnover of glucose and lipid metabolism-related enzymes, and dysregulation of this function has been shown to lead to significant alterations in liver metabolic homeostasis (3). However, CMA has only recently been identified in fish (4) and no data are currently available regarding its regulation as well as its role in the metabolic specificities of RT. In this work, we first assessed *in vitro* the existence of functional CMA in the RT. We then studied its regulation upon high glucose treatment and identified the underlying mechanisms. Finally, we studied the impact of silencing either of the two paralogous genes encoding the CMA-essential factor lysosome-associated membrane protein type 2A (LAMP2A) on the overall proteostasis of RT.

Material and methods

First, we established a trout hepatoma cell line (RTH-149) stably expressing a fluorescent reporter (KFERQ-PA-mCherry1) previously used to track CMA in mammalian cells (5), and more recently validated in medaka fibroblasts (4). Then, we exposed the cells either to mild-oxidative stress (H2O2, 25 µM), which activates CMA in mammals (6), or to high glucose (HG, 25 mM). We monitored by advanced imaging both the cellular localization and the half-life of the reporter. Subsequently, we used OxyBlot to analyze the oxidative stress status in cells incubated with HG or H2O2, and we explored the relationship between oxidative stress and CMA using antioxidants and specific mitochondrial inhibitors. Besides, we investigated the role of the nuclear factor erythroid-derived 2, like 2 (NRF2) on the effects of HG on CMA in RTH-149 cells using immunofluorescences, gene expression, *in silico* analysis, and siRNA-mediated knockdown approaches. Finally, to gain insight into the specific contribution of each of the two RT LAMP2As to the overall proteostasis of RTH-149 cells under an HG condition, we performed comparative quantitative proteomics after transfection with siRNAs designed to specifically target each of the *lamp2a* paralogs (hereafter referred to as si14 and si31, respectively).

Results

Our results showed that upon HG or H2O2 exposure, the KFERQ-CMA reporter accumulated in characteristic CMA-puncta that colocalized with lysosomes (**Fig. 1A**). Moreover, the half-life of the reporter was substantially shortened under these conditions compared to the CT, suggesting an active CMA-flux and supporting the existence of functional CMA in the RT. Then we observed that these mechanisms underlying the effects of HG on CMA in RTH-149 cells depended on the generation of reactive oxygen species (ROS) (**Fig. 1B**) at the mitochondrial level. In addition, the results showed that the NRF2 transcription factor mediates the upregulation of CMA by increasing the mRNA levels of both *Lamp2a*.

Finally, proteomics analysis reveals no significant alteration of any biological process in the cells transfected with si14 and exposed to HG, whereas different processes associated with cellular metabolism and its regulation were identified in si31 transfections (**Fig. 1C**), supporting a functional divergence between the two LAMP2As during hyperglycemia.

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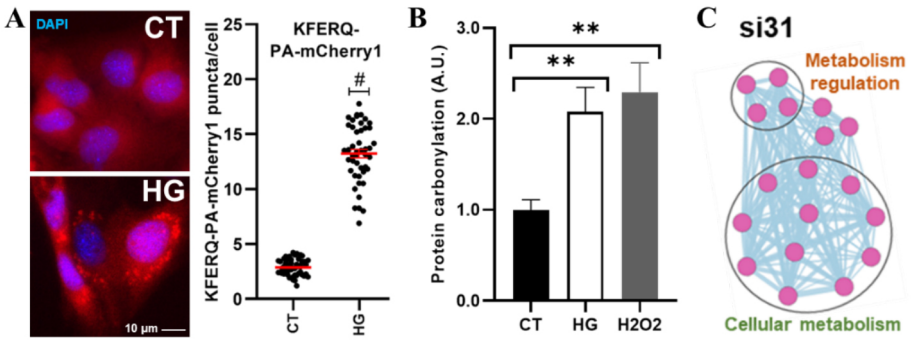

Figure 1. (**A**) Representative images of RTH-149 cells transfected with a KFERQ-reporter and incubated 16 h with CT (5 mM glucose) or HG (25 mM), and quantification of the number of puncta per cell. Values correspond to individual images with >15 images/experiment. N=3 independent experiments. (**B**) Protein carbonylation levels (OxyBlot) of cells incubated with CT, HG, or H2O2 for 16 h. N=4 independent experiments. (**C**) Network representation of the Gene Ontology Biological Processes modified by si31 transfection compared to negative siRNA control, and HG exposure.

Discussion

These sets of experiments univocally revealed that RT, like medaka, exhibits functional CMA activity, and emphasized, for the first time, the strong responsiveness of RT to HG exposure via the NRF2 pathway. Besides, the result highlighted the role of CMA, especially mediated through the LAMP2A paralogue 31, on the regulation of cellular metabolism upon glucose overload. Altogether, this work underlines the importance of considering this selective autophagy process to understand carbohydrate intolerance in carnivorous fish. Future studies will consider novel approaches for exploiting CMA metabolic impacts through aquafeed formulation to, in the last term, optimize the use of plant-derived carbohydrates and contribute to more sustainable aquaculture.

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DEVELOPMENT AND CHARACTERIZATION OF NOVEL RAINBOW TROUT (*Oncorhynchus mykiss*) CELL-BASED ORGANOTYPIC INTESTINAL PROTOTYPES

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Introduction

Cell-based models provide valuable support in the search for alternative feed formulations in the aquaculture sector. A powerful *in vitro* tool should consist of epithelial and fibroblast cells. Indeed, as we recently demonstrated[1], subepithelial fibroblasts modulate epithelial cell proliferation and differentiation in the rainbow trout (RT) intestinal mucosa. Moreover, growing data[2,3] showed that increasingly elaborated *in vitro* models that closely recapitulate the morphological and functional features of the native organ, could generate feasible data for the *in vitro* versus *in vivo* correlations studies. The H2020-FETOPEN project Fish-AI, aims to develop such an *in vitro* screening platform based on rainbow trout digestive enzymes and intestinal cells to evaluate nutritional and health values of novel aquafeeds. As part of this project, we developed, characterized, and compared four gradually increasingly complex RT intestinal *in vitro* platforms from a morphological and functional point of view.

Material and methods

Rainbow trout proximal intestine epithelial cells (RTpi-MI) have been seeded on 1) the culture inserts ThinCert™ (TC) (Greiner BioOne, 0.4 m pore size, cat. No. 665640); 2) the same inserts but coated with the solubilized basement membrane matrix Matrigel® (MM); 3) the same but with the rainbow trout fibroblast cell line RTskin01 previously embedded within the Matrigel® matrix (MMfb); 4) the highly porous synthetic scaffolding Alvetex™ (Reprocell) (AV) previously populated with the same fibroblast cell line (AV). The generation of effective epithelial barriers *in vitro* has been investigated measuring the transepithelial electrical resistance (TEER) and the apparent permeability (P_{app}) to 4kDa FITC-dextran (FD4). Moreover, epithelial cell functionality has been assessed by investigating the enzymatic activity of alanine aminopeptidase (ALP), leucine aminopeptidase (LAP), and alkaline phosphatase (AP), 3 well-known enterocytes' brush border enzymes. Thereafter, the 4 platforms have been characterized for their morphological properties using histological, and immunohistochemical techniques.

Results

All models successfully established an efficient barrier preventing the paracellular flux of FD4 and showing a significant increase of the trans-epithelial electrical resistance (TEER) compared to baseline values of their respective controls (inserts without cells). The value at plateau became higher going from the simple through the more complex models (TC-MM-MMfb-AV). Moreover, the activity of the brush border enzymes alanine aminopeptidase (ALP), leucine aminopeptidase (LAP), and alkaline phosphatase (AP), followed the same gradual pattern, suggesting that the presence of an epithelial and mesenchymal interface significantly boosted epithelial cells differentiation. Morphological analysis showed that RTpi-MI

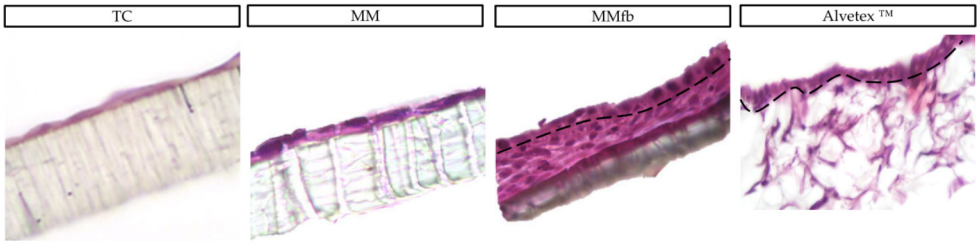

Fig. 1 Representative pictures of the four platforms after the formation of a fully functional epithelial barrier (TC = ThinCert™; MM = Matrigel®; MMfb = Matrigel® with fibroblasts; Alvetex™ with fibroblasts).

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had a flat shape when cultured alone onto TC and MM systems. Conversely, they assumed a more in vivo-like phenotype when fibroblasts were present, acquiring a cubic shape on MMfb and a cylindrical shape on AV with brush border enzymes neatly located on the apical membrane. In addition, fibroblast organization was also different depending on the culture support. While in MMfb they were densely packed with only limited extracellular space from one cell to the other, cells assumed a loose arrangement in AV more like what occurs in the connective tissue. Moreover, fibroblasts that populated the AV scaffolding synthesized and remodelled their own extracellular matrix, indicating a higher degree of cell differentiation.

Conclusion

The presence of fibroblasts boosted epithelial cell differentiation and polarization providing a more physiological environment. Therefore, our results indicate that reconstructing the epithelial-mesenchymal interface in vitro is important for the development of intestinal artificial platforms able to generate reliable, predictive data suitable for in vitro/ in vivo correlations studies. This observation suggests that, as we recently observed in vivo, also in vitro fibroblasts may represent an essential source of growth and signaling factors actively involved in the modulation of epithelial cell functions[1]. Overall, the platform based on the Alvetex scaffold better recreated the morphological and functional complexity of an artificial the intestinal mucosa therefore it is being tested for its ability to reliably predict the nutritional value of feed formulations.

Acknowledgements

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MICROPLASTICS UPTAKE OBSERVED IN A CELL-BASED ORGANOTYPIC RAINBOW TROUT (*Oncorhynchus mykiss*) INTESTINAL PLATFORM

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Introduction

Microplastics (MPs) are emerging as potential contaminants of aquafeeds that represent one of the main exposure routes for farmed fish. When ingested, MPs cross the gut epithelial barrier and are highly bioaccumulated in different tissues and organs [1]. This not only affects fish health but could be a threat for consumers. Several uptake mechanisms have been proposed for their absorption, including endocytosis, transcytosis, and paracellular diffusion. However, the knowledge of this phenomenon *in vivo* is still fragmentary and largely unknown, so that most pathways are only hypothesized. In this perspective, cell-based organotypic models represent a valuable tool to explore the molecular mechanisms at play. Therefore, a recently developed rainbow trout (RT, *Oncorhynchus mykiss*) *in vitro* intestinal platform, consisting of epithelial and connective cells [2], was used to evaluate MPs uptake.

Material and methods

Fluorescent MPs ranging from 1 to 5 μm (amino formaldehyde polymer, peak of emission at 636 nm when excited at 584 nm) were purchased from Cospheric LLC (Goleta, CA, USA). Some preliminary tests have been performed on RT proximal (RTpi-MI) and distal (RTdi-MI) intestine epithelial cells cultured directly onto plastic surface to: i) exclude any aspecific toxic effect; ii) verify MPs uptake; iii) identify the most suitable MPs concentration (12.5 mg/L^{-1}, 25 mg/ L^{-1}, 50 mg/ L^{-1}). MPs were diluted in L-15 culture medium and cells were exposed for 24 hours. Cellular viability was assessed through Neutral Red Uptake (NRU) assay. Since F-actin labelling defines cytoplasm extension and DAPI labels nuclei, their combination was used to determine MPs internalization. The organotypic intestinal platform was made of RTpi-MI and RTdi-MI cells seeded on the upper surface of an Alvetex™ (AV) insert, a highly porous synthetic scaffolding, previously populated with fibroblasts. Platforms were exposed to MPs for 2, 4 and 6 hours, after an effective epithelial barrier was established, as indicated by the trans-epithelial electrical resistance (TEER) value reaching its plateau. MPs uptake and distribution within the scaffolding were evaluated through histological and confocal (Nikon A1R confocal microscope) analysis.

Results

After 24 hours exposure, neither pathological changes nor alteration of cell viability were observed in both cell lines, regardless of the tested concentrations. MPs co-localization with F-actin around the nucleus, demonstrated that MPs internalization followed a dose-response pattern reaching the highest values at 50 mg/L^{-1} concentration (Figure 1). Since this corresponds to an environmental-relevant concentration [3] it has been selected as the exposure dose in the AV platform. After 2 hours of exposure, MPs crossed the epithelial barrier (Figure 2); after 4 and 6 hours, they progressively reached the deeper regions of the scaffolding, being absorbed in both epithelial and stromal cells (figure 2). Absorption appeared to be more effective in RT proximal intestine cell line than in the distal, suggesting that the latter discourages MPs absorption. This pattern reflects the function of the intestine *in vivo* whose proximal tract ensures 70% of nutrient absorption.

Conclusion

RT epithelial cells internalize MPs following a dose-dependent pattern. The organotypic platforms seem to replicate the regional absorption differences observed in the intestine, making them a reliable tool to investigate and explore the molecular mechanisms involved in MPs uptake. On this basis, we are now exploring the expression of specific transport-pathway transcripts to identify the one(s) actively involved.

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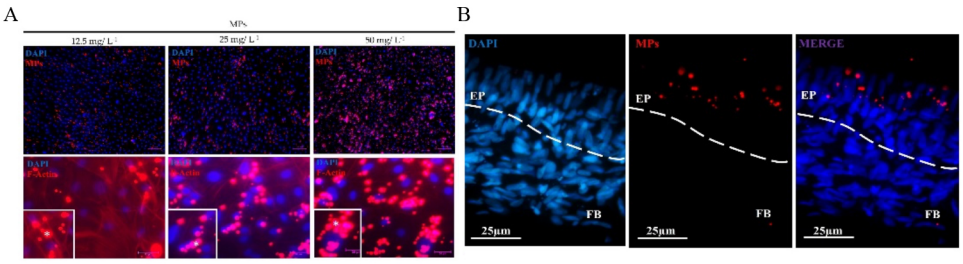

Figure 1. Panel A: F-actin staining showing MPs (red dots) absorption following a dose-dependent pattern in RT epithelial cells. Panel B: representative images of the AV platform showing that MPs cross the epithelial barrier *in vitro* and are absorbed by epithelial (EP, above the dotted line) and connective cells (FB, below the dotted line). Nuclei are counterstained with DAPI-blue signal.

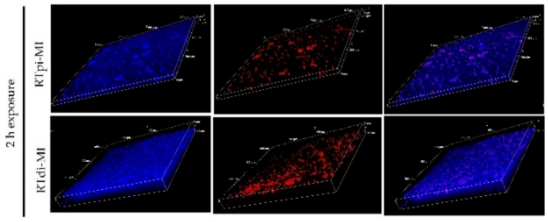

Figure 2. Representative confocal microscopy images showing MPs (red spots) distribution on the apical surface of the organotypic platforms after 2 h exposure. Note that MPs are more abundant in the RTdi-MI platform suggesting a slower absorption across the epithelial layer (nuclei are blue).

Acknowledgements

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BOLD-SHY PERSONALITIES IN AQUACULTURE: THE LINK BETWEEN BOLDNESS, STRESS TOLERANCE AND IMMUNITY IN EUROPEAN PERCH (*Perca fluviatilis*)

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Introduction

European perch, *Perca fluviatilis*, is of high commercial importance in European intensive aquaculture, with an annual production of over 900 t (FAO, 2019). However, the production still fails to meet market demands. One bottleneck is the European perch's high sensitivity to stress under captive conditions, which reduces immune resistance and at the same time increases the susceptibility to diseases (Milla et al., 2015). It has been hypothesized that the sensitivity to stress, and its impact on immunity, is related to the fish personalities. Therefore, beneficial personalities and stress-coping strategies may be promising targets for selective-breeding programs, which could help to overcome the breeding problems. According to recent research on farmed fish, it is known that bold/shy personalities correlate with behaviour variations in aggressiveness, recovery time, reaction to stress, and foraging (reviewed in Castanheira et al., 2017). Though, there are only limited reports on the relationship between personality and immune competence in fish, although this difference may magnify the resistance to diseases. We analysed the stress coping strategies of the bold/shy personality of European perch after exposure to a stressful condition. Furthermore, we investigated the link between both personalities and the defence against bacteria of the *Aeromonas* genus in vivo.

Material & Methods

One-thousand European perch juveniles were tagged with pit tag sized 7.0×x1.4 mm (Loligo Systems ApS, Denmark) and stocked in a separate white tank (net water volume 800 L) within the RAS of the Faculty of Fisheries and Protection of Waters, University of South Bohemia (Czech Republic). To assess the personalities, 900 individuals were subjected to a) open-field test and b) novel-object test (Fig.1.). For the open-field test the fish were transferred into white rectangular tanks (one fish per tank; 37 cm [H] × 57.5 cm [W] × 73.5 cm [L]) and the individual activity (=distance moved) was measured. The novel-object test started right after the open-field test. A small yellow Lego block (LEGO 6176 DUPLO Basic Brick) was placed in the centre of the tank, and

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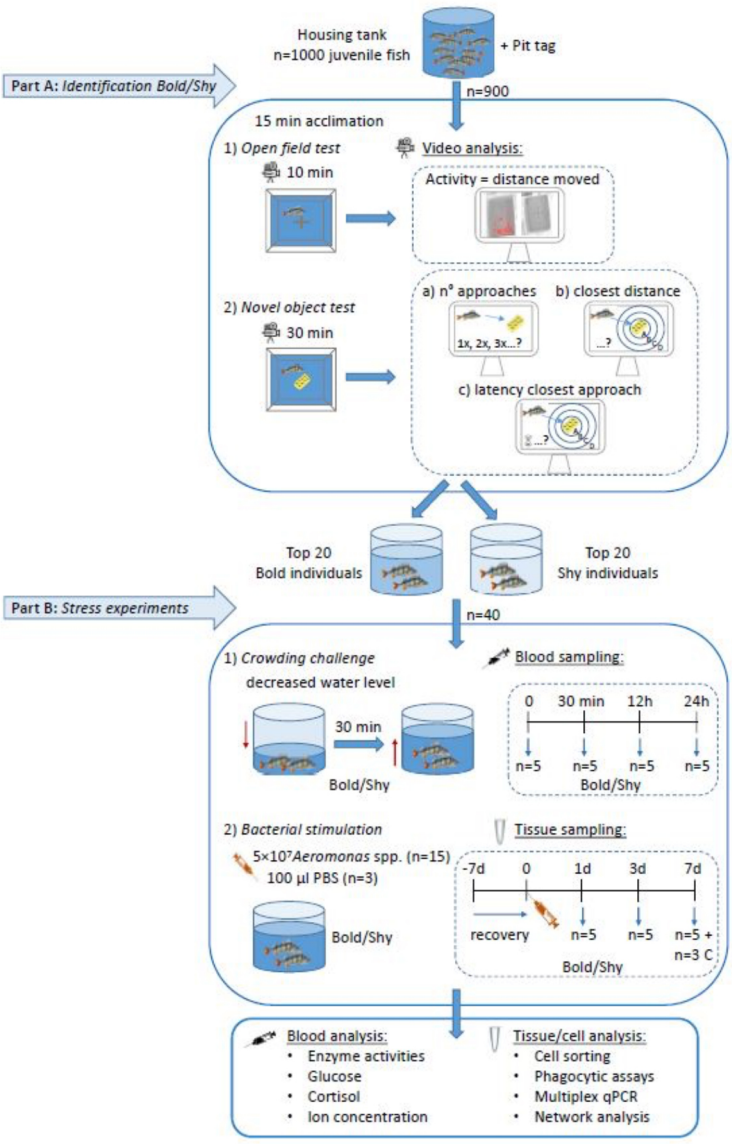

Fig. 1. Experimental design.

the behaviour of each fish was recorded for 30 min. Three parameters were determined from the video records: (i) the number of fish approaches to the Lego block, the closest distance from the Lego, and the latency of the closest approach to the Lego block. Somatic indices of each fish were calculated.

The top 20 boldest individuals (BI) and top 20 shiest individuals (SI) were subjected to a stress experiment in form of a crowding challenge. Water levels in the tanks were reduced to 5 cm above the dorsal fins of the fish for 30 min. Blood samples from five individuals per group (Bi, SI) were collected from the caudal blood vessels of anesthetized fish (MS-222; 200 µg in 10 L water) at four different time points: at 0 h, and 30 min, 12 h, and 24 h after the water level returned to the original level. Blood analysis included determination of enzyme activities, glucose and cortisol levels as well as ion concentrations. After seven recovery days a 100-µl suspension containing either 5×10^7 inactivated *Aeromonas* ssp. or sterile PBS was injected into the peritoneal cavity of fish from both groups. Head kidney and peritoneal leukocytes were sampled after 1-, 3-, and 7-days post-injection. Tissue/cell analysis comprised cell sorting, phagocytic assays as well as multiplex quantitative PCR (qPCR) of a panel of assays specific for 46 immune-relevant genes on two 48.48 Gene Expression biochips (Standard BioTools) using the BioMark HD system (Standard BioTools).

Results

Testing 900 European perch individuals in an open field and novel object test revealed the top 20 boldest and top 20 shiest individuals. The crowding challenge had only minor effects on levels of glucose, osmolality, and activities of several metabolic enzymes. Remarkably, bold perch had significantly lower basal and crowding-induced cortisol concentrations, in contrast to the elevated cortisol levels across almost all matching SI groups. The phagocytic potential of the head-kidney leucocytes was not statistically different between *Aeromonas* ssp/PBS stimulated BI and SI. The bacterial stimulus led to a proper transcriptional response of the immune system. Moreover, transcript levels of central immune genes were significantly increased in the head kidney (*cc125*, *ighm*, and *Cd74*) and in the peritoneal cells (*Cd74*, *ighm*, and *mmp9*) of BI compared to SI. Additional gene network analysis identified common and opposing patterns of upstream regulators for BI and SI, majorly comprising the typical nf-κb/rel-, stat- und irf-family members (and their associated regulators).

Discussion & Conclusions

We could affirm the existence of different personalities in juvenile European perch reflected by a clear discrimination between active individuals with pronounced exploration behaviour (BI) and rigid/immobile (SI) that took a freeze-hide position. Our data indicate that frozen activity and lower explorative behavior were positively correlated with lower ability in shy perch to cope with stress. We did not find any influence of personality on early innate immune mechanisms. Nevertheless, multiplex-gene expression profiling in the head kidney and peritoneal cells revealed that BI and SI responded differently to the intraperitoneal injection of inactivated *Aeromonas* spp and it seems that the immunity of the SI is at a deficit compared to BI. We assume that selective breeding for a specific fish personality, with careful consideration of possible trade-offs, can be a viable way to improve the production efficiency of commercial European perch farms.

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FREEZE-DRIED MICROALGAE PROVE A VALUABLE ALTERNATIVE FOR CULTIVATING EARLY LARVAL STAGES OF *Litopenaeus vannamei* SHRIMP

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Introduction

Early larval shrimp stages rely on microalgae as their main feed. Cultivating these microalgae can be a real burden for individual shrimp hatcheries that focus only on the reproduction and culture of the shrimp itself. Although this is the common practise, ensuring the availability of a constant and large supply of live microalgae is not without challenges. Microalgae cultivation requires constant maintenance, is often space-demanding and requires a considerable investment. Timing of the start and harvest of the culture of utmost importance. Over time, live microalgae cultures may also show variation in their nutrient profile or crash due to biological reasons or contaminating grazers.

Fortunately, advances in cultivating, processing and storing techniques offer promising alternatives. Freeze-dried (FD) microalgae are a reliable and external source that is available all-year-round. The long shelf-life allows an easy distribution from a central production site. The freeze-drying process preserves the biochemical composition of the microalgae and allows for single cell dispersion upon rehydrating the biomass. Today, only a handful of companies specializes in the production of processed microalgae from several species. Despite these advances, the use of processed algae-based products in shrimp aquaculture is still limited and instead focusses on the production and enrichment of live feed (*e.g.* rotifers and copepods) or inclusion as ingredient in formulated feed. Few publications mention the use of alternative processed algae-products for shrimp larvae.

In the current study, we investigated the potential of the FD form of a selection of microalgae species commonly used in shrimp hatcheries and that have a relevant nutritional profile, as feed for larval penaeid shrimp (*Litopenaeus vannamei*).

Materials and methods

L. vannamei nauplii (stage 1) were obtained from Imaqua BVBA (Lochristi, Belgium) and stocked in 100-L cylindroconical poly-ethylene tanks filled with 90L of filtered natural seawater, at a density of 140 larvae L^{-1}. Water temperature was set at 29°C, and the light-dark cycle at 12:12 hours. The larval tanks had a 200 **µm mesh sieve at their** outlet, and were further connected to a filter unit consisting of a protein skimmer and a biofilter, allowing to operate the tanks in batch and later-on in recirculation (RAS). All tanks were connected to the filter units once the larvae reached the mysis 1 stage. Water renewal rate was set at 300% of the tank volume per day. A standard feeding protocol was applied, providing microalgae at around 100 000 cells ml^{-1} for the zoea stages, and 100 000 towards 50 000 cells ml^{-1} for the mysis stages. FD microalgae were prepared through a series of blending and hydrating steps before administration to the tanks. *Artemia* instar I nauplii were introduced from the late zoea 3 stage onwards.

A series of experiments was conducted, wherein the following factors were investigated: microalgae species, microalgae form (live vs FD), rearing system (batch vs RAS) and feed inclusion rate (100% live, 100% FD or 50% of both). Depending on the experimental setup, treatments were performed in triplicate or quadruplicate. Trials were terminated when all treatments reached the postlarval stage (10-12 DPH). Evaluation criteria included survival to the postlarval stage, developmental stage index, fecal production, body fouling and water quality (TAN). All microalgae were supplied by Provion (Hemiksem, Belgium), including live *Chaetoceros muelleri*, *Thalassiosira pseudonana*, *Isochrysis* sp. Tahitian strain and *Tetraselmis chuii*, and their FD form, known under the product names ChaetoPrime, ThalaPrime P, IsoPrime and TetraPrime C, respectively.

Results

High survival to the postlarval stage was observed for shrimp larvae fed freeze-dried microalgae: 50.0 ± 8.8 % and 61.5 ± 7.6 % for FD *C. muelleri* and *T. pseudonana*, respectively. The highest survival, 70.3%, was observed under FD *T. pseudonana*. Both diets were supplemented with FD *T. chuii* for its antimicrobial effect. These survival rates were similar to those of larvae fed live microalgae.

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We also observed a substantial body fouling of the shrimp larvae during the zoea stages, more specifically on all antennae and the setae of the maxillipeds and the telson. The most severe fouling was observed on zoea 1 and 2 stages, and consisted mainly of small algae clumps. No more body fouling was observed from mysis 1 onwards. The fouling did not prevent the larvae from metamorphosing to the next developmental stage, as shed molts covered with microalgae clumps were observed. Visual observation showed that some heavily fouled larvae experienced a lower mobility compared to those free of fouling. Additionally, larvae fed FD microalgae also experienced a 1-day delay in development compared to larvae fed live algae. Whether this is the cause of the body fouling has not been be validated yet.

Conclusion, challenges and opportunities

Live microalgae can be substituted 100% by their freeze-dried counterparts in larval shrimp diets, without affecting survival, albeit with some side-effects including body fouling and a delay in developmental stage. Current and future studies are further investigating the cause of these observations and how further advances in microalgae processing and preparing could mitigate these side-effects.

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THE BLUEMARINE³.COM PROJECT: TOWARDS A MULTISPECIES HATCHERY AND NURSERY INCLUDING MACROALGAE, BIVALVES AND CRUSTACEANS

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Introduction

The sustainable extraction and production of food and biomass from the oceans is a priority with increasing attention and awareness. In line with this global interest, aquaculture-related activities in Flanders (Belgium) have been growing over the last two decades. Both industry and government are investing substantially in local aquaculture initiatives. However, they all face one common problem: the lack of starting material (bivalve spat, shrimp postlarvae and seaweed spores) in sufficient quantity and in desired quality, emphasizing their dependence on import or wild-catch. Within this project, we adapted hatchery and nursery concepts for molluscs, crustaceans and macroalgae to local (temperate) conditions, incorporated local North Sea species, and expanded our biological and technological knowledge on hatchery and nursery techniques with a strong emphasis on synergies and integration between the three species groups, in terms of infrastructure, rearing techniques and management.

The final goal was to design an integrated approach of a multispecies hatchery and nursery platform and knowledge center, and promote the development of aquaculture in Flanders, Belgium. The results were achieved by a team of academic and private partners with expertise in sustainable aquaculture (BlueGent), hatchery techniques and live feed (UGent-ARC), seaweed (UGent-Phycology), ecological risk assessment (UGent-GhEnToxLab), microalgae culture (Proviron), shrimp broodstock management and disease testing (Imaqua bvba), bivalve culture (Aquacultuur Oostende), food retailing (Colruyt Group), seaweed cultivation and advanced aquaculture textiles (SIOEN) and offshore solutions (DEME Group).

Approach

For each species group (macroalgae, molluscs and crustaceans), we started by collecting the baseline information and setting up the experimental infrastructure and biological material. We then investigated specific innovations (strain selection, RAS application, alternative feeds, disease control, …). Synergies in facilities and rearing techniques were identified and integrated concepts validated. Based on the experimental data, these synergies were then quantified and the gain in economic and ecological sustainability was valued through an integrated assessment tool. The final result is a first conceptual blueprint for a modular, integrated hatchery and nursery pilot, that forms the basis for a production unit for starting material, service center for experimental validation and knowledge hub for stakeholders.

Main results

A collection of local strains of five commercial seaweeds was built to serve as reliable source for the production of spores. The life cycle of the red seaweeds *Palmaria palmata* and *Porphyra umbilicalis* was further unravelled. Strains of *Ulva* sp. were selected, and the genomic diversity of favourable traits characterized, which resulted in an HD SNP map. Biodegradable cultivation substrates and binders for macroalgae spores were also investigated.

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A functional RAS was designed and successfully implemented for the nursery cultivation of two oyster species (*Crassostrea gigas* and *Ostrea edulis*), observing a growth rate close to that of commercial hatcheries. A model on the associated mineral consumption reveals that the addition of Ca and CO_3 is vital for culturing bivalves in a closed system. The microbial community profile associated with the mono- and multispecies setup was characterized. The first floating upwelling system (FLUPSY) in Belgium was successfully built and tested in the Spuikom, Ostend. The life-cycle and technical requirements for hard-to-culture microalgae species such as *Isochryis galbana* and *Skeletonema marinoi* were documented and the first steps towards the upscaled cultivation of these species was undertaken.

The life cycle control of feed requirements for both the tropical *Litopenaeus vannamei* and the indigenous cold-water *Palaemon* prawn has been optimized. Shrimp larvae were successfully reared in a closed recirculating system, with a survival equal to that in batch systems. Freeze-dried microalgae proved a valuable alternative to live microalgae for the cultivation of early larval stages of *L. vannamei*, with some treatments reaching up to 70% survival. This further reduces the dependence on and the investments in a local microalgae culture. The microbial community profile in systems fed with either of microalgae feed forms was characterized. New disease testing tools for shrimp were developed.

The synergies between monospecies cultures were identified to establish experimental multispecies setups that provided insight into the nutrient flow between the system compartments and cultured organisms. Multiple proof-of-concepts on different combinations of resource recycling and species co-culture (integrated multitrophic aquaculture; IMTA) were investigated. An environmental impact and economic feasibility assessment were done for the most relevant scenarios. With the collected knowledge from all mono- an multispecies assessments, a first conceptual blueprint for a modular multispecies hatchery and nursery pilot was developed.

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MAKING RESEARCH ACCESSIBLE USING A DIGITAL TOOL: COMMUNICATING FUTURE SCENARIO PREDICTIONS FROM RECURRENT NEURAL NETWORKS USING A LIGHTWEIGHT WEB APPLICATION

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Introduction

Norway is a global leader in salmon farming, with the aquaculture sector expecting to grow in the future to reach consumer demand. Advocates argue farm raised salmon have a low carbon footprint, and aquaculture can therefore play a pivotal role in reducing the carbon footprint of food production. The detractors, on the other hand, argue aquaculture poses an environmental threat as escaped salmon may hybridize with natural populations, and high population densities in pens lead to an increase in local pollution and sea lice occurrence. Furthermore, delousing of the fish is a challenge for fish welfare, the opponents argue. New production systems for salmon fish farming (i.e., land-based, floating closed, semi-closed, and open ocean aquaculture systems) aim to counter these challenges, as well as utilise new areas for production. More knowledge is needed regarding how these new production systems should be regulated, and how they will influence the public perception of the salmon farming industry.

In this study, variables relevant for exploring key future scenarios are co-developed during participatory stakeholder workshops using Fuzzy Cognitive Mapping (FCM), a soft computing framework. Industry stakeholders decide on relevant variables to include in models, how they influence each other, and discuss feedback mechanisms in participatory workshops. Data collected during workshops are then used as the basis for the creation of FCM models (Kosko 1986), which combine fuzzy logic and recurrent neural networks, to produce predictions of changes to the perception of salmon farming in response to potential future scenarios. The results from models (i.e., values that represent the strength and direction of change to the perception of salmon farming in response to different scenarios) were then made interactable and accessible through their integration into a lightweight web application as a first-generation policy action tool.

The application is developed to make the results from this study accessible to diverse stakeholder groups. Thus, a concise description of how the model functions and how to interpret results from the scenarios is included in the app. In addition, information on the relevant variables defined by stakeholders and the fuzzy cognitive map describing the relationship between variables stakeholders defined in workshops is provided to users for easier interpretation of results.

Methods

Stakeholders included in the participatory workshops included industry representatives working with new aquaculture systems in Norway, researchers, and representatives from trade organisations. The variables that stakeholders identified as relevant to Norwegian salmon farming and their interactions were visualized in the freeware Mental Modeler throughout workshops to facilitate the co-production of a map of the system. Connections between variables were quantified on a continuous scale between -1 and +1, where a negative value indicated that if one variable increased, the variable on the receiving end of the "negative" connection would decrease. -1 indicated a strong decrease in the connected variable (and it follows +1 indicated an increase in one variable would also result in the direct, strong increase in the connected variable and values near zero describe weak relationships between variables). These values served as the basis of a semi-quantitative model we used to describe our system and the basis of the creation of the projected future outcomes that were possible to interact with in the web application. Variables and their relationships were calibrated and validated in subsequent workshops.

Models were developed using the FCM package (Dikopoulou and Papageorgiou 2017) in R. Models were fit with the rescale inference rule which is preferred where there is not previous information about a concept-state, and we did not have information on the initial state of the system (Papageorgiou 2011). A sigmoidal transformation function was applied as sigmoidal (continuous) FCMs are recommended for quantitative and qualitative scenarios with complex feedback structures as we found in our system (Tsadiras 2008). The weight matrix consisted of the values obtained from the participatory

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workshops. The application was built using Shiny (Chang et al. 2023), an open-source framework for building interactive web applications. One interactive element of the application is in the form of a drop-down menu where users can select what variables to have a positive value in the activation vector. Visualizations and the table of values predicted for each variable in the application show values reached once the model has reached convergence and the system has reached an equilibrium point. The model outputs shown in visualisations from the user's chosen scenario are based on the stakeholders' perception of the system if there is a change to the current system. Feedback from stakeholders have been and will be implemented to make the app more user friendly. Specifically, similar variables were grouped together to simplify the interpretation of the results and the app is being developed in both Norwegian and English to match the language needs of different users (from local industry actors to academics). Stakeholders will be invited to give feedback on the application to ensure a user-friendly interface is created as development of the app continues.

Results & Discussion

Following the axiom "all models are wrong, but some are useful," the outputs of the workshops and FCM models in the web application will not be used as predictions of how perceptions will change as Norwegian salmon farming changes. Indeed, the data collected from stakeholders is limited due to the small number of actors it is possible to include in workshops. Outputs from the models may be useful 1) in the context of engaging non-experts in research and 2) for serving as conversational focal points for ideating scenarios and informing decision making. The FCM developed in workshops also serves an important purpose as a simplification of the challenges faced by the Norwegian salmon farming industry, which allows for the visualisation of important and sometimes surprising relationships between identified variables. The app's value comes from its ability to bridge gaps between researchers and stakeholders and between stakeholders themselves who may have differing opinions on how different variables will affect other variable under future scenarios. Indeed, the app may be used as a policy action tool, and to improve the communication between resource managers, regulators, policy makers, and actors from industry who make critical choices and researchers beyond a traditional scientific article or report. The conversations that result from interaction with the digital tool can thus be used to inform decision makers on how to develop Norwegian salmon farming in coming decades under different scenarios.

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AQUAPONICS: SUSTAINABLE, HEALTHY AND PROFITABLE SOLUTION TO ALLEVIATE THE OVEREXPLOTATION OF THE NATURAL RESOURCES

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Climate change and the overexploitation of the natural resources of the oceans and land for cultivation drive the need for innovative food production within a sustainable, healthy and profitable framework according to EU guidelines. Organic and Innovative technologies, such as aquaponics which is a perfect symbiosis between aquaculture and hydroponics is undoubtedly a solution to alleviate this type of problems. Tilamur is an SME that since 2012 has been working on the implementation of innovative technologies for efficient aquaponics solutions. Since 2021 Tilamur is participating in one of the most ambitious projects in the application of innovative technologies for aquaculture and precision agriculture called Pestnu which has been financed with funds from the EU (Nº 101037128) . Over these first 18 months of project execution, Pestnu project has implemented two digital automated analyzers for nitrites, nitrates and ammonium into the fish tank and the nutrients tank for the plants, which provide us in situ with real-time measurements of these types of parameters that are so essential in aquaculture and agriculture. The implementation of this type of technology has provided us with a 60% saving in reagents and analyzes from external laboratories as well as a reduction in labor when collecting daily samples. In the field of agriculture we have installed automatic traps capable of monitoring pests of different insects in real time, an autonomous robot capable of detecting and treating infected plants, satellite technology to monitor the state of water stress and health of the plants at all times. floors. Following the EU guidelines in the field of biostimulant use, the Pestnu project has developed this type of biofertilizers through waste from residual water treatment plants and microalgae cultivation, managing to strengthen the immune system of plants and a saving of almost 50% in fertilizers.

FATTY ACID PROFILE OF RAINBOW TROUT (*Oncorhynchus mykiss*) SUPPLEMENTED WITH DIETARY TRIBUTYRIN: PRELIMINARY APPROACH BY GAS CHROMATOGRAPHY

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Introduction

The production of short-chain fatty acids (SCFA) results from anaerobic bacterial intestinal fermentation of soluble fibre carbohydrates leading to an intricate functional cross-talk between diet, gut microbiome and host metabolism.

While most studies on aquaculture species focus on the supplementation of SCFA in their salt forms, SCFA delivered as their equivalent triacylglycerol (TAG) forms has huge potential. Tributyrin (TB), the TAG forms for butyrate, rapidly generates the release of 3 fatty acids (FA) along with glycerol following intestinal lipolysis. TB has been well studied, showing the potential to improve animal performance and productivity in land-farmed animals when added as dietary supplement, however its applicability in aquafeeds is less studied (Palma et al., 2023). According to earlier research, supplementing fish and shrimp diets with TB may help to mitigate some of the drawbacks of including plant-based ingredients on aquafeeds. This will be especially challenging for carnivorous species such as rainbow trout (*Oncorhynchus mykiss*), which require the replacement of a significant amount of fishmeal with plant-based proteins to improve diets sustainability. Previous results on *O. mykiss* supplemented with variable percentages of TB did not improve growth or feed efficiency, nor did they reduce feed intake (Espirito Santo et al. 2022). As a preliminary approach, gas chromatography (GC) was used as methodology to assess the effects on fatty acids profile of digesta, intestine, liver and muscle of *O. mykiss* supplemented with TB.

Material and Methods

Four hundred eighty juvenile trout (19.0±0.4 g, mean±SD) were randomly distributed in 12 tanks, connected to a RAS system under controlled conditions (16±1ºC; 0.30±1‰; pH 7±1; >95% air saturation; 12 h:12 h photoperiod). Four experimental diets: basal diet (control; TRB0) including 10% fishmeal and 70% plant-based ingredients (44% crude protein, 18% crude fat, gross energy 21.8 MJ kg^{-1}) with inclusion levels of 0.1 (TRB1), 0.2 (TRB2) and 0.4% (TRB4) of a product containing 55% TB (Lucta S.A.) in replacement of silica. After 44 days, three fish per treatment were sampled after 6 h and 24 h of feeding to evaluate the impact of dietary TB supplementation on the FA profile.

Muscle, liver and intestine were collected and tissue extraction was performed by MTBE method (Matyash et al., 2008). Digesta was processed according to Louvado et al. (2020) followed by MTBE extraction. Dried samples were resuspended in 1 mL of hexane and 0.5 mL of methanol. After vortex, 400 µL of sodium methoxide were added. The top layer was filtered with a nylon membrane and 150 µL of the filtered solution was placed in a vial and added 100 µL of the internal standard methyl nonadecanoate (C19:0) (Sigma-Aldrich) with a final concentration of 0.3 mg/mL. The GC was performed in a NEXIS GC-2030 (Shimadzu) chromatograph equipped with a flame ionization detector and a TR-CN 100 capillary column (60 m × 0.25 mm × 0.20 µm). Helium was used as carrier gas at a pressure of 150 kPa at the top of the column. The temperature of the injector and detector was 260°C and the split ratio was 1:25. The initial temperature of the column was maintained at 90°C for 7 min after the injection, increasing 5°C/min to 220°C and held for more 15 min. The data were acquired and analyzed using Lab Solutions data analysis software. FA were identified by comparing the relative retention times with an authentic external standard, Supelco 37 component FAME mix (Sigma-Aldrich). The quantification of FA was based on the internal standard method. The results were expressed in percentage of the total FAME (%).

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Statistical analysis and graphic design were applied to fatty acid relative abundances values using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). A two-way ANOVA was conducted on these values with a Sidak's correction and multiple comparisons (ntotal=24, nreplicates=3).

Results and Discussion

GC analyses were able to identify and relatively quantify sixteen FA from C14:0 to C22:6n-3 in muscle, thirteen in intestine and liver, and eleven on digesta. SCFA (C1:0 – C5:0) were not detected. Similarly to our results, SCFA were found below the limit of detection on the intestine of zebra fish (*Danio rerio*) (Cholan et al., 2020). Considering this is a preliminary study, no major effects were found on intestine, liver, and digesta samples ($p > 0.05$, two-way ANOVA). In muscle, after 6 h, C14:0 and 18:1w9 cis were the only FA that presented some variations between experimental groups. C14:0 was higher in TRB2 when compared to TRB0, while 18:1n-9 cis was higher in TRB1 when compared to TRB0. Other FA were found only in certain groups, such as C20:0 that was found only in the 3 replicates of the fish with TRB4 diet at 6 h. In digesta, the FA found in higher percentage were C16:0, C18:0 and 18:1n-9 cis. The lack of significant differences between experimental groups in this particular sample could be explained by the high variability observed within individuals.

Conclusion

With this preliminary approach GC could not detect SCFA (C1:0 – C5:0) in muscle, liver, intestine and digesta of *O. mykiss* supplemented with variable percentages of TB. However, we were able to perform a FA profile on these samples, which did not reveal significant differences in most FA after dietary TB supplementation.

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SUPPORTED COVALENT ORGANIC FRAMEWORK TO REMOVE GEOSMIN FROM WATER IN AQUACULTURE

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Geosmin, a metabolite of gram-positive bacteria actinomycetes and fungi, is a bicyclic alcohol detected by humans as earthy and mud taste at very low concentrations (from 5 to 50 ng/L, depending on the matrix) [1]. Therefore, for the aquaculture sector, the presence of this compound implies high production costs due to the low acceptance by the consumers.

Covalent organic frameworks (COFs) are porous crystalline materials with long-range order [2,3], which, due to their small pore size (1–5 nm), tuneable structure, and large surface area, have been demonstrated as efficient materials for the capture of different molecules from water, e.g., different types of pharmaceuticals [3,4]. In this work, different COFs with varying pore size and functional groups were tested for their capacity to adsorb geosmin. The conditions were optimized for the material to be able to adsorb concentrations of geosmin far above the detection limit of humans. In addition, geosmin desorption was studied and optimized to allow for the reuse of the COF adsorbent.

Among the tested materials, the COF with best efficiency could adsorb 78 mg of geosmin /g of COF, reaching equilibrium after an incubation of only 30 s. TpBD-Me_2 was selected for further characterization. Additionally, geosmin could be removed from each COF by a simple incubation in organic solvent. At least five cycles of reuse were demonstrated without significant efficiency loss.

However, COFs are powders as synthesized and should not be used directly in water as their recovery would be extremely difficult and their release into the environment could lead to some ecotoxicity [5]. To overcome this, TpBD-Me_2 was supported on alumina pellets, keeping high adsorption capacity for geosmin.

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EFFECTS OF DIETARY IRON SUPPLEMENTATION ON BIOSYNTHESIS OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN THE NEREID POLYCHAETE *Hediste diversicolor*

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Introduction

Multiple aquatic invertebrates have the necessary enzymatic machinery for *de novo* biosynthesis of long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA) including the so-called "omega-3" EPA (20:5n-3) and DHA (22:6n-3). Two distinct types of fatty acyl desaturases, namely methyl-end (ω des) and front-end desaturases (Fed), are known to be involved in animals' LC-PUFA biosynthesis [1]. Iron (Fe) has a prominent role in LC-PUFA biosynthesis since it is a cofactor of desaturases, and is present in the active di-iron centres of all aerobic desaturases [2]. Since expression of desaturases involved in LC-PUFA biosynthesis can be increased by feeding a low LC-PUFA diet, an innovative strategy to enhance the endogenous production of LC-PUFA in aquatic organisms consists of dietary Fe supplementation to guarantee adequate supply under conditions resulting in desaturase activation. Supplementation of Fe has been shown to positively influence LC-PUFA biosynthesis in salmonids [3] but, to the best of our knowledge, has not been yet investigated in invertebrates. The present study aimed to assess dietary Fe supplementation as an enhancer of LC-PUFA biosynthesis in the nereid polychaete *Hediste diversicolor*, a commercially important species with great interest for aquaculture.

Materials and Methods

First, an *in vitro* trial was carried out by growing transgenic yeast expressing the *H. diversicolor* desaturases (two ω des and two Fed) in the presence of specific fatty acid (FA) substrates. For each desaturase, the following treatments were tested: no Fe supplementation (control), supplementation with $FeSO_4$ (inorganic Fe), supplementation with ProPath® **Fe (organic Fe)**, and supplementation with an Fe chelating agent (chelator). The desaturase activity of transgenic yeast was estimated by calculating the conversion of the FA substrate into the FA product. Second, an *in vivo* trial with *H. diversicolor* juveniles was carried out. Briefly, 20 worms (25-50 mg ww) were randomly distributed in nine experimental units (3 units x 3 diets). The worms were fed for 7 weeks on an experimental diet with low LC-PUFA (control), which was supplemented with either $FeSO_4$ (inorganic Fe) or ProPath® **Fe** (organic Fe). Worms were fed to 4% of the biomass 5 d per week. Survival and specific growth rate (SGR) were recorded. After 7 weeks, the animals were starved for 24h prior sampling for lipid analysis. Total lipids were extracted and quantified gravimetrically, with an aliquot being transmethylated to fatty acid methyl esters (FAME), and analysed using gas chromatography. The results were processed using principal component analysis (PCA). FA analyses from yeast (*in vitro* assay) were carried out as described above for worm samples.

Results and Discussion

In the *in vitro* trial all the *H. diversicolor* desaturases converted the specific FA substrate into the corresponding product with inorganic and organic Fe treatment exhibiting higher enzyme activity than control. The Fe chelating agent reduced the activity of al desaturases (Figure 1a). These results suggest that Fe can effectively enhance desaturase activity as previously reported in yeast [4]. At the end of the experiment, no significant differences ($p>0.05$) in SGR (average of 0.084) nor survival (96.1±4.2 %) were found among treatments. Moreover, the results from the *in vivo* trial did not show any clear effect of organic and inorganic Fe supplementation on the FA composition of *H. diversicolor* whereas a clear segregation of the day 0 samples was found (Figure 1b). High levels of 18:1n-9 and 18:2n-6 (23.6 and 19.3%, respectively) on the FA profile of all treatments were found, suggesting a strong dietary effect (34.4 and 15.4%, respectively). Besides, in all treatments, the FA composition of polychaetes showed high levels of PUFA (33.4%) such as 20:4n-6 (ARA), EPA and DHA, indicating bioconversion and trophic upgrading. The reasons underlying the apparent discrepancy between the enhanced desaturase activity observed *in vitro* and the lack of increased LC-PUFA biosynthesis in worms fed on Fe supplemented diets (*in vivo* trial) remain unclear. However, it is reasonable to speculate that the Fe enhancing effect on LC-PUFA biosynthesis could not be detected in the present study due to an insufficient capacity of experimental diet to increase the expression of fatty acyl desaturases in vivo. Further analyses on gene expression, as well as FA composition of the polar and neutral lipid fractions, will contribute to clarify the role that dietary Fe supplementation may play as enhancer of LC-PUFA biosynthesis in *H. diversicolor*.

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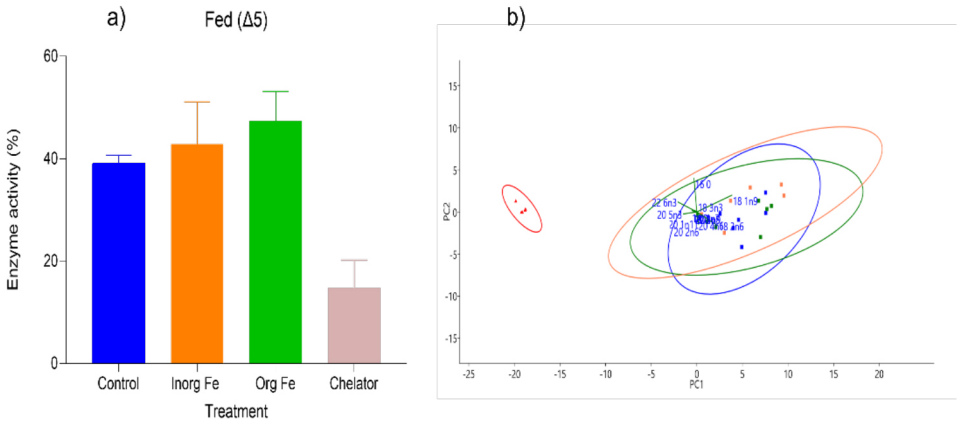

Figure 1. a) *In vitro* assay testing the desaturation capacity of the *H. diversicolor* Fed (Δ5 desaturase) towards 20:3n-6 under no supplementation (Control), supplementation with inorganic and organic iron (Inorg Fe, Org Fe), and supplemented with an Fe chelator. b) PCA of the fatty acid profiles of *H. diversicolor* fed with different supplemented diets (*in vivo* trial). Blue (control), orange (inorganic Fe) green (organic Fe) and red (day 0). Fatty acids responsible for the grouping pattern are displayed in the biplot (blue vectors); 95% confidence ellipses are shown.

Acknowledgements

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LESSONS LEARNED: CHALLENGES AND SOLUTIONS FOR FISH HANDLING IN LAND BASED AQUACULTURE

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Introduction
In traditional sea-based salmon farms, fish is vulnerable to sea lice, predators and various diseases, to mention a few. The current open-pen solutions are not possible to grow further in many countries due to these issues. Land-based aquaculture solves many of these challenges as well as offering other benefits to the fish and environment, but at the same time new risks are present.

Challenge 1: High capex need
For land-based aquaculture, there is undoubtedly a much higher capex demand than sea-based alternatives. To ensure that the project is profitable, it is required to utilize the production capacity optimal. The solution is to design the system to be flexible, allowing fish to be moved from any tank in the system, split on size, and moved back to any two tanks in the farm – and at the same time minimize the required piping.

Challenge 2: Gentle fish-handling solutions
Handling the fish as it grows larger is increasingly complex. Pumping a full-grown salmon is much more challenging than a smolt, making technology from traditional smolt farms not suitable for the larger fish. The solution is to learn from the wellboat industry (live fish carriers) that has evolved over decades to handle big fish. This includes crowding grids, pumping without impellers, avoid sharp bends, keep the fish in water as much as possible, to mention a few.

Challenge 3: Good water quality
Increasing the biomass density and at the same time moving the fish often between tanks will stress the water quality in the system. With such a high value in the tanks, giving the fish proper water quality is crucial. The solution is to ensure good water circulation in the tanks, and stable and efficient water treatment. Using circular tanks and correct diameter/height ratio ensures even water distribution and minimized risk of H2S or low oxygen areas. Traditional biofilters are complex and difficult to grow, but there are alternatives with either a hybrid flow-through system, or electrochemical water treatment. A typical hybrid flow-through system will not include biofilter, but CO2 degassing and particle removal, and a higher exchange of water than RAS system. Lately, electrochemical water treatment solutions is proven as an excellent alternative as well.

DAILY CROWDING STRESS AFFECTS WOUND HEALING, WELFARE, AND TRANSCRIPTOMIC RESPONSES IN SURGICALLY TAGGED ATLANTIC SALMON (*Salmo salar*)

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Introduction

A 20-year average grow out site mortality rate of 17% in Norwegian aquaculture has been observed (DOF, 2022). While approximately 400 aquatic species are being farmed and aiding species diversity, only 25 species have been the subject of five or more published welfare studies (Franks et al., 2021). These findings underline the need to improve and expand our understanding of aquatic animal welfare. Using smart tags embedded in fish offers a novel real-time avenue to monitor and document species-specific welfare (Macaulay et al., 2021). However, the tagging process and the presence of the tag must not disrupt the fish's natural biological and behavioural patterns. Thus, our study aims to evaluate the potential stress effects over time on the healing process by analysing gene expression in the skin and head kidney. We also present previous observations on physical wound changes and physiological alterations from the same sampled fish (Virtanen et al., 2023).

Materials and methods

In nine 1.0 m^{-3} indoor seawater tanks, 30 ~1kg Atlantic salmon per tank were kept under constant conditions. Sixty-eight days of acclimation were given, and the three experimental groups consisted of: Control, Wound, and Wound + Stress ($Stress^+$). Starting on day 7 up until day 56, nine fish from each group were sampled weekly (three fish per tank, three tanks per group). The Control group was untouched, the Wound group received an abdominal dummy tag, and the $Stress^+$ group had the tag plus a daily crowding stress, where the water level was drained significantly in the tank and then, after 30 seconds at its lowest point, brought back to normal levels. Skin samples were taken weekly from a location 1.5 cm away from the incisional wound (and from a similar location in the control group), along with head kidney samples. Both tissues were analysed using rt-qPCR to examine 14 genes related to cell signalling, immunity, wound healing, and stress. Based on rt-qPCR results skin samples from weeks 1, 4, and 8 were selected and underwent RNAseq analysis.

Results

Regarding physical wound changes, external wounds were sealed by week 5 in both treatment groups, while external inflammation was larger in $Stress^+$ fish than in only wounded fish. Internal wound healing was only seen sealed at week 8 for two individuals in the Wound group; generally, the wound was larger in the $Stress^+$ group. No significant effects were seen on stress responses in the wound group. In contrast, significant increases in ACTH and cortisol production were visible starting at weeks 4 and 6 respectively, and fin erosion was worse in the stressed group starting at week 3. Regarding head kidney transcription, a possible early immune suppressive effect was seen in the $Stress^+$ group compared to the wound group, specifically during week 2 and consistently for il-2 for the first three weeks. For the skin samples, limited differences were seen between the $Stress^+$ and Wound groups, however week four showed the biggest difference within mmp9 and mmp13 being significantly expressed in the $Stress^+$ group. In the head kidney samples, mmp 9 and 13 were still upregulated at the end of the study likely due to internal wound healing. Regarding the RNAseq data, no differentially expressed genes were significant between the two treatment groups at week 1, however by week 8, a major disruption in genes associated with cellular and metabolic processes was observed. Additionally, the most differentially expressed genes were seen at week 1 when comparing treatment groups to the Control group.

Discussion & conclusion

The results show that introducing stress delays wound healing, amplifies the inflammatory response (not seen through cytokines), dysregulates the stress response, and significantly alters gene expression as the combined stress and healing process progresses. Conversely, the tagging process does not create physiological stress (with the first sampling being 7 days post-tagging), and wound healing proceeds in a regulated manner. Practical implications from the study are that tagging can be seen as non-stressful 7 days post-tagging, tags should be positioned away from the wound incision due to the prolonged internal healing process, transcriptional markers identified through RNAseq can aid in early detection of potential issues while possibly suggesting possible avenues to enhance fish skin health and welfare post-tagging. Furthermore, even though some results (like those from rt-qPCR) show only limited effects, all results underscore the importance of providing a stress-free environment for fish during wound healing and tagging where the first three weeks are seen as the most critical time.

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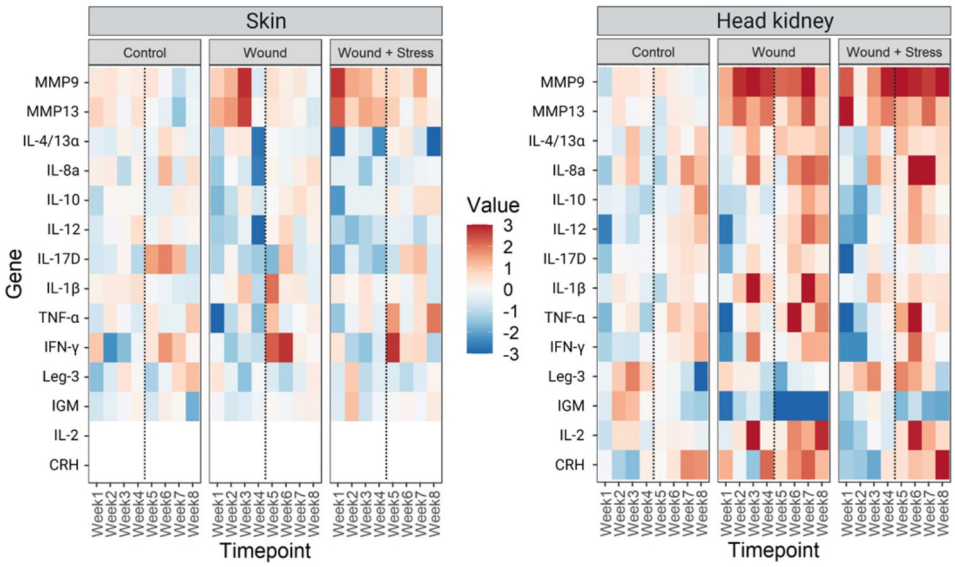

Figure 1. Heatmap displaying the mean log2 normalized expression divided by the standard deviation (SD) for 14 genes across three groups and two tissues in arbitrary units. The colour intensity represents the degree of deviation from the mean expression level of the control group (values above 3 and below -3 are represented with maximum intensity).

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IMPROVEMENTS UNDERLYING GROWTH, IMMUNITY AND RESILIENCE REVEALED IN SALMON AND SHRIMP USING PROTEOMICS AND METABOLOMICS

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Introduction:
Enhanced growth, immunity, and temperature stress resilience are highly sought-after phenotypic traits in aquaculture, yet our understanding of their genetic regulation is extremely poor. Omics approaches, such as proteomics and metabolomics, are powerful tools to understand the effects that nutrition and environmental conditions exerts upon cultured animals, and how the animal responds to external stimuli. Application of omics technologies in aquaculture can enable manufacturing of advanced diets that provide specific nutrients intended to attain enhanced growth, immunity, and resilience that lead to increased production yield while ensuring environmental sustainability and promoting animal welfare and social license.

Methods:
In shrimp hepatopancreas and haemolymph, data independent acquisition (DIA) proteomics and metabolomics were used to elucidate metabolic pathways activated by the inclusion of the microbial biomass Novacq™ compared with a control group fed a fishmeal diet. In salmon, a rapid targeted proteomics method was developed and used to quantify stress in liver of salmon subjected to increased temperatures. In addition, targeted metabolomics was applied to detect modifications to central carbon metabolism in the plasma of thermally stressed fish.

Results:
A strong signature of glycoconjugate metabolism driven by hexosaminidases and arylsulfatases A and B was observed in DIA results for Novacq™ fed shrimp. This was complemented by metabolomics that revealed a significant ten-fold increase in the abundance of the chitin amino sugar precursor N-acetyl D-galactosamine. Joint-pathway analysis indicated that Novacq™ fed shrimp preferred using energy from carbohydrates through activation of the amino- and nucleotide sugar metabolic pathways. In salmon exposed to high temperatures, a multiplexed 55-peptide thermal stress panel revealed many are significantly changed by temperature, with abundance of two different serpin peptides significantly correlated with increased temperature. Targeted metabolomics of plasma revealed significant reductions in key amino acids and vitamin precursors, while significant increases were recorded in purine and pyrimidine nucleotides.

Conclusions:
In shrimp fed Novacq™, combined DIA proteomics and metabolomics pathways revealed mechanisms of energy utilisation, enhanced growth and immune defence stimulation. In salmon at high temperature, two serpin peptides were identified as highly sensitive temperature responsive biomarkers in liver, along with changes to amino acid and nucleotide metabolism in the plasma. Detection in non-invasive tissues such as plasma or mucous will facilitate monitoring the effectiveness of strategies to reduce impacts of stress and improve farmed salmon welfare.

DEUTERATED WATER STABLE ISOTOPE ENRICHMENT AND METABOLIC FLUX ANALYSIS TO TRACE NUTRIENT UTILISATION IN CRUSTACEANS

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Introduction:
The efficient utilisation of dietary ingredients is of primary importance in aquaculture nutrition, in order to optimise feeding efficiency, profitability and reduce nutrient loss into the environment. Apparent digestibility of diets and their ingredients are traditionally measured as an indication of ingredient and feed quality, but this does not guarantee the residual absorbed nutrients are utilised adequately. Special consideration of the method in shrimp is needed to account for potential nutrient losses from leaching. Tissue composition and the impact of various dietary assimilated lipid sources has also been extensively studied, but this sheds little light on the metabolic capacity of the animal once the nutrients are ingested.

More recently, the impacts of feed ingredients have been measured using various molecular (gene expression) and omics (RNAseq, proteomics, metabolomics) techniques. A complementary technique is to incorporate inert, non-radioactive stable isotopes into the water (i.e., $^{2}H_2O$ or deuterated water), and/or enriched within specific feed ingredients (^{13}C or ^{15}N), to track the metabolic transformations over several days of feeding. This technique has been highly successful in fish, to calculate the fractional synthesis rate (FSR) of glucose, glycogen, triacylglycerides (TAGs) and free fatty acids (FFA), or muscle protein synthesis by using alanine as proxy, and define the impact of dietary starch or other ingredients on hepatic metabolism.

Methods:
In this study, the fundamental ability to track nutrient assimilation in crustaceans using stable isotope enrichment was assessed in black tiger shrimp, *Penaeus monodon*, using seawater enriched with 5% $^{2}H_2O$. In the first phase, body water (as estimated from hemolymph) ^{2}H-enrichment during residence time in seawater was tracked over time in unfed shrimp. Aqueous metabolites were extracted from shrimp hepatopancreas, muscle and hemolymph from the 48 h group and quantified by ^{1}H NMR. Subsequently, 12 shrimp were fed twice daily a standard commercial diet over 3 consecutive days in 5% $^{2}H_2O$ enriched seawater. The lipid phase was extracted from hepatopancreas and muscle tissue and subjected to both proton (^{1}H) and deuterium (^{2}H) NMR to calculate FSR. Protein FSR was estimated from ^{2}H incorporation into protein bound alanine extracted from muscle tissue.

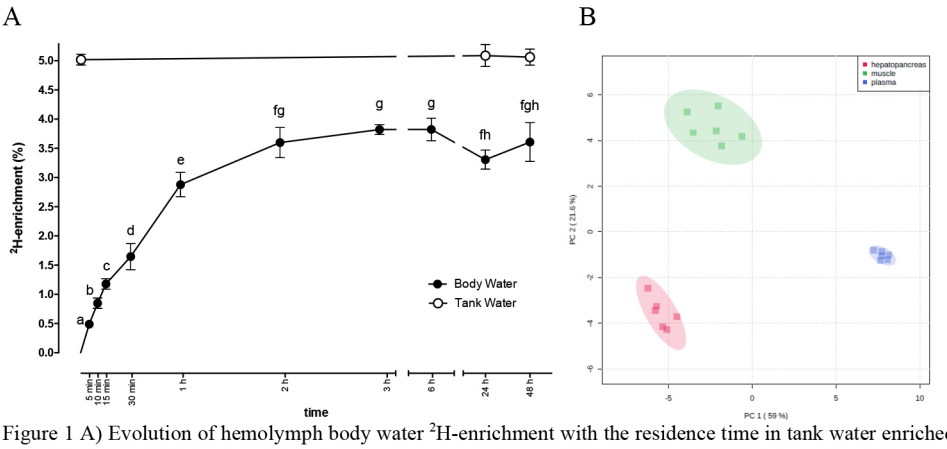

Figure 1 A) Evolution of hemolymph body water ^{2}H-enrichment with the residence time in tank water enriched with 5% (mean ± SD; 5, 10 and 15 min n = 8, the remainder n = 6). One-way ANOVA followed by Tukey test ($p<0.05$), with significant differences indicated by different letters. B) Partial Least Squares (PLS) scores plot computed with the metabolites concentration in plasma, hepatopancreas and muscle samples (Q2 = 0.983; R^2 = 0.991; 1000 permutations: P = 0.001).

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Results:
Results showed that body water rapidly equilibrated with tank water after 2 hours, which ensured the 3-day feeding duration in the second phase was sufficient for metabolite enrichment. The ^{1}H spectra from the 48 h samples quantified 34, 49 and 49 metabolites from hemolymph, hepatopancreas and muscle, respectively, which clearly displayed tissue-dependent distribution. Metabolites detected were mainly amino acids and their derivatives, along with simple sugars, with important roles for succinate, butyrate, choline and amino acids defining tissue clusters.

Using comparative $^{1}H/^{2}H$ spectra from hepatopancreas extracts, fractional synthesis rate (FSR) of TAG bound FA (de novo lipogenesis, DNL), was calculated at 0.16 ± 0.09 % / day. Meanwhile glycerol turnover was high calculated at 3.13 ± 0.9 % / day, demonstrating an enhanced role of glycerol as a metabolic intermediate in shrimp. FA elongation was calculated at 0.43 ± 0.14 % / day while FA desaturation was calculated at 0.32 ± 0.33 % / day. Using comparative $^{1}H/^{2}H$ spectra from muscle extracts, DNL was calculated at 0.08 ± 0.03 % / day. Meanwhile there was no enrichment detected in muscle glycerol, or evidence of elongation or desaturation, supporting the concept that muscle has very limited role in metabolising dietary nutrients in shrimp, where the primary metabolising organ is the hepatopancreas. While the $^{1}H/^{2}H$ NMR analysis quantified low values of ^{2}H-enrichment in Ala (0.01-0.07 %) compared with other species, muscle protein FSR was calculated at 0.9 ± 0.3 % / day.

The combined ^{1}H NMR-based metabolomics and ^{2}H NMR analysis of metabolite ^{2}H incorporation while resident in $^{2}H_2O$ allows for a comprehensive, undisturbed, non-invasive and non-radioactive analysis of metabolic flux. This involves shifts in metabolite concentrations in different tissues as well as a dynamic interpretation of nutrient utilization, accumulation and turnover.

Conclusions:
While the use of omics methods can provide a snapshot of the impact of dietary ingredients on the animal at the time of sampling, stable isotope enrichment in deuterium captures a movie of all metabolic transformations occurring over several cumulative feeding events and post-feeding transformations. This study establishes a new method for defining metabolic requirements in crustaceans and assessing the impact of dietary ingredients. The collection of $^{1}H/^{2}H$ NMR spectra simultaneously from a single sample and subsequent *in vivo* metabolic flux analyses (MFA) across multiple tissues places this technique above others for integrated nutritional analyses.

THE ROLE OF FISH AND SEAFOOD IN THE FIRST 1000 DAYS OF LIFE

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Summary
Quality nutrition is of critical importance during the first 1000 days of human life, conception until a child's 2nd birthday. Sufficient micronutrition is vital for supporting early childhood development and minimising long term health risks. Fish and seafood products can contain elevated concentrations of micronutrients in comparison to other protein sources, but the potential of this food group is underexploited in the UK. Understanding of the role of fish and seafood in early childhood development could act as an important step towards informing and improving public health. To further contemporary understanding of the role that fish and seafood currently plays in the first 1000 days of life in high income countries, the following objectives are being investigated:

Objective 1- To analyse the quantity and type of seafood expectant mothers and young children in the UK currently consume in order to identify the scope for improved seafood consumption;

Objective 2- To determine commercially available fish and seafood products with the greatest micronutrient concentrations per GBP (£) to identify accessible products for meeting the key nutritional needs of different income groups;

Objective 3- To examine the level of understanding of the UK fish and seafood safety guidelines for pregnant and breastfeeding women;

Objective 4- To identify the key barriers to seafood consumption amongst expectant mothers and young children and potential mechanisms that might remove these barriers.

Background and Significance
Quality nutrition is of critical importance during the first 1000 days of life. The first 1000 days of life refers to the time from conception through to a child's second birthday. During this period, significant neurocognitive and physical development occurs that shapes the future health of a child. Micronutrients such as iron, zinc, calcium, magnesium and vitamin D are vital for supporting this early childhood development and minimising long term health risks. Consequently, the demand for micronutrients is increased for mothers supporting the beginning of a child's life. Severe micronutrient deficiency is rare in the UK and other high income countries but mild deficiencies can still have adverse effects on the health of mothers and babies. There is also a positive correlation between a lack of micronutrient intake and a low socio-economic status so ensuring widespread accessibility to nutritious foods is imperative to ensuring optimal early development for the next generation.

Recognising and informing the public about micronutrient rich, sustainable foods is vital for encouraging healthy diet habits. In the UK, pregnant women receive supplements for folic acid and vitamin D. However, micronutrients are more beneficial to humans when consumed in food, rather than in a supplemental pill. This is due to the surrounding health benefits of food consumption, such as the displacement of less healthy food alternatives, increased protein intakes, and the collective increase of a variety of micronutrients. For example, fish oil supplements do not offer the same health benefits as the consumption of real fish. Seafood products can contain elevated concentrations of micronutrients in comparison to other protein sources. Bivalve shellfish are particularly high in micronutrients, for example containing up to 20 times the amount of vitamin A than beef, pork or chicken. In addition to this, the environmental impact of fish and shellfish production can be lower than other animal production systems. Despite this, in 2019 the National Diet and Nutrition Survey (NDNS) identified the mean consumption of oily fish was 56g in UK adults aged 19-64. The NHS recommends around 140g of oily fish per week. Highlighting the potential for seafood to be a quality, sustainable source of nutrition could be important in improving its consumption.

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The barriers inhibiting fish and seafood consumption during the first 1000 days of life have been hypothesised but rarely quantified. Cost, safety, health and environmental reasons will all be investigated in this study. There are many myths and misconceptions surrounding the safety of seafood during pregnancy, particularly the risk of heavy metal poisoning. This research aims to understand these further and ascertain what women themselves believe to be the barriers. This novel approach will also enable direct insight into how to encourage optimal, safe seafood consumption, and in turn informed and improved public health.

Data and methods

In order to address objective 1, the National Diet and Nutrition survey (NDNS) database will be utilised to quantify what women of childbearing age are currently consuming. The NDNS is a database of survey responses on quantitative information of food consumption, nutrient intake and nutritional status of the general population in the UK. This dataset will provide an accurate depiction of dietary choices in the UK which is important because there are upper limits as to how much seafood is recommended during pregnancy and whilst breastfeeding. Advice to women needs to balance encouraging optimal consumption with acknowledging the potential health risks.

Artificial intelligence (AI) tool Beautiful Soup will be used to webscrape data from 10 leading UK supermarkets. This model will enable rolling analysis of fish and seafood prices in the UK and their associated nutritional values. In order to assess product affordability, household food spending needs to be considered. DEFRAs family food dataset details how much of household income is spent on food shops. This dataset includes information on how much UK equivalised income decile groups spend on their food shop and how much of that is currently spent on seafood products. This will enable the analysis of cost as a barrier to improved fish and seafood consumption during the first 1000 days of life.

An online survey has been granted ethical approval and will be used to assess the changes of diet for expectant and new mothers as well as identify the barriers to improves nutrition and women are encouraged to share their thoughts on potential resolutions. The target is to survey greater than 100 expectant mothers and parents/careers to children under 2 years from a diversity of socio-economic backgrounds. Questionnaires distributed to 50 active Facebook groups will gather data quantifying fish and seafood intake, guideline understanding level and key barriers to fish and seafood consumption for mothers. The survey also asks women to identify mechanisms that they believe have the potential to remove fish and seafood consumption barriers.

Conclusion

The potential for fish and seafood to help supply expectant mothers and children under the age of 2 with the essential micronutrients required for healthy development is significant. Current under exploitation of nutritious seafood in high income countries, such as the UK, means that there is an opportunity to sustainably improve public health. Understanding how to improve seafood consumption in this precious period could help enhance positive health outcomes for a future generation.

VACCINATION CAUSES SALINITY-DEPENDENT GROWTH IMPAIRMENTS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Background

Vaccination enhances survival in farmed rainbow trout (*Oncorhynchus mykiss*) exposed to certain pathogens, but vaccinated fish may develop mild-to-severe side-effects[1]. Indeed, industry experience testifies that growth may be impaired, resulting in up to 20% lower harvest mass. The growth impairments stem from an initial reduction in appetite, leaving less energy available for growth[2]. This might be mediated through cross talk between the endocrine systems regulating growth and immunity[3]. In addition, the reduced growth may result from increased metabolic demands from immune activation or adjustment of homeostatic imbalances. Vaccine-induced effects have primarily been studied in fresh water, while less is known about the effects in sea water, even though salmonids are often transferred to and reared in sea water after vaccination. This knowledge-gap warrants attention, especially considering the observed negative salinity-dependent effect on growth in trout that likely relates to effects on metabolism and appetite[4]. Thus, as both vaccination and seawater-acclimation may interfere with processes underlying growth, we hypothesized that their combination may lead to additive effects. We therefore analysed the effects of vaccination on growth in rainbow trout acclimated to fresh water or sea water, and compared the effects on metabolism, osmoregulation, and endocrine growth-regulation.

Materials and methods

Rainbow trout were either immunized with Alpha Ject 3000, or sham-injected with phosphate-buffered saline. The fish were fed daily until satiation with ≤2% of body weight. Starting 12 days post injection (dpi), salinity was gradually increased to 31 ppt over 10 days for half of the vaccinated and unvaccinated fish, while the other half remained in fresh water. During acclimation, feeding was limited to ≤1% of body weight, and then returned to initial levels until the experiment ended at 52 dpi. Length and weight were recorded at 0, 12 and 22 dpi. At 44-50 dpi, the metabolism and aerobic scope of the fish was examined by manually chasing the fish for 5 minutes to elicit a maximal metabolic response, before measuring standard metabolic rate after 48 hours of recovery. Fish were then euthanized, length and weight measured, and blood plasma acquired for measuring concentrations of growth hormone (GH) and insulin-like growth factor I (IGF-I). In addition, the kidney, gill, and intestine were sampled for analyses of Na^+/K^+-ATPase (NKA) activity.

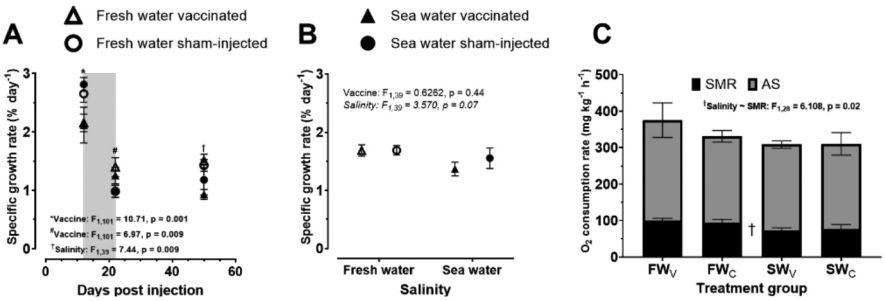

Figure 1. Effects of vaccination and salinity on growth and metabolism. A. Specific growth rate for weight across treatment groups during the three phases of the experiment (0-12 dpi; 12-22 dpi; 22-50 dpi). Fish were vaccinated at 0 dpi, and the shaded area represents the period of seawater-acclimation between 12 and 22 dpi. B. Specific growth rate for weight across treatment groups over the entire experimental period (*i.e.*, 0-50 dpi). C. Standard metabolic rate (SMR; black) and aerobic scope (AS; gray) at 50 dpi in vaccinated (subscript V) and sham-injected (subscript C) trout acclimated to fresh water (FW) or sea water (SW). Maximum metabolic rate is represented as the combined SMR plus AS. Data were analyzed by two-way analysis of variance and are shown as means ± s.e.m.

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Results and discussion

Size was initially uniform across treatment groups (weight and length for all fish [mean ± s.e.m.]: 32.8±0.8 g; 14.3±0.1 cm). Vaccinated fish grew less than sham-injected fish for two weeks after injection (Fig. 1A), which is in line with research showing that initial appetite inhibition can limit growth for two weeks after vaccination[2]. However, during the seawater-acclimation phase, the vaccinated fish grew faster than non-vaccinated fish (Fig. 1A), compensating for the initially reduced growth. When the fish were fully acclimated and their feeding rates increased, freshwater trout grew faster than seawater trout (Fig. 1A). This is in line with research showing a negative relationship between growth and salinity in rainbow trout[4]. During this phase, growth rates were numerically lowest in the vaccinated trout acclimated to sea water, although this was not significant. Yet, there was no interaction effect of salinity and vaccination on growth rates when analysed across the entire experimental period (Fig. 1B). However, there was a strong trend for lower growth in sea water ($p = 0.07$), where vaccinated trout again had the numerically lowest growth rates (Fig. 1B). While this pattern was similar for both weight and length growth, the effect on length appeared to be more pronounced (data not shown). Over 50 days, this did not cause any differences in condition factor, but may indicate ongoing spinal deformation, which is a known risk of vaccination in Atlantic salmon[5].

We did not find conclusive support for our hypothesis that metabolic differences could explain differences in growth. For example, aerobic scope was similar across treatment groups (Fig. 1C). However, fish in seawater had significantly lower standard metabolic rate than fish in freshwater, and it was numerically lowest in vaccinated seawater-acclimated trout (Fig. 1C). This could be associated with reduced growth rates in fish fed *ad libitum*[6]. The analyses of endocrine and osmoregulatory effects are ongoing and will be complemented later. In conclusion, we show that the growth of rainbow trout is compromised following vaccination with Alpha Ject 3000, and the effect was more pronounced in seawater where it appeared to persist for at least 50 days. This may partly relate to metabolic changes, and it remains to be seen if the differences in growth can be explained by endocrine and/or osmoregulatory changes.

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A NOVEL VIRTUAL SENSOR FOR RECIRCULATING AQUACULTURE SYSTEMS TO BETTER CONTROL THE DENITRIFICATION PROCESS

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Introduction

Recirculating Aquaculture systems (RAS) are gaining importance for a sustainable future seafood production. Reasons are the increasing threat to coastal ecosystems and climate change that is imposing thermal distress on organisms in conventional systems.

RAS could be a solution if the water circulation can be largely closed. This requires control over nutrient concentration such as nitrate from ammonia excretion of fishes converted in the biological nitrification. Nitrate accumulating in RAS process water is often controlled by strong water exchange contradicting the sustainable concept of RAS. Without denitrification RAS pollute the environment as open aquaculture installation do.

Heterotrophic denitrification operated with an organic carbon source is an efficient but potentially instable process. The carbon supply to denitrification is crucial to avoid accumulation of nitrite that is an unwanted intermediary product in biological denitrification. By detecting the occurrence of elevated nitrite concentrations in the denitrification effluent the dosage of organic substrate could possibly be adjusted to meet with the demand.

This abstract summarizes company-based research. It describes the discovery of a virtual nitrite sensor that is capable to detect a deficient carbon supply to denitrification. With that a new opportunity is revealed for more sustainable RAS technology.

Material and methods

The investigations were carried out in a precursor model of a SEAWATER Cube. For details refer to Orellana and co-workers (2014, Aquacultural Engineering 58, 20–28). The RAS was fully automated. Process data were saved for later use and evaluation.

The 8 m^3 RAS included a subsequent treatment of the denitrification effluent in a floatation to remove excess bacteria biomass, to replenish oxygen, and to oxidise unwanted inorganic and organic residuals (Fig. 1).

The denitrification process was maintained by feeding acetate as carbon source. The acetate dose was estimated from literature data. It was continuously fed into the denitrification by pulses of constant volume (Fig. 1, metering pump).

Nitrate-rich process water was fed into the denitrification whenever the Oxidation-Reduction-Potential (ORP) in the denitrification decreased to values of - 110 mV and below. Water flow stopped at - 90 mV ORP. The effluent water from denitrification was passed into the flotation for clarification and removal of excess bacteria biomass.

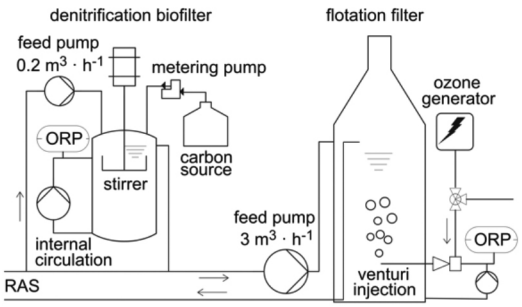

Fig. 1: The experimental set up of the denitrification filter and flotation.

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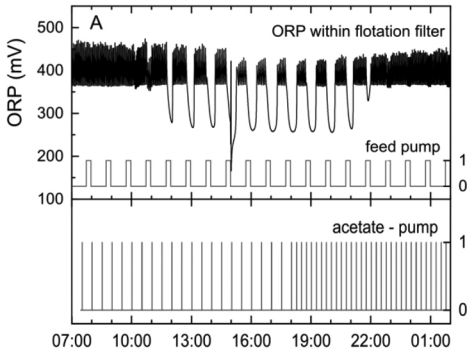

Fig.2: A: ORP in the flotation filter and process water flow (operational state 0/1). B: C/N ratio and operational state of the acetate -pump delivering carbon into the denitrification biofilter (operational state, 0/1). The daytime is shown on the abscissa.

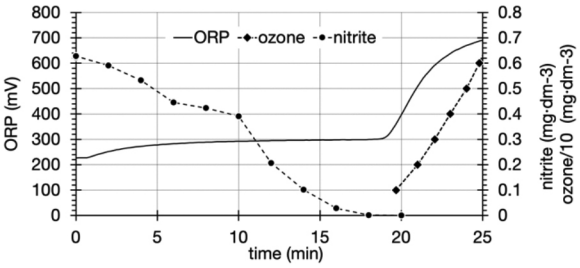

Fig. 3: ORP, ozone and nitrite concentration in the disconnected flotation after an addition of nitrite at time zero that was increasing the concentration to above 0.6 $mg \cdot dm^{-3}$.

The flotation was operated with ozone. The two-point regulator adjusted the ozone feed to maintain an ORP at around + 400mV in the flotation (Fig. 2). In this experimental set up a reductive milieu was followed by an oxidising milieu in the flotation delivering nitrate free and oxygenated process water back into the RAS (Fig.1).

During experiments ORP was continuously monitored. To investigate the effect of ozone, nitrate, nitrite, and carbon feed on the denitrification process and ORP the concentrations were either determined discontinuously (O_3, NO_3^-, NO_2^-) or calculated from mass flow data (carbon source).

To investigate the effect of elevated nitrite on the ORP within the flotation the filter was disconnected from the RAS and nitrite was dosed into the flotation. Subsequently ORP, ozone, and nitrite concentrations were measured.

Results

Figure 2 shows a short time section of the registered process data revealing two recurring patterns of the ORP signal in the flotation filter. The typical up and down movement of the ORP signal was caused by switching on and off the ozone generator according to the measured ORP within the flotation filter (Fig. 1, A).

Time by time it was observed that the ORP unexpectedly decreased in the flotation filter. This was likely linked to an incomplete denitrification process. Parallel measurements of nitrite concentrations proved that nitrite concentration in the denitrification filter always had increased when a drop in ORP was recorded.

The untypical ORP pattern slowly disappeared if carbon dosing was upregulated (Fig. 2 A, 17:30). The elevated nitrite concentrations disappeared from the denitrification effluent meaning that the full denitrification process had been restored.

Fig. 3 shows the result of an additional experiment to investigate the effect of elevated nitrite concentrations on the ORP in the flotation.

The results (Fig. 3) shows that ORP in the flotation remained on low levels (≦ 300 mV) when nitrite was present in the water. Nitrite concentrations of around 0.6 $mg \cdot dm^{-3}$ already impeded an increase in ORP. After 18 minutes nitrite had disappeared. Subsequently ORP and ozone concentration abruptly increased at a constant feeding rate.

Discussion

Coincidental observations in a process chain of a denitrification biofilter and a flotation led to the discovery of a virtual sensor that allows to observe the function of a microbial denitrification process. The release of nitrite from denitrification due to an insufficient carbon supply led to a marked change of pattern in the ORP signal of the downstream flotation. The virtual sensing of elevated nitrite marks a next level in the control of denitrification which is inevitable in future RAS operations. No other solution is seen as direct measurements of carbon, nitrate, or nitrite concentrations would be more costly.

EFFICIENT GENOME EDITING IN AN *Oreochromis mossambicus* CELL LINE USING RIBONUCLEOPROTEIN COMPLEXES

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Introduction:
Aquaculture is the fastest-growing food production sector worldwide (FAO 2020). Nile tilapia (*Oreochromis niloticus*) has the third-highest production of all finfish species (FAO 2020). However, the recent emergence of Tilapia Lake Virus (TiLV) presents major threats to tilapia production, mortalities up to 90% at several stages of the production cycle (Dong et al. 2017). There are currently no effective treatments. Genetic and genomic technologies have the potential to increase the resistance of Nile tilapia stocks to TiLV. Recent advances in genome editing via CRISPR/Cas technologies have facilitated genome-engineering approaches that have the potential to characterise genes involved in resistance and generate fully resistant animals. Also, extensive progress has been made in utilizing cell lines for studying fish pathogens and host responses, and the potential for enhancing fish health research through the manipulation of these cell lines is significant. The effectiveness of CRISPR/Cas editing using ribonucleoproteins (RNP) in Omb cell lines remains uncertain. Exploring this approach could offer valuable insights into creating models for investigating TiLV diseases in tilapia.

Methods:
This experiment employed two cell lines, namely the Omb wild-type (WT) and Omb-GFP cell lines. TracrRNA-ATTO550 was utilized to assess the transfection efficiency under various electrical stimulation conditions in the Omb wild-type cell line. Cell survival of Omb-GFP cells under different electrical setting was determined using the CellTiter Glo assay. On Day 3 and Day 7, knockout efficiency was evaluated through Sanger sequencing. Additionally, after 14 days post-electroporation, the disappearance ratio of EGFP protein was assessed via flow cytometry.

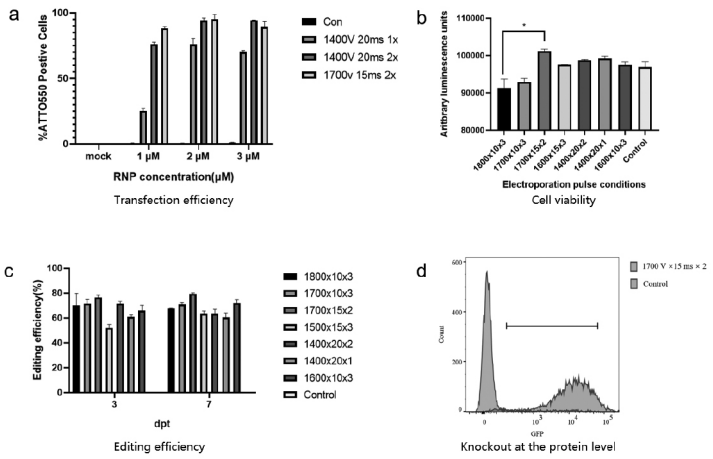

Figure 1: Efficient editing of Omb cell line by electroporation of Cas 9 RNP. a, Using tracrRNA-atto550 test the best efficiency, test by flow cytometry after 24 h. b,c,d, efficient knock out of GFP in Omb-GFP cell line. The optimal concentration (2μM) of RNP, different electroporation configurations were employed for Omb-GFP cell knockout. Cell survival 24 hours after electroporation was measured with CellTtiter-Glo (b), editing efficiency was assessed by Sanger sequencing and ICE analysis at 3 and 7 days post electroporation (c), GFP fluorescence knockdown was measured after 14 days using flow cytometry in experimental (edited) group: 6.62% and control group: 90.3% (d).

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Result:

Electroporation of Cas9-gRNA complex leads to efficient editing in OmB (Figure 1). The highest tracrRNA transfection efficiency was 2 **μM** group (Fig 1a), using that concentration, we optimised electroporation conditions using a GFP expressing cell line. We measured fluorescence as a proxy of cell survival, and observed that 1700V 15ms 2 pulses settings resulted in the highest cell survival (Figure 1B), editing efficiency was measured by Sanger sequencing and loss of eGFP signal also showed 1700V 15ms 2 pulses is best setting (Fig 1c and 1d).

Conclusion

In the current study, the best setting for RNP electroporation was 1700V 15ms 2 pulses, tracrRNA concentration was 2 **μM**. Editing efficiency was higher in day 7 than day 3, so the Cas9 protein must be still active at room temperature for over one week. Through Cas RNP complex, we can efficiently edit the Omb cell line, needing just about 2 weeks from design to experimental testing of the edits.

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EVOLUTION OF THE STOCKING DENSITIES FROM INTENSIVE TO HYPERINTENSIVE CULTURE OF *Litopenaeus vannamei* IN BFT SYSTEM

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Introduction

In subtropical and temperate areas, low temperatures limit shrimp culture to summer and fall. The shrimp biofloc culture rearing system (BFT system) in an enclosed greenhouse is a technological alternative aimed at increasing the culture period in these regions. The shrimp farming sector has developed great interest in the BFT, due to the ability of the bioflocs provide a stable culture environment by the maintenance of water quality, low feed-conversion rates (FCRs) through the natural food source and high productivity reaching up to 9 kg.m-3 (Samocha et al., 2017). In order to achieve the highest productivity rates, several authors have been testing the effects of high stocking density rates in order to determine which is the most appropriate (Da Silveira et al., 2020; Krummenauer et al., 2011). However, the production efficiency in culture tanks can be boosted by increasing the culture tank's carrying capacity, which can be described as the maximum biomass of aquatic cultivated organisms that can be maintained in a rearing system indefinitely, usually described as unit of mass per volume unit (kg.m-3) (Timmons and Ebeling, 2010). Anyway, with the system carrying capacity known, the definition of the most adequate stocking density must consider the shrimp desired final individual weight. In addition, for a better efficiency in the use of the available culture structure, it is also possible to use strategies that maintain the shrimp stocking densities closer to the system carrying capacity throughout the production cycle like multi-phase production system (Van Wyk, 1999). The BFT system at high stocking density requires a vigorous and efficient aeration to keep the oxygen concentration within the desirable concentrations, and maintain a high particle density and organic matter in suspension. The dissolved oxygen is the most critical water quality variation in aquaculture. In this scenario, several technologies and practices has been tested and adopted in order to increase the productivity, for example Samocha et al. (2017) described a system with carrying capacity of 9 kg.m-3 with the possibility of using pure oxygen if it needs. These technological advances have greatly increased the aquaculture system's carrying capacity in terms of maintaining desirable water quality parameters. Considering that, the multi-phase system permits the maintenance of stocking densities closer to the system's carrying capacity, and the system's carrying capacity in terms of water quality maintenance is no longer a limit. The present study intended to show the evolution of the stocking densities from Intensive to Hyperintensive culture of shrimps in BFT systems and determinate the most adequate maximum shrimp biomass in each stage of a multi-phase system.Material and methods

The present study aims to define the most adequate highest stocking densities for each stage of a multi-phase system, considering that the system's carrying capacity is able to maintain the water quality parameters at desired levels. This study was divided into four phases according to the size: Phase 1, shrimp were stocked with initial weight of 0.002 g; Phase 2, initial weight of 1.04 g; Phase 3, initial weight of 6.09 g; and, Phase 4, initial weight of 12.51 g. Each phase lasted for 40 days, and the treatments applied were the different stocking densities. Shrimp for all stages were cultured at high stocking densities in biofloc technology system (BFT). The water quality parameters were maintained within the optimum levels for the *L. vannamei* development.

Table 1: Stocking density, survival and Biomass of *L. vannamei* in different phase.

	Density (shrimp/m^3)	Survival (%)	Biomass (Kg/m^3)
Phase 1	11,250	94.3	4.05
Phase 2	1,500	88.0	7.52
Phase 3	1,000	91.1	9.35
Phase 4	750	95.9	12.59

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Results and discussion

The results of the present study confirm the *L. vannamei* density dependent growth dynamic in all stages of development as reported by several authors (Da Silveira et al., 2020; Krummenauer et al., 2011). The phase 1 or pre-nursery has already been considered intensive culture when the densities reach 2,000 to 5,000 shrimp.m^{-3}. The utilization of the BFT technology in the early stages of *L. vannamei* can generate shrimp juveniles of excellent quality due to the nutritional benefits taken from the presence of bioflocs. In different experiments survival and growth in all treatments and were significantly different ($P<0.05$). Better results of each experiment are shown in table 1. However, for phase 4 the quadratic model ($y = -1.292e\text{-}05x^2 + 0.02852x - 1.513$) indicate that the support capacity reaches the max biomass (14.22 kg) at 1,125 shrimp.m^{-3}.

Conclusion

In summary, the present study confirms the shrimp density dependent growth pattern even when maintaining optimum levels of water quality parameters. In addition, proposes a biological limit of 14 kg.m^{-3} for the shrimp culture in BFT system. However, the water quality parameters deterioration is the limiting factor for the system's carrying capacity, and the shrimp stress behavior generated by the lack of space is the biological limiting factor for the shrimp culture maximum biomass (biological limit). In addition, further studies are needed for better understanding of the shrimp biological limit and for technology development aiming to increase the shrimp culture productivity.

Acknowledgements

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GROWING NATIVE OYSTERS (*Ostrea edulis*) IN OPEN WATER IMTA SYSTEMS– A PILOT STUDY

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Introduction

Integrated Multi-Trophic Aquaculture (IMTA) is defined as a practice that combines appropriate proportions of fed aquaculture species, with organic extractive species and inorganic extractive species to create an environmentally sustainable, economically stable and socially acceptable balanced aquaculture system (EUMOFA 2023). In temperate regions, IMTA systems are uncommon and research into establishing economic and environmental IMTA value chains with appropriate and available species and technologies is essential to achieve commercial scales (Barrington et al 2009). The ASTRAL project is a European Union Horizon 2020 collaborative that focuses on IMTA, it aims to support and promote sustainable production across the Atlantic. ASTRAL IMTA production is carried out in five IMTA labs involving partial inshore recirculation (South Africa), inshore recirculation (Brazil), two open coastal systems (Ireland and Scotland) and one prospective lab (Argentina).

In Ireland, the Marine Institute runs a pilot-scale IMTA lab based in a sheltered bay on the west coast. The lab is exploring the cultivation of Atlantic salmon (*Salmo salar*), native oysters (*Ostrea edulis*), seaweeds (*Alaria esculenta, Saccharina latissima*) and sea urchin (*Paracentrotus lividus*). In Scotland, SAMS is managing an experimental low-trophic aquaculture site exploring the economic and practical feasibilities synergies of growing seaweeds (mainly kelp: *Alaria esculenta, Saccharina latissima*) and shellfish (*Ostrea edulis, Pecten maximus*) alongside within an integrated system designed to reduce material and capital requirements.

Both sites have incorporated native oysters into their IMTA value chains to assess this species suitability for cultivation in an open water IMTA system. Growth performance, mortality and operational constraints were measured as part of this assessment.

Methodology

At the Marine Institute's Marine Research Site in Ireland, juvenile native oysters were sourced from an oyster bank situated in the same bay as the research site. Approximately 300 oysters were collected by dredging under license, spat was identified and 2 grades were chosen (6 – 18 months old). Oysters were deployed into three locations the Low-Trophic Grid (LTG) and Long-Line (LL), locations enriched by the effluent produced by salmon in adjoining fish pens and a control (Ctrl) location away from the nutrient load provided by fed species. Oysters were mixed and stocked evenly in 6mm mesh round oyster tumblers (AP6 baskets). At SAMS site, 2000 juvenile oysters were sourced from a commercial hatchery and split into two batches – one for subtidal cultivation at SAMS LTA site and the other for intertidal cultivation at a neighboring traditional oyster monoculture site. Oysters were also deployed using the AP6 baskets. At regular intervals (approximately 8-10 weeks), both sites followed the same protocol for the monitoring of progression in shell and tissue growth and recording any mortality.

Results

Initial results suggest that oysters can be successfully grown as part of the open coastal IMTA system. Although growing slowly, native oysters grown in an IMTA system can provide good yield and would be of value in a well-established market.

Oyster survival differed substantially between subtidal and intertidal culture, with mortality rates exceeding 70% in summer in the intertidal.

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Practical considerations: The floating AP6 oyster baskets were selected to replace buoyancy required to maintain cultivation materials at the appropriate depth. These baskets must be routinely (every two weeks during the summer months) inverted to expose biofouling to the air for extended periods to minimize the impact of biofouling on buoyancy and flow rates within the baskets. This may not always be possible and careful selection of floating basket types that allow for biofouling to be managed easily whilst maintaining efficient husbandry of oysters is essential to develop efficient cultivation practices. At the more exposed SAMS LTA site two failure modes were identified whilst using the AP6 baskets and these must be corrected if this system is to be scaled.

Potential remediation of these extractive species systems is under investigation and will form part of the outcomes of the ASTRAL project.

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Acknowledgements

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PHENOLOGICAL STUDY OF *Palmaria palmata* ALONG THE WEST COAST OF IRELAND

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Introduction

The phenology of the red alga *Palmaria palmata* is described for a number of locations on the west coast of Ireland from January 2020 to March 2021. Increasing demand of *P. palmata* in Ireland for wild collection and cultivation required further understanding of the reproductive cycle and phenology of this species. At present, wild populations are hand-harvested at low tide; this is labour-intensive and does not always provide a consistent product. Hand-harvesting can put pressure on wild populations, with the increasing demand areas can be overharvested. This suggests that the development of cultivation methods such as tetraspore release and on-growing at sea could help ease this pressure on wild populations. This phenological study aims to understand features of selected populations of *P. palmata* along the Irish coastline and the timeline of their reproductive cycles to understand the optimal time to collect fertile biomass for tetraspore release and to increase the chances of a higher harvest return.

Material and methods

Over the year, monthly sampling of approx. 100 individuals from populations of *P. palmata* in two locations in County Galway and additional regular (not monthly) sampling from six populations in Counties Cork, Kerry, Clare, Galway, Leitrim and Donegal. The total length of each individual was measured to the nearest 0.1mm and weighed to the nearest 0.1 g. Each individual was also inspected for condition, epiphytes, reproductive status, and if reproductive, the percentage of unspent sori remaining in the sori. An estimation of the amount of released ('spent') tetraspores from the sori of individuals was made visually inspecting each specimen in a well-lit area to compare the dark, raised areas of sorus (containing fully mature tetraspores), compared with the pale areas of sorus that had already released tetraspores ('spent sorus'; Figure 1).

Results

An estimation of the proportion of mature tetrasporophytes and males was recorded, with the majority of individuals (71-100%) reproductive from November to April. There was a seasonal trend to the proportion of fertile individuals found, with overall high fertility in the winter and low in the summer. Asynchronous reproductive maturity was observed, with mature tetrasporophytes developing reproductive sori in October, and most abundant in populations by February (79% ± 10.76 SD). Whereas males were developing reproductive sori in November with lower numbers of mature males abundant in January (31% ± 6.86 SD). This was found to be similar across sites. Measurement of the percentage of unspent sori indicates that most tetrasporophytes have a synchronised short (two week) peak release of tetraspores in November to December (Figure 3).

Discussion

To cultivate *P. palmata* in an ethical and sustainable way, wild material used for seeding or vegetative culture ideally would be collected from abundant and healthy populations. Monitoring these populations over time to understand their fertility and reproductive cycles is beneficial knowledge for the harvesters and cultivators. Overall, on the West of Ireland there was a clear seasonal development of the different life phases of *P. palmata* as identified with reproductive sori of tetrasporophytes and males. According to this phenological study, the best time to find fertile material in Irish west coast populations occurs from December to March. By sampling regularly and measuring the amount of unspent tetraspores in sori, a short period of just over two weeks in November into December emerged as the peak release event of tetraspores in almost all tetrasporophytes; this has not previously been observed for this species.

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Figure 1: Example of a tetrasporophyte with spores that have been released (white star) versus tetraspores still remaining in the sori (yellow star). Individual sampled from Spiddal, Co. Galway, (27/3/2020).

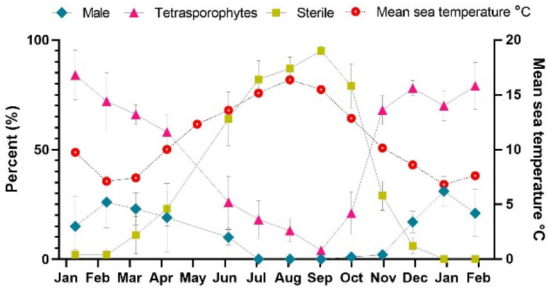

Figure 2: Proportion of all *P. palmata* populations expressed as males, tetrasporophytes, and sterile individuals present in samples along the west coast of Ireland between January 2020 and February 2021 (n=6527). Error bars represent standard deviation (SD).

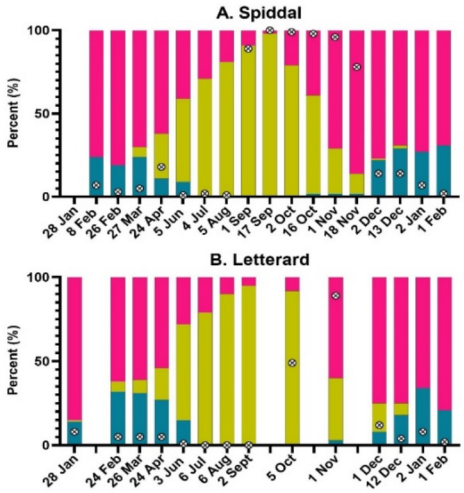

Figure 3: A and B : Proportion of males ( ), tetrasporophytes ( ), and sterile ( ) *P. palmata individuals* and the percentage of remaining reproductive sori ((⊗ Unspent tetraspores) present found on the tetrasporophyte present in Spiddal (A) and Letterard (B) between January 2020 and February 2021.

EFFECT OF DIETARY PROTEIN ON LIVER ENZYME ACTIVITY AND MORPHOLOGY OF EUROPEAN PERCH (*Perca fluviatilis*)

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Introduction

Due to the development of intensive fish production and the increasing global consumption of aquaculture products (FAO, 2022), European perch (*Perca fluviatilis*) is currently considered an interesting species for the diversification of European aquaculture (Fontaine and Teletchea, 2019; Gebauer et al. 2021). Furthermore, it can serve as a complementary species to wild stocks in niche alpine markets, thus reducing pressures on wild individuals of the same species (Cooney et al. 2021. In the wild, European perch are opportunistic, schooling predators feeding on a diverse diet (Thorpe, 1977). In contrast, the nutritional requirements under perch aquaculture conditions are currently not fully defined (Bochert, 2022). It therefore becomes necessary to develop formulations for the production of efficient feeds to produce nutritionally complete commercial fish.

The aim of this experiment was to determine the effect of dietary protein content on morphology and enzymatic activity in liver of the European perch (*Perca fluviatilis*).

Materials and methods

The study was carried out as part of the project "Diversification of the productive function of earthen ponds based on semi-intensive rearing of Perca fluviatilis - PROPERCH (no. 00002-6521.1-OR1400004/17/20) co-financed by the European Maritime and Fisheries Fund.

A 12-week feeding experiment was carried out, during which the fish were fed a feed with fish meal (FM) levels of: 44%, 52%, 60% and 68%. On the last day of the experiment, the fish were sacrificed, measured and weighed, and biological material in the form of liver sections was collected from 12 individuals from each of the 4 groups for analysis. Tissue sections were fixed in Bouin's fluid and then subjected to a standard histological procedure. The sections for biochemical analysis were placed in liquid nitrogen and later homogenates were prepared from them. Paraffin-embedded tissues were sectioned and stained with haematoxylin and eosin (HE) and AB/PAS (Alcian blue - Schiff's reagent with periodic acid). The stained slides were subjected to microscopic analysis. In addition, biochemical analysis of the activity of the following oxidative stress enzymes was performed: alkaline phosphatase, acid phosphatase, superoxide dismutase (SOD) and glutathione peroxidase. The obtained results were analysed using statistical methods.

Results

The highest body weight was observed in the fish meal-fed group at 68% and the lowest in the group with 44% FM (Kruskal-Walis ANOVA; $p<0.05$). In contrast, the most favourable FCR values were achieved in the FM52 and FM60 groups, which translated into the Protein Per Growth Rate (PCR), which was also the most favourable in these two groups. Increase in phosphatase activity in individuals fed diets with a fish meal content above 52%. The group with the highest proportion of fish meal in the diet showed increased superoxide dismutase activity. In addition, all study groups had high glutathione peroxidase activity.

Conclusions

The results obtained indicate that the most favourable protein content in the feed for common perch kept in RAS are feeds with a fish meal content above 52%.

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RESEARCH TO OPEN UP UNDEREXPLOITED OPPORTUNITIES FOR INNOVATION IN AQUACULTURE

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Summary

We present research which aims to help improve the efficiency of thriving aquaculture sectors, create new opportunities in underexploited aquaculture sectors, and provide methodologies to reach a larger consumer market with sustainable and nutritious fish and seafood. Key topics include: nFIFO as a new metric to assess the micronutrient efficiency of fed aquaculture, value chain exploitation and new species opportunities in the bivalve sector, and food preparation and marketing approaches to drive consumption of sustainable fish and seafood.

Improving the efficiency of thriving aquaculture sectors [1, 2]

Aquaculture is the world's fastest growing food sector, and many industries such as salmon farming are thriving. The salmon industry grew by 270% since 1998 to become the most valuable sector in aquaculture at USD $23 billion in 2018. Salmon farming has perhaps unfairly received bad publicity on its use of wild-caught fish in feed, and has in reality made remarkable progress, with Fish-in Fish-out (FIFO) ratios now close to 1:1, having been 4:1 in the 1990s. However, FIFO does not capture the micronutrient content of feed and farmed species.

We have developed a new tool, nutritional Fish-in Fish-out (nFIFO), which enables practitioners to assess the micronutrient efficiency of fed aquaculture systems, and identify scenarios to enhance nutrient rich seafood production. For example, for salmon farmed in Norway in 2020, nFIFO exceeded one for seven of nine dietary micronutrients that are essential in human diets, indicating salmon farms consume substantially more micronutrients than are produced as seafood. We identified a reallocation system by which directing one-third of edible fish in feed for human consumption, we could increase seafood provisioning for people and create new by-products to further support the salmon sector. Application of this research to the salmon industry and to other fed aquaculture sectors can allow us to make more efficient use of limited global fishmeal and fish oil supplies, support further growth in fed aquaculture, and provide a greater quantity of micronutrients from seafood to people.

Creating opportunities in underexploited aquaculture sectors [3, 4, 5]

Bivalves are rich in protein, essential fatty acids and key micronutrients, and have a lower environmental impact than other sources of protein. However, conventional bivalve farming has limiting problems making the sector financially less attractive than others such as finfish or crustacean farming. Specifically, problems include food safety in increasingly polluted open marine environments, slow growth rates versus fish, costly processing, transport and storage. Bivalves are also far less popular as a consumer product relative to other meat or finfish products. Aside from a few select nations such as Portugal, most of European food manufacturer, retail and consumer interest in bivalves is weak compared to southeast Asia.

We have performed value chain analyses in collaboration with Europe's largest frozen food manufacturer, and identified key opportunities that could enable bivalve to become a popular mass-market consumer food. This includes new innovations in depuration, storage, food processing and technology. We have also carried out laboratory trials on terenids, bivalves which could potentially offer an order of magnitude faster growth rate than mussels, clams or oysters. There may be an opportunity to create a new sector in terenid aquaculture, and we have begun to gather critical data including nutritional profile and growth data that underpin further concept development.

Methodologies to reach a larger consumer market [6]

Finfish and seafood are one of our richest sources of key nutrients such as omega-3, which play a critical role in child development, brain, and cardiovascular health. Yet consumption of fish and seafood varies greatly by global region, and there are many areas where consumption is markedly lower than recommended or nutritionally beneficial. For example, while consumption of bivalves is as high as 100 kg per capita in Shandong in China, the EU average is below 1kg per capita. While the NHS in the UK recommends around 140 g of oily fish per week, NDNS data show the mean consumption of oily fish in adults aged 19-64 is just 56 g per week.

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We are performing research to identify several methodologies which could enable a greater proportion of the consumer market to access and consume nutritious seafood, and most critically in sustainable way. National surveys have allowed us to identify key behavioural levers which could help drive the consumption of sustainable bivalve meat in place of less sustainable meat products. Catering trials have allowed us to investigate the impact of 'hiding' bivalve meat in popular consumer dishes in place of conventional meat on consumption patterns. We are assessing ways by which we could get consumers to consume 'under loved' components of fish and seafood, such as heads, bellies and viscera, that would otherwise go to non-human uses. In addition, we have performed focus studies to look at how we could provide pregnant mothers and babies, a group who could benefit greatly from the nutrients in fish and seafood, with greater knowledge about and access to fish and seafood.

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DIETARY SELENIUM AND VITAMIN B6 IN THE GLUTATHIONE METABOLISM OF RAINBOW TROUT *Oncorhynchus mykiss* EXPOSED TO PERIODIC HYPEROXIA

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Introduction

Dietary selenium (Se) supplementation is increasingly discussed in modern fish feed formulations due to its low availability from plant raw materials and its essential role in the antioxidant system of fish (Fontagné-Dicharry et al., 2015). Thereby the effective biosynthesis of selenoproteins from organic Se is dependent on pyridoxal-phosphate as an enzymatic co-factor (Soda et al., 1999). A deficiency of vitamin B6 has been associated with impairments in the glutathione metabolism and antioxidant system (Taysi, 2005). Recent changes in the recommendation for dietary pyridoxine levels in plant-based aquafeed formulations (Hansen et al., 2015) raise the question, if the limited availability of the two micronutrients Se and pyridoxine may induce interactive effects in the glutathione metabolism of fish, especially when exposed to stress. Therefore, a feeding trial was conducted to investigate such possible interactive effects between dietary Se and pyridoxine supplementation in rainbow trout (*Oncorhynchus mykiss*).

Material and Methods

Four diets were designed: CTL, without any Se or pyridoxine supplementation; SEL, supplemented with 4 mg selenomethionine / kg diet; PYR, supplemented with 50 mg pyridoxine hydrochloride / kg diet and SEPY, co-supplemented with similar Se and pyridoxine levels. Groups of 50 juvenile rainbow trout (28 ± 3 g) were randomly distributed in triplicate tanks per treatment and fed one of the experimental diets for eleven weeks. At the end of the feeding period, 8 fish per tank were euthanized and tissue samples dissected. The remaining fish were exposed to oxygen stress (periodic hyperoxia) prior to sampling. Therefore, the dissolved oxygen (DO) level in the tanks was elevated for 8h (09:00-17:00h) per day from 8.5 ppm to 13 ppm (=168 %) DO over a one-week period. The liver samples collected of stressed and non-stressed fish were analyzed for metabolites and gene expression associated to the glutathione metabolism. The collected data was analyzed by repeated three-way ANOVA and is presented as mean ± standard error of means.

Results

Neither Se nor pyridoxine supplementation had any significant effect on fish growth performance (182 ± 3 g). Total liver glutathione levels were significantly lower in stressed compared to non-stressed fish (1351 ± 76 vs 927 ± 57 pg/mg protein). Fish fed diets supplemented with Se showed lower cysteine (Cys) levels in liver tissue compared to fish fed diets without Se supplementation (1522 ± 67 vs 1112 ± 57 pg/mg protein). Homocysteine (hCys) and total glutathione levels were observed to increase with pyridoxine supplementation (8.1 ± 0.7 vs 9.9 ± 0.9 and 1003 ± 66 vs 1275 ± 97 pg/mg protein). However, in stressed fish a significant interaction between Se and pyridoxine was detected as hCys levels were the highest in fish fed PYR, but the lowest when fed SEPY (12.9 ± 1.1 vs 5.7 ± 0.6). In addition, Cys levels were the lowest in stressed fish when fed CTL, but the highest with SEL (1587 ± 257 vs 806 ± 127). The gene expression of antioxidant enzyme glutathione peroxidase (Figure 1) and that of other selenoproteins was only affected by Se but not by pyridoxine supplementation.

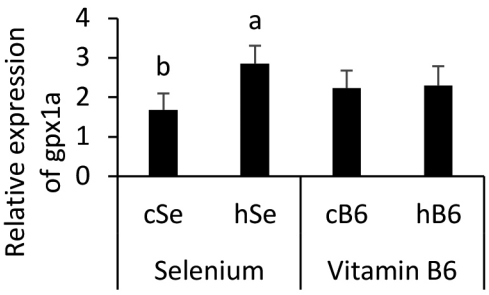

Figure 1. Relative gene expression of *gpx1a* measured by real-time qPCR (n=6). Data are normalized to fish fed the CTL diet before subjected to hyperoxic stress. Unlike superscript letters indicate significant main effects ($p < 0.05$) according to three-way ANOVA. Interactive effects were not significant.

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Discussion and conclusion

The decrease in Cys levels detected with dietary Se supplementation might be a result of the co-metabolization of selenomethionine and methionine through the transsulfuration pathway (Dalto and Matte, 2020). On the contrary, dietary vitamin B6 supplementation seems to elevate transsulfuration as indicated through higher liver hCys and glutathione levels. This might relate to pyridoxal-phosphate being a co-factor of transsulfuration enzymes (Soda et al., 1999). Although vitamin B6 acts as a co-factor for the biosynthesis of selenoproteins through the delivery of Se to the translational machinery, in the present study, no effect of pyridoxine supplementation on selenoprotein gene expression was observed, suggesting that the pyridoxine levels in the non-supplemented treatments might have been sufficient to support selenoprotein synthesis. Nevertheless, interactive effects of Se and pyridoxine on transsulfuration metabolites in stressed fish indicate that both micronutrients play an important role to maintain glutathione homeostasis under stressful conditions similar to observations in mammals (Dalto et al., 2015).

Acknowledgement

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RECOVERY AND STABILISATION OF VERTEBRAL DEFORMITIES IN ATLANTIC SALMON

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Introduction

Farmed teleost fish commonly suffer an increased rate of vertebral deformities. Vertebral deformities have an adverse impact on fish welfare, growth, and swimming behaviour. Under unfavourable conditions (inappropriate handling stress, increased mechanical load, increased water temperature) vertebral deformities are likely to progress and worsen over time[1-2]. However, an increased amount of recently published studies observe that deformities can recover and disappear or stabilise over time[3-5]. Here we present a detailed description of the process of recovery and containment of deformities in Atlantic salmon (*Salmo salar*).

Materials and Methods

Individual animals of Atlantic salmon were followed by x-ray imagining to analyse the development of vertebral deformities in two studies. The first study[4] followed 200 PIT-tagged farmed animals which were x-rayed at three time points: prior to seawater transfer (100 g), six months after seawater transfer (1.1 kg), and 12 months post-seawater transfer (2.3 kg). The second study[3] followed 135 PIT-tagged Atlantic salmon. These animals were x-rayed prior to seawater (50 g), seven months post-seawater transfer (720 g), and 16 months post-seawater transfer (4.5 kg). Further analytics included whole mount Alizarin red S staining, mineralised and non-demineralised histology, and mineral content analysis.

Results and Discussion

Studies detect two broad categories of vertebral deformity which determine further development. Category I. comprises deformities that affect the bone of the vertebral centra. These are characterised by either bent bone trabeculae and replacement of adipose tissue with ectopic cartilage in the bone marrow spaces (hyper-dense vertebrae), by vertical displacement of the vertebral centra, or by mild compression of the vertebrae. Importantly, the intervertebral space between the vertebral centra remain non-deformed and thus category I deformities were observed to recover.

Category II. comprises deformities with alterations in both, vertebral centra and intervertebral spaces. Two studies[3,4] show that these deformities can stabilise as long as no more than three vertebrae are affected (stabilisation through vertebral fusion). Stabilised vertebral fusions do not further aggravate, do not affect animal welfare, and are also common in wild Atlantic salmon[6]. In cases with damage to more than three adjacent intervertebral spaces, recovery has not been observed. Instead, extended vertebral fusion centra develop.

Conclusions

These studies provide detailed insights into the development of vertebral deformities based on tracking individual animals from freshwater up to harvest size. Surprisingly, several types of vertebral deformities can recover and stabilise over time under favourable rearing conditions.

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RESIFARM: TOWARDS RESILIENT ROBOTIC AUTONOMY FOR UNDERWATER OPERATIONS IN FISH FARMS

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Introduction

Aquaculture is an established industrial sector of progressively increasing importance for food production as an alternative that minimizes negative impact on climate change. Continuously growing demand of aquaculture infrastructure introduces important yet-to-be-addressed technological and logistical challenges, with reliance solely on human operators becoming unattainable in the future. Robotics and automation could become a leading force on expanding aquaculture operations and resolving scalability issues in a consistent and sustainable way [1]. However, exposed aquaculture settings are among the most challenging domains for robots to operate, due to uncertainty introduced from limited and low-quality sensor readings, lack of static reference caused by changing surroundings due the constant deformation of the fish cage structure, and necessity for real-time decision in environments with currents, surge, moving obstacles, and uncertainty. The ResiFarm project, aims to address such problems regarding scalability and safe operations in exposed aquaculture settings by providing fundamental technologies enabling underwater robots to operate safely, accurately, and resiliently. The potential of different types of autonomous robots is explored for the produced technology, such as ROVs and Underwater Swimming Manipulators (USMs), Figure 1.

Approach

Resilient autonomy depends on solving robustly two fundamental problems in robotics: State Estimation, which is the problem of localizing the robot with its surroundings, and Motion Planning, which is the problem of deciding on efficient and safe actions that the robot could take towards accomplishing a task. Unfortunately, both are becoming more challenging to solve in the underwater domain, and especially in aquaculture settings. The focus of this paper lies on safe and robust motion planning.

Even assuming perfect state estimation — a prerequisite for motion planning — safe autonomous aquaculture operations require addressing simultaneously and in real-time a combination of unique challenges, such uncertainty, unexpected surge and currents, potential control errors, moving obstacles, and moving deformable nets. Several motion planning approaches exist, such as sampling-based, lattice-based, and optimization-based motion planning. We focused on path optimization, due to its computational efficiency, quick replanning frequency, and guarantees, as shown in past work [2].

Such techniques can provide very quickly locally optimal solutions, which are sufficient given the limited on-board sensing range. Though, the most important advantage of these techniques is that user-defined cost functions could be implemented making them highly adjustable to different platforms, and for different tasks, such as for inspection [3].

Towards enabling autonomy, a novel framework, called ResiNav, was developed that guarantees safe navigation in the presence of motion errors due to currents, waves, or imperfect controls, localization and map uncertainty, and detected dynamic obstacles. ResiNav [4] deals with all these challenges in real-time in a holistic perspective by informing the path optimization process with the past experienced conditions, adjusting automatically the clearance needed. The necessary clearance is computed analytically with minimal information and provides a tight worst-case boundary that guarantees safety.

Results

Currently, several motion planning concepts have been tested in simulation. The AquaVis [3] pipeline was applied for cage inspection. Virtual visual objectives were placed on the net, enabling the robot to inspect it from a desired proximity. The robot inspected the net, while also avoiding safely dynamic obstacles executing unknown trajectories in simulation, Figure 2. Additionally, ResiNav has been implemented and rigorously tested for different conditions, environments, and robot velocities in simulation. An example of one test case with strong side current is shown below in Figure 3. We speculate that, almost certainly, a motion planner with such capabilities will be at the core of future safe and robust autonomy applied for operations in exposed aquaculture infrastructure.

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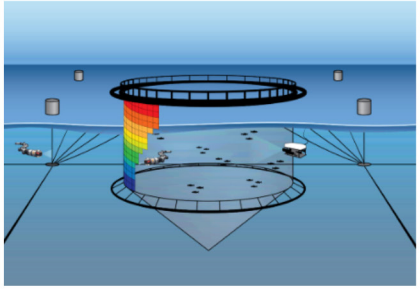

Figure 1: Abstract illustration of different robots operating in an aquaculture setting, the desired outcome of ResiFarm.

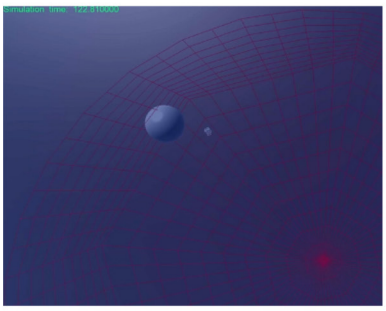

Figure 2: An ROV inspecting net in simulation with moving obstacles

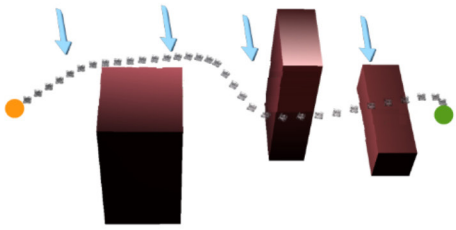

Figure 3: An executed safe path utilizing ResiNav, in the presence of currents

Conclusion and Future Work

A collection of approaches is investigated to realize the ambitious goals of ResiFarm, towards robust motion planning, with a subset of them described briefly above. Future focus will be directed towards data collection and testing of robust motion planning concepts in real fish farms (infrastructure provided by SINTEF ACE) using ROVs and investigating extensions to different platforms, such as the Eelume USM. We aspire that the result from ResiFarm would provide fundamental technologies and insights to support scaling aquaculture operations in the future, safely, accurately, and resiliently.

Acknowledgements

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AQUAPONICS PRODUCTION OF WHEATGRASS (*Triticum aestivum*) IN VERMICULITE SUBSTRATE WITH DIFFERENT STOCKING DENSITIES OF AFRICAN CATFISH (*Clarias gariepinus*)

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Introduction

The direct production and marketing of aquaponic vegetables is to be considered critical due to the quality and quantity as well the labour costs compared to the competition of industrial vegetable production. However, there are possibilities to generate profit from vegetable ingredients and metabolites (e.g., vitamins, proteins, essential oils) via secondary value chains that are relatively independent of the expiry date compared to fresh vegetables. Larger quantities can be accumulated by extracting and drying secondary plant constituents and by storing and hoarding them in a pre-stored state. Such extracts can either be used fresh (e.g., wheatgrass smoothies) or dried as powders or tablets as food supplements or cosmetics. Fast-growing plant species are particularly predestined for such an aquaponic value chain, such as herbs (Knaus et al., 2020) or functional- / super-foods like wheatgrass (*Triticum aestivum*; van den Driessche et al., 2018).

Wheatgrass (*T. aestivum*) has been tested with different growing media in aquaponics with African catfish (*Clarias gariepinus*) before (Xu et al., 2022). Results indicated that fish effluents had beneficial effects on wheatgrass growth, especially in improving nutritional quality for organic produce. It is necessary to reduce the use of synthetic fertilizer in aquaponics. Hence, the aim of the present study was to test the influence of nutrient solution from African catfish recirculating aquaculture systems (RAS) with different stocking densities on the growth of wheatgrass.

Material and methods

Wheatgrass was cultivated in an ebb-and-flood commercial aquaponic system from 23.8.2021 to 03.9.2021 (12 days) in the FishGlassHouse of the University Rostock, Germany. In triplicate, wheatgrass was irrigated with two distinct effluents from intensive aquaculture unit (IAU: 140fish/tank; 138.78 ± 120 kg/tank) and extensive aquaculture unit (EAU: 35fish/tank; 25.80 ± 23.29 kg/tank) recirculating aquaculture systems of African catfish (*C. gariepinus*) and tap water with fertilizer (Universol Basis, ICL, Israel) as control. Plant growth parameters and nutrient contents were measured and analysed in order to assess the growing performance of wheatgrass. The system design was identical to Xu et al. (2022).

Table. 1: Vitamin levels of wheatgrass (*T. aestivum*) irrigated with different nutrient water sources (control [C], extensive aquaculture unit [EAU], and intensive aquaculture unit [IAU]) and cultivated in pure vermiculite substrate. Different letters within a row indicate statistically different mean values at $p < 0.05$.

Vitamins	Control (C)	Intensive (IAU)	Extensive (EAU)
Vitamin B_1 (mg/kg)	0.87 ± 0.04 [a]	0.72 ± 0.08 [a]	0.79 ± 0.08 [a]
Vitamin B_2 (mg/kg)	2.30 ± 0.11 [a]	2.68 ± 0.91 [a]	2.19 ± 0.12 [a]
Vitamin B_3 (Niacin) (mg/kg)	8.15 ± 1.13 [a]	8.15 ± 1.57 [a]	7.77 ± 1.11 [a]
Vitamin B_5 (mg/kg)	1.30 ± 0.10 [b]	1.59 ± 0.43 [a,b]	2.10 ± 0.24 [a]
Vitamin B_6 (mg/kg)	0.89 ± 0.4 [a]	0.73 ± 0.28 [a]	0.55 ± 0.15 [a]
Vitamin B_7 (Biotin) (µg/kg)	10.67 ± 3.06 [a]	10.00 ± 2.65 [a]	10.00 ± 4.36 [a]
Folic acid B_9 (µg/kg)	1033.33 ± 20.82 [a]	1067.33 ± 65.43 [a]	1153.33 ± 127.41 [a]
Vitamin B_{12} (µg/kg)	2.34 ± 0.20 [a]	3.05 ± 1.70 [a]	3.82 ± 0.56 [a]
Vitamin E (mg/100 g)	0.25 ± 0.02 [a]	0.44 ± 0.35 [a]	0.26 ± 0.01 [a]

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Results and discussion

The shoot length reached the highest value in the IAU (27.07 ± 3.51 cm), comparable to the EAU (26.44 ± 2.81 cm), and significantly lower in the control (23.61 ± 2.89 cm). Furthermore, the SPAD index was greatest in the IAU (27.55 ± 5.78 %) and was lower in the control (23.86 ± 5.91 %) and the EAU (23.34 ± 4.41 %). Generally, experimental groups with fish water irrigation had better growth performance of wheatgrass although the pH value in control was the lowest (6.62 ± 0.06; EAU: 6.69 ± 0.12; IAU: 7.10 ± 0.34), and the EC value was the highest (control: 1701.80 ± 35.81µS/cm; IAU: 1439.20 ± 56.64 µS/cm; EAU: 993.50 ± 32.57 µS/cm). A significantly higher nitrate level in the IAU (434.50 ± 62.03 mg/L) was assumed to be credited to optimal wheatgrass growth with intensive fish effluent, which had distinct lower values in both the EAU (177.62 ± 52.42 mg/L) and control (177.82 ± 45.79 mg/L).

The majority of the plant nutrients in the wheatgrass had no significant difference among all the groups in the present study. Noticeably, vitamin B_5 (pantothenic acid) reached a significantly higher value in the EAU (and IAU) than the control (Table 1). The difference from the control was probably attributed to the significantly higher pH value in the IAU (slightly above 7), and EAU. This pH value in the IAU may have had a positive effect on the microbial communities and thus positively influenced vitamin production (Ratzke & Gore, 2018) and plant growth.

In conclusion, both aquaculture effluents (IAU and EAU) were beneficial to the growth and vitamin (B_5) content of wheatgrass, although some growth conditions, such as pH and EC value, were better in the control. It was assumed that high nitrogen levels, abundant organic matter, organic metabolites, and certain beneficial microbes in the aquaculture effluents significantly promoted plant growth; meanwhile, microbes that can biosynthesize vitamin B_5 should be investigated and verified further in future experiments.

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DISCOVER NOVEL TRAITS FOR BREEDING USING IMAGE ANALYSIS AND CLASS ACTIVATION MAPS IN FISH

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Introduction

In recent year, there is an increasing number of applications of computer vision in agriculture and aquaculture. The applications include individual tracking, behavior monitoring, species identification, phenotyping and prediction of complex traits based on images or video (Vo et al., 2021). Image data has the benefits of being high-throughput and non-invasive. In image data analysis, one of the most popular methods is neural network, especially convolutional neural network (CNN). However, as CNN has been criticized for its 'black box' nature, the lack of interpretability of the image-based neural network can make the results questionable for real-life application. Some methods attempt to understand how CNNs learn by revealing the imaginal features they use to establish the prediction. One of the most intuitive methods is class activation map (CAM). The core function of CAM is to reveal the regions in an image that are most relevant for the prediction. Field experts can therefore use CAM-revealed features as a reference to judge the reliability of the CNN prediction. Ideally, class activation map can make CNN more transparent and promote data driven decisions.

The study aims to investigate the validity of the predictive features of CNNs revealed by CAM from a genetic perspective with a case study. The case study is interested in the physical characteristics of fish that contribute to their critical swimming speed () (Brett, 1964). We use individual 3D images and CNN to predict in rainbow trout. With fish physiologist we then defined two new traits based on the CAM features derived from the prediction. At last, we calculated the genetic properties of these new traits in relation to swimming speed and body weights.

Materials & Methods

We conducted swimming test and acquired individual records on for 1037 rainbow trout. Each fish was weighted and measured for length manually and allowed time for recovery. Afterwards, all fish were subjected to imaging through an imaging equipment. Each individual fish was placed towards the same direction when its lateral side was captured simultaneously in two images: one RGB image, and one depth image where each pixel contains the distance information between the camera and the lateral surface of the fish (Figure 1). By combining these two images, we obtained a 3D-colored hologram of each fish with its lateral side up (Figure 2, left).

We also corrected the recorded for body length for each fish. For corrected prediction we used part of the analytical framework for image data proposed by Xue et al. (2023). Predictive features were visualized using gradient weighted class activation map (GradCAM) (Selvaraju et al., 2019). We included the interpretation from fish physiologists and refined the visualized predictive features into two swimming traits. We then annotated these traits on the original RGB and depth images for each individual (Figure 3). We estimated the heritability of these traits and their genetic correlation with corrected using an animal model: , where are the measurements of the traits, is the overall mean of each trait, are the additive genetic effects and the residuals.

Results

The correlation of the CNN prediction with the real corrected was 0.23. The right side of figure 2 shows the 3D predictive features revealed by GradCAM. The regions highlighted in red correspond to the contour of the fish, the volume of the head, the caudal fin and the volume of a narrow region along the dorsal side. Based on the location of these features and the biological function of the corresponding regions in a fish, two swimming traits were defined in consultation with fish physiologists: One trait is the volume of the head. Another one is the volume of epaxial muscle corrected by body weight, hereafter refer to as the ratio of epaxial muscle. The heritability of head volume and ratio of epaxial muscle were 0 and 0.23, respectively. No significant genetic correlation was found between the volume of the head and corrected . However, the ratio of epaxial muscle had a genetic correlation of 0.35 with corrected .

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Discussion

In this study, we built a CNN using 3D images to investigate the relationship between the physical characteristics and the swimming speed of rainbow trout. The predictive features from CAM were narrowed down to two traits by annotation: head volume and ratio of epaxial muscle. Image-based CNN explained only 23% of the variance within corrected but this is enough for the activation map of the trained CNN to provide valuable information for pinpointing novel predictor traits. The results of genetic analysis validate the intrinsic relationship between and the ratio of the epaxial muscle - a trait derived from the activation map features of the trained CNN. The positive genetic correlation makes intuitive sense; if two fish are of the same weight, the one with a higher volume of epaxial muscle swims better. Head volume, however, shows no significant genetic relationship with . The highlighted region of head in CAM might due to variation in its other properties like shape or its relative distance to other highlighted parts. Future study will continue the interpretation of predictive features, also with different animal models.

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EFFICACY OF DIFFERENT RATIO BETWEEN VITAMIN C PHOSPHATE AND PHYTOCEE (HERB EXTRACT) IN DIETS ON THE PERFORMANCE OF WHITE SHRIMP, *Penaeus vannamei,* UNDER DENSITY STRESS CONDITION, AND DISEASE CHALLENGE AGAINST *Vibrio parahaemolyticus*

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Introduction

Disease outbreaks and climate change have been an ongoing challenge for some large Asia shrimp aquaculture producers, particularly in Thailand and China. In Thailand, shrimp diseases are the most important causative agents. The currently pathogenic bacteria and viruses are *Vibrio harveyi* and *V. alginolyticus* as well as acute hepatopancreatic necrosis disease (AHPND) and early mortality syndrome (EMS) caused by *V. parahaemolyticus* (Lightner, 2011). Furthermore, stress is universal challenge in animal and it can be defined as the nonspecific response of the body to any demand made upon it. Stress can cause adverse effects in animals like low productivity, susceptibility to disease, organ damage, retarded growth and death. (Chen,2015). Dietary vitamin C is essential for penaeid shrimp, and its deficiency would induce severe damage, such as the "black death" syndrome. It also acts as a promising nutritional supplement, since it has been already demonstrated that this vitamin, besides functioning as a potent antioxidant such as an immunostimulant in fish and crustacean (Maggioni et al., 2004). Many herb extracts have the function related to vitamin C on animal physiology. Phytocee, herb extract, has the adaptogenic effect which could be understood by examining its ingredient. *Withania somnifera*, one of the key ingredients has been shown to have anti-stress effects and improve immunity in animals (Singh, 2011). Therefore, the aim of this study was to evaluate the effects of ratio between vitamin C phosphate (vit-C phosphate) and Phytocee on growth performance and survival after pathogen challenge test.

Material and Methods

The experiment was a factorial design with 2 factors of A: 2 levels of vit-C phosphate 35% (0 and 1000 ppm) and B: 2 levels of Phytocee (1000 and 2000 ppm) with 4 treatments and five replication as Table 1.

Shrimp with initial mean weight of 1.4 g/shrimp were randomly distributed into 240L glass aquarium (Total 20 aquariums) that contained 120L of 15 ppt seawater and stocked 30 shrimp per aquarium (250 pcs/m^3). During 8 weeks of feeding trial, shrimp were fed experimental diets 3-5%BW, 4 times a day and weighted on week 0, 4 and 8 for determined growth performance and after challenge test by bath treatment against $6.2X10^5$ CFU/ml of *Vibrio parahaemolyticus* AHPND for 12 days, mortality was recorded.

Results

After 8 weeks of feeding trial, the growth performance of shrimp fed different ratio between vit-C phosphate and phytocee (herb extract) in diets showed that shrimp fed diets of vit-C phosphate 1000 ppm had better growth performance than group of shrimp fed vit-C phosphate 0 ppm ($p<0.05$). Moreover, shrimp fed diets of Phytocee 2000 ppm had better growth performance than shrimp fed Phytocee 1000 ppm ($p<0.05$). Hence, shrimp fed Phytocee 2000 ppm exhibited the better performance than group of shrimp fed diet of Phytocee 1000 ppm both with vit-C phosphate 0 and 1000 ppm ($p<0.05$) when focusing on final weight, average daily gain, and shrimp production (T4>T3,T2,>T1). Feed utilization in term of feed conversion ratio in shrimp fed Phytocee 2000 ppm was lower ($p<0.05$) than shrimp fed Phytocee 1000 ppm both in shrimp fed vit-C phosphate 0 and 1000 ppm (T4<T2<T3<T1). The results of growth performance and feed utilization were presented in Figure1. The survival rate were not significantly differences ($p>0.05$). Shrimp fed vit-C phosphate 0 ppm have tended to present the poor survival rate than shrimp fed vit-C phosphate 1000 ppm ($p=0.063$) both in shrimp fed Phytocee 1000 and 2000 ppm and Phytocee 2000 ppm demonstrated the better survival rate than 1000 ppm. The survival rate was 82.67, 85.33, 86.00 and 88.67% in T1, T2, T3 and T4, respectively. The levels of Phytocee did not affect the survival rate under high stocked density of 250 shrimp/m^3 in clear water of indoor condition due to proper culture management resulting exhibited on promote growth performance and shrimp production. After disease challenge by *V. parahemplyticus* AHPND immersion treatment, the survival rate of shrimp fed vit-C phosphate and Phytocee showed that shrimp fed vit-C phosphate

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TABLE 1 : Supplemented description in shrimp feed trial

Treatment	Diet Description		
1	0 ppm vitamin C phosphate	+	1000 ppm Phytocee
2	0 ppm vitamin C phosphate	+	2000 ppm Phytocee
3	1000 ppm vitamin C phosphate	+	1000 ppm Phytocee
4	1000 ppm vitamin C phosphate	+	2000 ppm Phytocee

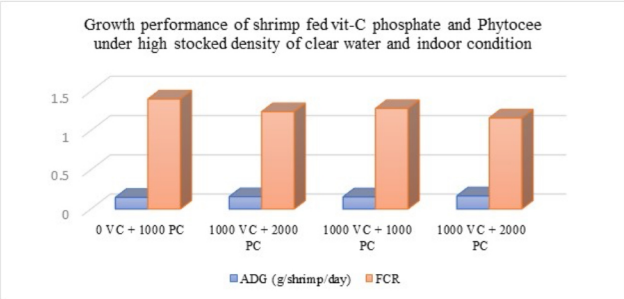

Figure 1 : Growth performance of shrimp fed vit-C phosphate and Phytocee

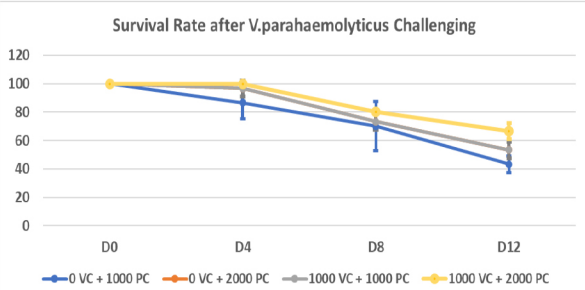

Figure 2 : Survival Rate after *V. parahaemolyticus* Challenging

1000 ppm and phytocee 2000 ppm had highest survival rate follow by shrimp fed vit-C phosphate 1000 ppm and phytocee 1000 ppm ($p<0.05$) and the poor survival rate exhibited in shrimp fed vit-C 0 ppm (T4>T3>T2, T1). There, the results indicate the efficacy of vit-C and Phytocee on improving growth performance and survival rate under high stress condition of stocking density and disease challenge and imply that Phytocee has the adaptogenic effect on reducing stress resulting promoting growth performance and survival rate under stress condition.

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UNLOCKING THE POTENTIAL OF THE RAW MATERIAL BASKET - FROM MARINE BASED DIETS TO 2.0 FISH DIETS

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Feed accounts for up to 70% production costs in aquaculture. The increase in the use of vegetable and other alternative protein sources, together with the recent aquafeed price increases of up to 30%, calls for the further innovation in lower cost ingredients. However, the optimal nutritional value of the feed should be maintained, and the use of dietary enzymes in aquafeeds have potential to improve the nutritional value of the feed as well as contributing to the sustainable development of aquaculture.

Accordingly, the hunt for new raw materials of sufficient quality and affordable price is very challenging across aquaculture species and their growing conditions. In addition, stakeholder concerns are not limited to the nutritional value of individual raw materials but also include important factors such as sustainability, welfare, and the effect on the environment A reminder on the benefits of available enzymes to support aquaculture as it grows sustainably is of major interest.

Data from trials with phytases, proteases and enzymes improving the digestion of non-starch polysaccharides, have shown to have significant effects in the performance of the species, the cost of the feed and reduction in phosphorous excretion for example (salmonids, warm water species, Mediterranean species, and shrimp).

A few recent studies show that the use of phytase enzymes (release the phosphorus contained in phytate form in the vegetable ingredients: Phytate P) impact the digestibility and absorption of phosphorus, other minerals and protein. The effect of phytase on the phytate-P, which is poorly digested by animals and is also an antinutritional factor, can improve growth performance in fish species up to 5% because of an increase in phosphorus digestibility up to 65% in rainbow trout and 60% in tilapia. At the same time, these studies demonstrated a reduction in environmental emissions of phosphorous.

Proteases are a key tool to improve the digestibility of ‘low value’ ingredients, increasing the flexibility of the raw material basket: protease inclusion in shrimp and tilapia feeds can maintain animal’s performance with a significant decrease on the use of marine proteins or soy protein concentrates, contributing to control the costs of the feeds and reducing the feed and farm footprints.

Studies demonstrate that the in-feed inclusion of a combination of enzymes, such as phytase, protease and xylanase, can improve energy utilization up to 8% in tilapia when modern formulas are used.

This paper will review the existing data in the use of enzymes in aquaculture feeds, as well identifying current gaps and future needs to support the sustainable growth of aquaculture.

Chlorella vulgaris AS SOURCE OF VITAMIN C FOR PIKEPERCH (*sander lucioperca*) LARVAE

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Introduction

Most animals can synthesize Ascorbic Acid from glucuronic acid, but fish and crustaceans lack the enzyme gluconolactone oxidase necessary for the last step in this biosynthesis (Chatterjee, 1973; Dabrowski, 1990). Therefore, they depend on constant supplies of acceptable amounts of vitamin C through the feed. Currently, fish larvae in aquaculture rely primarily on commercial live feed enrichments to supply the required amount of Vitamin C. Due to the high amount of vitamin C found in *Chlorella vulgaris*, we looked at the potential replacement of commercial enrichment diets during the first 21 days post-hatching with *Chlorella vulgaris*.

Materials and Methods

This trial tested the use of *Chlorella vulgaris* on pikeperch (*Sander lucioperca*) larvae during first feeding. The trial included three live feed enrichment treatments and one control, fed to the larvae for the first 21 days post-hatching. The first treatment exposed the live feed to the "Selco Spresso" enrichment diet by INVE, while in the second treatment, Ascorbic acid was used on the live feed. The third treatment used *Chlorella vulgaris* as the live feed enrichment, and the control treatment used *Nannochloropsis occulata*. After 21 days post-hatching, a representative sample of larvae (100 per treatment) from the four treatments was challenged for 180 minutes in high salinity (15ppt) to test resilience and cortisol levels. Growth, survival, and bladder inflation data were also collected.

Results

Significant differences were found between treatments regarding stress resilience to the salinity challenge and cortisol levels compared to the control treatment and between enrichments. Significant differences were also found in larvae Vitamin C concentration after 21 dph. No differences were found in the growth or survival (average 65%), although *chlorella* treatment had the highest survival (72%).

Conclusions

The results suggest that using *Chlorella Vulgaris* in live feed during the pikeperch larval stage, positively enhances stress resilience and could potentially replace the use of more expensive commercial enrichment diets.

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EFFECTS OF A HEALTH-PROMOTING ADDITIVE ON THE IMMUNOCOMPETENCE OF RAINBOW TROUT (*Oncorhynchus mykiss)*, UNDER FIELD CONDITIONS

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Introduction

Health feed additives are designed to enhance animal immunocompetence and reduce the dependence on chemical treatments, such as antibiotics, in fish farming. This study aims to evaluate the effects of a phytogenic-based additive, SANACORE® GM (Adisseo, France), on the immunocompetence of rainbow trout (*Oncorhynchus mykiss*) in farm testing.

Material and methods

Rainbow trout with an average individual weight of 230g were fed the functional additive (0, 1 and 2 kg/mt) in raceway compartments. Each compartment was stocked at a density of approximately 3 kg/m3 in triplicates. After a cultivation period of 60 days, the fish's health status was assessed by measuring serum glucose, total protein, albumin levels, serum alternative complement activity, lysozyme activity, and antioxidant activity. Lastly, the additive effect on the community of microorganisms in the fish's digestive tract was also evaluated.

Results

Supplementation of the functional feed resulted in significant increased antioxidant enzyme activities, serum globulin production, lysozyme activity ($P < 0.05$), and in term complement system activity although showed increase tendency but there was no significant discrepancy ($P > 0.05$). Additionally, the intestinal bacterial counting showed significant reduced populations of opportunistic bacteria such as *Aeromonas*, *Streptococcus*, and *Yersinia* while significantly promoted the population of probiotic bacteria *Lactobacillus* ($P < 0.05$). In conclusion, the findings of this study demonstrated that the SANACORE® GM enhanced the immunocompetence of rainbow trout by elevating immune enzyme activities and antioxidant defense, making fish better prepared to combat infections. A healthier gut microbiome is important to prevent dysbiosis and better deal with stress and disease.

FIRST REPORT OF NATURAL *Nematopsis* sp INFECTION IN *Chamelea gallina* IN BULGARIAN WATERS OF THE BLACK SEA

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Introduction

Chamelea gallina (Linnaeus 1758) is a benthic species which has a wide area of spread including the East Atlantic Ocean coasts, the Adriatic Sea, the Black Sea and the Mediterranean (Tanrıverdi et al., 2019). This wedge clam is found intensively in the Western Black Sea and the Marmara Sea, where they play an important economic role (Dağtekin et al., 2023). In addition, *Chamelea gallina* are dietary sources of n-3 LC-PUFA, trace elements (Cr and Fe) and protein (Peycheva et al., 2023) There are significant gaps in knowledge about population and health status of clams in Bulgarian waters of the Black Sea.

In this study we present the first record of *Nematopsis* sp. infection in the wedge clam *Chamelea gallina* along the Bulgarian Black sea coast**.**

Material and Method

The study was carried out in the North Bulgarian Black sea coast near Cape Shabla. The samples were collected by scientific scuba diving, consisting of 30 clam *Chamelea gallina* twice a month, in the period between April 2021 and May 2022. The biometric measurements were undertaken with digital caliper (nearest 0.1 mm) including anterior-posterior length (L), dorsal-ventral length (W), the distance between two valves (D), total weight (TW), wet weight of the soft parts (WWSP), and weight of shell (WS). A total of 720 specimens have been analyzed to detect the changes occurring during reproductive processes by classical histology techniques. First, a section from the tissue (mantle, gonad, gill, digestive gland, and foot) was fixed in 10% neutral buffered. In the next step samples were dehydrated with graded series of ethanol, cleared in xylene and embedded in paraffin wax. Paraffin blocks were cut (4-5 µm) with a microtome (Leica RM2125, Germany) and stained with haematoxylin and eosin (H&E). Slides were examined and described regarding the presence of morphological alterations under a light microscope (Olympus BX51, Germany) equipped with a digital camera (Olympus DP72, Germany).

Results and Discussion

In routine classical histological examination among the analyzed clam organs parasites were observed in gills in three of them. The identified as *Nematopsis* sp. parasites were found as gametocysts, and the effects on infected individuals presented tissue damage, intense hemocyte infiltration, especially disruption of the structure of the gill tissue (Figure 1).

Conclusion

To our knowledge, this is the first record of *Nematopsis* sp. infection in the wedge clam *Chamelea gallina* along the Bulgarian Black sea coast. Further research is needed for the process of parasitism that may occur simultaneously with other pathogens in different environmental conditions.

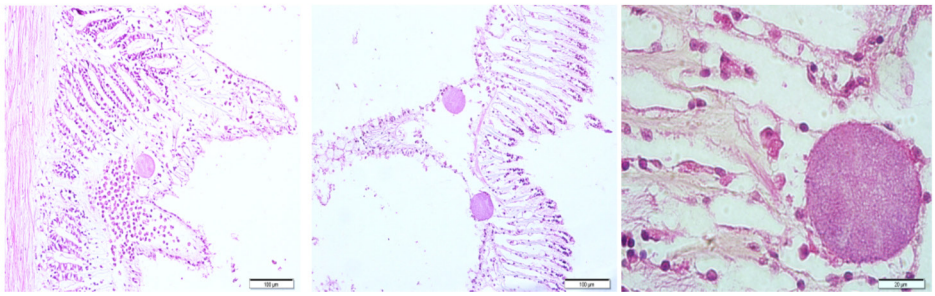

Figure 1: Tissue damage and extensive hematocyte infiltration in the gills

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STATUS AND OUTLOOK OF VOCATIONAL TRAINING OF AQUACULTURE IN SAUDI ARABIA

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Due to sustainable economic development and food security for Saudi Arabia, aquaculture is essential for the national development sectors. In addition, mariculture is more appropriate than freshwater culture due to the limitations of factor policy and natural environment. Therefore, the Saudi Arabian Fisheries Authority (Ministry of Environment, Water and Agriculture) has been promoting mariculture to support sustainable development and food security of national policies. However, the Saudi Arabian aquaculture industry has faced many problems related to the lack of a wider range of aquaculture species, the absence of local hatchery facilities or inadequate production, diseases, and the lack of well-trained personnel. In Saudi Arabia, few institutions and organizations provide vocational training for aquaculturists. In addition, most field work is dependent on foreign labor. Sustainable strategies can be developed to support the aquaculture industry, including the national training center for the private sector and improving vocational training in the public sector. In addition, vocational training can focus on hatchery managers and technicians and the development of new cultured species.

GROWTH, FRY PRODUCTION, AND COST ANALYSIS FOR SABAKI TILAPIA *Oreochromis spilurus* PRODUCTION SYSTEMS

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This study estimated the survival, growth, fry production, and farming costs of Sabaki tilapia (*Oreochromis spilurus*) broodstock in the following five types of outdoor production systems: arena, raceway, cage, concrete pond, and earth pond. Experiments were conducted in triplicate with 300 broodstock per production system. Survival rate, growth performance, fry production and farming cost were monitored. Our results indicated that Sabaki tilapia fry production was feasible under high salinity conditions in the production system types studied; however, the systems showed significant differences in terms of survival rate, growth performance, and fry production. The arena and raceway systems were the most effective and had the highest fry production.

Table 1. Sabaki tilapia (*Oreochromis spilurus*) survival rate, growth performance, and fry production at production systems

Systems	**Survival rate (%)**	**FCR**	**Specific growth rate**	**Mean fry production per female (21 days)**
Arena	97.3 ± 0.5[a]	1.48 ± 0.12 [a]	1.99 ± 0.05 [a]	458 ± 78[a]
Raceway	97.8 ± 1.5[a]	1.47 ± 0.14 [a]	1.97 ± 0.07 [a]	449 ± 55[a]
Cage	79.4 ± 1.9[b]	1.37 ± 0.12[b]	2.21 ± 0.05[b]	344 ± 61[b]
Concrete pond	94.3 ± 1.4[a]	1.48 ± 0.20 [a]	1.93 ± 0.05[a]	287 ± 12[b]
Earth pond	90.1 ± 1.3[c]	1.24 ± 0.08[c]	2.20 ± 0.17[b]	337 ± 12[b]

In each column, different letters indicate a significant difference ($P < 0.05$).

Table 2. Sabaki tilapia broodstock culture annual costs at production systems (USD [$])

Systems	**Feed**	**Labor**		
Arena	$447.84[a]	$86.22	$22.48	$22.84
Raceway	$435.96[a]	$86.22	$32.78	$20.5
Cage	$382.06[c]	$92.69	$43.56	$33.24
Concrete pond	$459.13[a]	$73.74	$27.64	$19.74
Earth pond	$418.13[b]	$62.45	$60.25	$19.69

Operating costs include drugs, utilities, maintenance, equipment, pond preparation, and part-time labor.
In each column, different letters indicate a significant difference ($P < 0.05$).

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UTILIZING PHOSPHORUS SMARTLY FOR SUSTAINABLE BONE HEALTH IN SALMON FARMING: A GLIMPSE INTO THE FUTURE

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Introduction

Bone health is an essential aspect of overall health and well—being of fish. Bones provide support to the body, protect vital organs, and store minerals such as calcium and phosphorus. All vertebrates need sufficient minerals for their Ca—P based skeletons. Fish mainly obtain calcium from the water, but not phosphorus as ocean is empty of phosphorus. Dietary phosphorus deficiency is considered a nutritional risk factor for the development of vertebral deformities in farmed Atlantic salmon (*Salmo salar*, L). Bone health is important not only for fish welfare, but also for farmers, as bone defects (vertebral deformities) may lead to bad fillet quality and eventually downgrading of the fish at slaughterhouses.

Results

Our recent studies have shown no correlation between dietary phosphorus levels and growth in freshwater Atlantic salmon (Drábiková et al., 2021, 2022). The growth of post-smolt Atlantic salmon deficient in phosphorus (with a 50% lower dietary P content) was restricted compared to those reared in seawater with a sufficient phosphorus content, despite no significant differences in the condition factor or feed conversion ratio between the experimental groups (Witten et al., 2016, 2019). Fish fed a low phosphorus diet develop extended areas of non-mineralised bones without having significant effects on the prevalence of vertebral deformities in Atlantic salmon (Drábiková et al., 2021, 2022; Witten et al., 2016, 2019). Excessive dietary phosphorus intake (50% above the dietary P requirement) has no additional benefits in terms of bone mineralization, but ultimately results in water waste. Furthermore, elevated CO2 levels in water can prevent dietary induced osteomalacia (bone softening) in Atlantic salmon (Drábiková et al., 2023).

Conclusion

In conclusion, fish need an optimum phosphorus diet and environment to keep their bones healthy. This is critical for closed aquaculture systems where excess nutrients (phosphorus) build up in the water.

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CLIMATE CHANGE AND MICROSCOPY – HOW CELLS MAY TELL US ABOUT THE FUTURE OF AQUACULTURE

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Introduction

Fluctuating conditions, more extreme events and an unpredictable environment are impact from climate change. There is an urgent need to understand how farmed aquatic organisms respond to these challenging conditions. Aquatic animals farmed in the ocean are in direct contact with the environment, without any possibilities to escape unfavourable conditions. These animals are part of an intensive production regime, and stress related to production management are further inducing stress on the animals. How production procedures in combination with fluctuating environmental conditions exert stress on the animals and how the stressors work in combination, is important knowledge when making climate adaptation strategies.

The presented results provide insight into how cells from different organs in Atlantic salmon and Atlantic cod can be used to understand how changing climate conditions in combination with secondary stressors affect fish health.

Methods

Using climate projections from the Intergovernmental Panel on Climate Change (IPCC) we evaluated potential future temperatures at selected Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*) farms in Norway. Based on these results we designed a temperature study with Atlantic salmon to evaluate biological impact from temperature alone and in combination with secondary stressors.

Atlantic salmon post-smolt (50g, N=600) and Atlantic cod (60g, N=400) were exposed to different temperature regimes, optimal: 12°C, high: 17°C or fluctuating (only salmon): 12/17°C, conditions. The trial was run for two (cod) and three (salmon) months, with the fluctuating temperature cycling in three rounds. All groups were exposed to different combinations of secondary stress, such as reduced oxygen, pathogens, algae or jellyfish.

Samples of skin, gills, and olfactory organs were collected at different time-points, and microarray, histology, scanning electron microscopy and immunohistochemistry used to evaluate the impact of temperature alone, and in combination with the secondary stressors. *In vitro* models of skin biopsies and scale explants were used to further investigate the functional biology of increased temperature. Migration assays and immunohistochemistry with markers for stress induced genes were used to increase our understanding of the combined stress.

Results and Discussion

Results show no growth different between fish from the different temperature regimes and few differences when looking at the operational welfare scores (OWIs). However, comparing cellular responses in the skin, gills and olfactory organs from fish reared at the three different temperatures revealed altered morphology, changes in immune response and reduced regeneration capacity in fish from high and fluctuating conditions. High temperature and fluctuating conditions were shown to reduce skin integrity, thereby increasing the risk for wounds and secondary infections. After exposure to secondary stressors, such as algae and jellyfish, fish from higher temperature showed increased damages in gills and further reduced migration potential in skin. Results indicate that fluctuating conditions and constant high temperature are challenging for salmon health, but that the biological responses are different.

Conclusion

Fish in production systems are exposed to changing environmental conditions, pathogens, and operational procedures, that may expose the animals to stress either individually or in combination. Our results indicate that biological alterations caused by high and fluctuating temperatures may reduce Atlantic salmon's and Atlantic cod's resilience to diseases and production related stress. Understanding how multiple stressors affect the biological performance of farmed species is essential when planning for robust production. Looking into the cellular responses to multiple stressors may help us understand when the fish is vulnerable, what the tolerance thresholds are, and accordingly how the fish may be handled to sustain good welfare and survival.

Acknowledgements
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FARMING PERFORMANCE COMPARISONS OF TETRAPLOID-BASED RECIPROCAL TRIPLOID *Crassostrea gigas* AND *C. angulata,* FROM SEED TO MARKETSIZE

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As the leading role of mollusk industry in China, oyster aquaculture has witnessed a rapid popularity and sparkling development of triploid seed production and farming over the past few years. In this field the method of triploid production has been the mainstream one by mating tetraploids and diploids. When tetraploidy-inducing approach has been the popular one, the production of tetraploid oyster is still a great technical difficulty. In the past few years, we've successfully developed a new technique of oyster tetraploid-induction, and then applied it to commercial production of triploid seeds.

The triploidy performance and characteristics of tetraploids were evaluated using complete inter-ploidy breeding between diploid and tetraploid Pacific oyster or Portuguese oyster. In the Pacific oyster, experiments on two intra-ploidy groups (DD: 2n♀ × 2n♂, TT: 4n♀ × 4n♂) and two reciprocal triploid groups (DT: 2n♀ × 4n♂, TD: 4n♀× 2n♂) were carried out successfully in duplicates. High fertilization rates and D larvae rates were observed in all groups, while the TT and TD groups have a lower hatching rate due to a dysfunction in tetraploid eggs. In grow-out stage, DT hybrids had a higher survival rate than intra-ploidy groups during the whole life history stage. Growth advantage of reciprocal triploid hybrids was evident in both, while DT progeny was always larger than TD in the whole process except in the D larvae stage. As for the two intra-ploidy groups, all tetraploid progeny was smaller than the progeny of diploids, and some tetraploid progeny showed the ploidy loss phenomena as aneuploids and triploids at the grow-out stage.

In the *C. angulata,* aritificial breeding was made between diploid (D) and tetraploid (T) to produce DD, DT, TD and TT groups. The results showed that the fertilization rate, cleavage rate, D larval rate and larvae survival rate of DT group were significantly higher than those of TD and TT groups, and the larval growth of DT group was significantly faster than that of the other three groups. In cultivation period, the growth and survival rate of DT in the 2 sites were significantly higher, and the 100% triploid rate was stable. In addition, some triploids in DT and TD groups were fertile and could produce functional gametes. But the proportion of female and hermaphrodite in fertile triploids was higher than that in diploids and tetraploids, and the fertility of triploids was still poor overall. However, some tetraploids in TT group would lose chromosomes during growth and become triploids, diploids or aneuploids. Tetraploid had the slowest growth and the lowest survival rate at the both sites, but its fertility was normal and could produce a large number of functional gametes, which could provide sufficient sperm for large-scale production of triploids. All our data/results demonstrated that the diploid ♀× tetraploid ♂ group have excellent commercial production capacity of triploid for both species in the industry.

Posıdonıa oceanıca AND FISH FARMING IN THE GÜLLÜK GULF

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Introduction

Seagrass habitats are important ecological ecosystems. *Posidonia oceanica*, a native species, is essential to the welfare of the marine ecosystems of the Mediterranean Sea (Duarte 2002). Human activities such as eutrophication, coastal tourism and anchoring, destructive fishing methods, effects of coastal development, uncontrolled aquaculture development, coastal constructions and industrial wastes are the prime cause for the decline in density and surface area of *P. oceanica* meadows over the past three decades (Pergent, G. 2006). This study aims to map the fish farm areas and the spatial distribution of *P. oceanica* in the Güllük Gulf using sonar, remote sensing techniques and GIS (Geographic Information System) in an effort to increase the ecological sensitivity of the gulf.

Material and method

The study area lies east of the Aegean Sea. This 670 km^2 Güllük Gulf is a coastal area with a high concentration of touristic activities, fish aquaculture, and second homes. In the study, remote sensing (the Sentinel 2 satellite image 2018) was utilized to illustrate the distribution of fish farms. *P.oceanica* data was collected by using side-scan sonar and Stratabox Sub Bottom Profiler. *P.oceanica* data and fish farms data was converted to create a GIS map.

Result

Figure 1 illustrates the fish farms and *P.oceanica* distribution in the Güllük Gulf. Meadows are more common in the northern regions of the Gulf. The legislation Turk (MEF, 2007) defines an aquaculture area as Allocated Zone Aquaculture (AZA), which encompasses 21.2% (139.8 km^2) of the Güllük Gulf. There are 61 fish farms in the AZA. There is no *P. oceanica* in the AZA region.

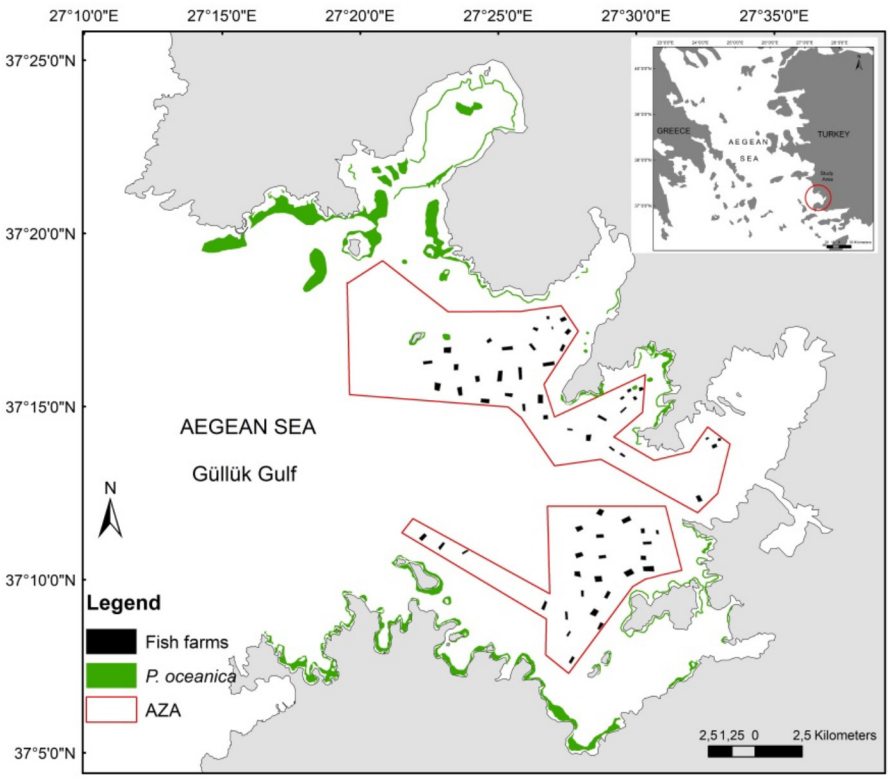

Figure 1 Distribution of *P.oceanica* and fish farms in Güllük Gulf

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Discussion and Conclusion

The study location, Güllük Gulf, is also located in the Aegean Region and is a coastal area where human activity, tourism, fisheries, agriculture, secondary houses and Güllük port activities are intense. The rapid development of fish aquaculture and tourism has paralleled the urbanization of the Güllük Gulf (Yücel-Gier et al., 2013). Due to the conflict between aquaculture and other sectors, fish farms had to be relocated to areas a minimum distance of 0.6 miles from the land and at the minimum depth of 30 meters according to Turkish legislation passed in 2007 (MEF, 2007). After the relocation aquaculture production increased to 88.000 tons in 2008, present production is 121.000 tons (TUİK 2021). *P.oceanica* is seen outside the AZA in Figure 1 (2018), the aquaculture farms were relocated in 2007. Mapping was done in 2018 so it is difficult to determine the effects of aquaculture on *P.oceanica* prior to 2018.

With the development of remote sensing and sonar imaging techniques, *P.oceanica* data can be collected and processed via GIS; this method enables the making of maps common and widespread. In addition, such maps can be used as an important tool in the sustainability of activities such as aquaculture by selecting the right location and integrating coastal planning.

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METABOLIC AND DIGESTIVE CONSEQUENCES OF ONGROWING GREATER AMBERJACK (*Seriola dumerili*) JUVENILES AT DIFFERENT TEMPERATURES

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Introduction

A good knowledge of the potential effects of water temperature on feeding and on-growing process is of primary importance to optimize the fish production. Temperature modulates ingestion, transit, digestion, assimilation, metabolism and ultimately feed utilization and growth rate. These two last parameters, together with the cost of feeds and energy, are necessary data for producers to make decisions. The production in indoor facilities with temperature control is depending on energy cost while in outdoor facilities (ponds and cages) it depends on environmental temperature. The effect of temperature is more noticeable in fast growing fish, as the greater amberjack (*Seriola dumerili*), a species of great interest for the aquaculture industry worldwide. Currently, its cultivation is expanding in the Mediterranean countries. Therefore, it constitutes a good model to study the consequences of an increased temperature. In this study, we examined the ingestion, digestive enzyme activities, metabolites in plasma and liver, antioxidant response in the liver and the integrity and electrogenic amino acid transport in the intestine, in greater amberjack juveniles growing at three different temperatures. The final aim was to elucidate the mechanisms behind feed utilization that justify the growth differences and to provide a useful informative basis to the productive sector.

Materials and methods

Greater amberjack juveniles (weight 23.35 ± 5.07 g; mean and SD) were randomly distributed in three independent RAS units set to 18, 22 and 26ºC of temperature, each one with three 900-L tanks. Juveniles were reared for 58 days under a photoperiod of 12h-light/12h-dark and fed until apparent satiation 3 times a day with a commercial diet (Skretting). Every two weeks, 5 fish per tank were sampled to check their body weight. At the end of the experiment, 16 fish per tank were sampled for assessing feeding and growth performance (weight gain, feed intake, feed conversion ratio) and analytical determinations. Luminal pH was measured in stomach and middle intestine. Activity of digestive proteases (trypsin, chymotrypsin, leu-aminopeptidase, pepsin) was analysed at the actual physiological temperature and pH. Oxidative stress biomarkers (protein carbonylation PC, catalase activity CAT, lipid peroxidation LPO, total antioxidant capacity TAC and mitochondrial reactive oxygen species mROS) were analysed in liver concomitantly with plasma cortisol; metabolites (triacylglycerol TAG, lactate, protein, glucose, glycogen, cholesterol) were also evaluated in plasma and liver. Intestinal integrity, permeability and electrogenic amino acid transport were assessed in using chambers. Differences were checked by ANOVA followed by Tukey's test.

Results

Voluntary feed intake, final body weight and weight gain increased with the temperature increase from 18 to 26°C ($p<0.05$). Feed conversion ratio was higher at 18°C than at the other temperatures ($p<0.05$) (Table 1). Overall, the activities of trypsin and chymotrypsin were higher at 22°C than at 18ºC when analysed at their physiological temperature and pH (Fig. 1). Contrarily, pepsin was not detected at the actual physiological pH. In liver, TAG was higher at 18°C, whereas hepatic glucose and glycogen (Table 2), as well as plasma protein and cortisol levels were higher at 26°C. Oxidative stress parameters were higher at 18°C than at the other temperatures, excepting mROS that was similar among treatments. Epithelial electrophysiology showed that tissue resistance was temperature-dependent and the electrogenic amino acid transport was higher at 26°C than at the other temperatures.

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Table 1.- Final body weight (BW), weight gain (WG), voluntary feed intake (VFI) and feed conversion ratio (FCR) of *S. dumerili* juveniles on-grown at the three water temperatures. Data are presented as mean ± SD. Different superscript letters indicate significant differences among treatments (one-way ANOVA; $p < 0.05$).

Temperature	18 °C	22 °C	26 °C
BW	74.25 ± 13.54^{c}	157.79 ± 30.06^{b}	189.88 ± 60.84^{a}
WG	51.09 ± 1.56^{b}	123.65 ± 13.54^{a}	166.61 ± 28.67^{a}
VFI	3.29 ± 0.09^{b}	3.78 ± 0.27^{b}	5.37 ± 0.41^{a}
FCR	1.47 ± 0.11^{a}	1.02 ± 0.07^{b}	1.05 ± 0.09^{b}

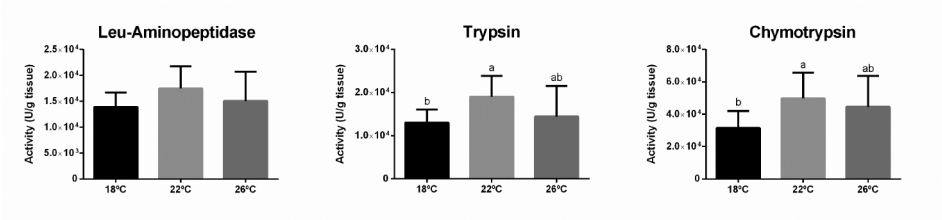

Figure 1. Digestive enzyme activities in *S. dumerili* juveniles on-growing at the three water temperatures. Data are presented as mean ± SD. Different superscript letters indicate significant differences among treatments (one-way ANOVA; $p < 0.05$).

Table 2.- Hepatic metabolites in *S. dumerili* juveniles on-growing at the three temperatures (mean ± SD). Different superscript letters indicate significant differences (one-way ANOVA; $p < 0.05$).

Temperature	18 °C	22 °C	26 °C
Triacylglycerol (mM TAG g liver^{-1})	100.44 ± 15.83^{a}	82.35 ± 18.42^{ab}	84.34 ± 13.58^{b}
Lactate (mM lactate g liver^{-1})	2.42 ± 0.48	1.95 ± 0.38	2.33 ± 0.77
Glucose (mM glucose g liver^{-1})	13.24 ± 2.75^{b}	14.86 ± 2.71^{a}	14.35 ± 3.68^{ab}
Glycogen (mM glycogen g liver^{-1})	33.77 ± 11.95^{c}	39.06 ± 9.69^{b}	46.20 ± 11.82^{a}

Discussion

The tested temperatures are within the tolerance range described for this species. Ingestion clearly increased with the increase of temperature, but growth and FCR were similar at 22 and 26°C, although weight values were higher at 26°C. Interestingly the level of enzymatic activities was also similar at 22 and 26°C. In addition, the temperature increase modifies the intestinal epithelium selectivity and improves electrogenic amino acid transport from the lumen at least in the mid-intestine. At 26°C, fish are using primarily TAGs from liver for covering the energetic demand, while at 18°C switched to carbohydrates. The higher plasma protein level reflects a higher synthesis activity for growth. TAGs were less utilised at 18°C, with risk of liver steatosis, or even favouring damage by oxidative stress at the lowest temperature, despite activating antioxidant defences. From a practical view, 22 and 26°C appear as optimal temperatures for on-growing the species, although surely better growth would be obtained at 26°C in longer periods. At 18°C, the low ingestion and the damage derived from the oxidative stress impair the growth capacity.

ONGROWING EUROPEAN FISH SPECIES WITH FEEDS FORMULATED WITH A BY-PRODUCT DERIVED FROM CEREAL PROCESSING

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Introduction

The cereal industry generates large amounts of residual by-products with high potential as feed ingredient for aquafeeds. Such raw materials could help to substitute protein and lipids from less sustainable sources such as soy concentrate, wheat flour or oils and reduce the competitiveness between the production of aquafeeds and the use of food for human consumption. In this study, one of these by-products, corn gluten feed, has been tested in dose-response experiments as ingredients in on-growing feeds for two European species (greater amberjack, *Seriola dumerili*; gilthead seabream, *Sparus aurata*). The final aim was to examine to what extent this more-sustainable and low-cost product can be used as alternate ingredient for fish feeds.

Materials and methods

Corn gluten feed was provided by Roquette (Corex, 18% crude protein). The experimental diets had increasing proportions of this novel ingredient. Diets were manufactured by SPAROS Lda and were tested in greater amberjack and gilthead seabream. The inclusion level increased from 0 to 25% on weight basis (control: 0%, 12.5%; and 25%) replacing wheat flour and rapeseed oil. All diets contained 47% of crude protein and 20% of crude fat. Greater amberjack juveniles (average body weight 92.25 g) were distributed in 9 tanks in RAS and fed until apparent satiation three times a day for 97 days at 22 ºC. Gilthead sea bream juveniles (average body weight 93.36 g) distributed into 9 tanks in RAS were maintained for 57 days at 24 ºC and fed in excess with automatic feeders twice a day. Feeding and growth performance were measured at the end of the trials with both species. Intestinal integrity, oxidative stress biomarkers (catalase activity CAT, lipid peroxidation LPO and mitochondrial reactive oxygen species mROS) in liver as well as fillet composition and organoleptic characteristics were also analyzed in greater amberjack. Statistical differences were tested by one-way ANOVA followed by Tukey's test ($p < 0.05$).

Results

Experimental diets were well-accepted by both fish species and the fish grew up in all cases. In greater amberjack, the voluntary feed intake (VFI) was lower, but not significantly different, in fish fed diet with 12.5 inclusion of corn gluten feed than in fish fed the other two diets. Growth parameters and feed conversion ratio (FCR) with the two experimental diets were similar to those of the control diet. The hepatosomatic index (HSI) was also similar among treatments. However, the viscerosomatic index (VSI) was higher with diet containing 12.5% of corn gluten feed (Table 1). Oxidative stress parameters (CAT and mROS) were higher with 12.5%-diet than with the other two diets, but LPO was similar among treatments though with a trend to be lower with the control-diet (Table 2). Organoleptic analysis of fresh and cooked fillet samples revealed no differences among treatments for all sensory attributes and a good acceptance for fillets coming from fish fed diet with the new ingredient. No histological alterations associated to inflammatory processes in the intestine were observed in fish fed the experimental diets. In gilthead sea bream, VFI was significantly lower in fish fed on diet with 12.5% of corex inclusion, but growth parameters and FCR were similar in juveniles fed on the three diets. Likewise, no statistical differences were detected for VSI (Table 3).

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Table 1.- Final body weight (FBW), weight gain (WG), specific growth rate (SGR), voluntary feed intake (VFI), feed conversion ratio (FCR) and viscerosomatic index (VSI) of *S. dumerili* juveniles fed on three diets (mean ± SD). Different superscript letters indicate significant differences among treatments (one-way ANOVA; $p < 0.05$).

	Control	**12.5%**	**25%**
FBW	269.37 ± 74.82	228.93 ± 72.70	257.39 ± 39.34
WG	177.12 ± 1.54	136.68 ± 3.40	165.14 ± 6.28
SGR	1.09 ± 0.22	0.94 ± 0.07	1.06 ± 0.07
VFI	1.31 ± 0.21	1.20 ± 0.25	1.16 ± 0.10
FCR	1.33 ± 0.46	1.56 ± 0.43	1.22 ± 0.32
VSI	6.01 ± 0.53^{b}	6.90 ± 0.55^{a}	6.31 ± 0.68^{b}

Table 2.- Oxidative stress biomarkers in liver of *S. dumerili* juveniles fed on three diets (mean ± SD). CAT: catalase activity; mROS: mitochondrial reactive oxygen species; LPO: lipid peroxidation. Superscript letters as in Table 1.

	Control	**12.5%**	**25%**
CAT	0.93 ± 0.18^{b}	1.22 ± 0.28^{a}	0.97 ± 0.17^{b}
mROS	4.43 ± 3.26^{b}	12.00 ± 10.03^{a}	5.88 ± 5.51^{b}
LPO	11.43 ± 3.79	16.41 ± 6.78	15.23 ± 6.44

Table 3.- Final body weight (FBW), weight gain (WG), specific growth rate (SGR), voluntary feed intake (VFI), feed conversion ratio (FCR) and viscerosomatic index (VSI) of *S. aurata* juveniles fed on three diets (mean ± SD). Superscript letters as in Table 1.

	Control	**12.5%**	**25%**
FBW	221.34 ± 34.23	225.65 ± 32.19	217.80 ± 28.71
WG	128.05 ± 6.20	129.43 ± 3.96	124.43 ± 4.00
SGR	1.52 ± 0.05	1.53 ± 0.03	1.48 ± 0.03
VFI	1.75 ± 0.02^{a}	1.69 ± 0.02^{b}	1.74 ± 0.02^{a}
FCR	1.21 ± 0.05	1.15 ± 0.03	1.23 ± 0.04
VSI	5.37 ± 1.94	5.34 ± 1.48	4.86 ± 1.72

Discussion

The inclusion up to 25% of corn gluten feed in diets for greater amberjack and gilthead seabream allowed similar growth and feed utilization than the control, even a tendency for a more favorable utilization of the diet with 25% inclusion was observed in greater amberjack. With the 12.5%-corex diet, fish required to activate higher antioxidant defences to prevent damage by oxidative stress. In addition, fish fed with the new ingredient maintained the integrity of the intestinal mucosa as well as the flesh quality. These results support the potential of corn gluten feed to be used as an ingredient for fish diets. This by-product has a good protein content and is also rich methionine, a limiting amino acid in feeds with high proportion of plant derived ingredient. The use of this ingredient would increase the sustainability of growing stage in the aquaculture industry without affecting the zootechnical performances and reducing competition with human food chain resources.

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ENHANCING RISK EVALUATION IN FISH FEED INCORPORATING CEREAL BY-PRODUCTS: UTILIZING EARLY RESPONSE HISTOLOGICAL BIOMARKERS IN GREATER AMBERJACK (*Seriola dumerili*)

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Introduction

The increasing incorporation of plant-based products in fish feed warrants consideration of potential unintended factors, including the presence of new plant toxins. To enhance risk assessments in animal studies, the utilization of early response biomarkers has been proposed. This study aims to explore the interaction between an experimental diet and the gut integrity in juveniles of greater amberjack (*Seriola dumerili*), focusing on the effects of corn gluten feed as a plant-based ingredient obtained as by-product from the cereal industrial processing. Various biomarkers of exposure and effects were evaluated, including histochemical properties of goblet cells, morphological changes in the posterior intestine (villus length, variations in size and number of goblet cells), and alterations in cell proliferation using the proliferating cell nuclear antigen (PCNA) approach.

Materials and methods

In this study, a cereal by-product, corn gluten feed (Corex, Roquette; 18% crude protein), was evaluated as a novel ingredient in experimental diets for greater amberjack. The diets were formulated by SPAROS Lda and consisted of increasing proportions of corn gluten feed, ranging from 0% to 25% on a weight basis (control: 0%, 12.5%; and 25%). This ingredient replaced wheat flour in the experimental diets maintaining a consistent crude protein content of 47% and crude fat content of 20%. Greater amberjack juveniles with an average body weight of 92.25 g were distributed into nine tanks in a recirculating aquaculture system (RAS). The fish were fed three times a day until apparent satiation over a 97-day feeding period at a water temperature of 22 ºC. At the end of the trials, samples of the distal intestine were collected, fixed in a formaldehyde solution, and processed for histological analysis. Histochemical properties of goblet cells, intestinal integrity, and changes in cell proliferation were assessed in greater amberjack fed with the experimental diets. Statistical differences among the different diet groups were analyzed using one-way ANOVA, followed by Tukey's test ($p < 0.05$).

Results

Concerning the histological organization of the intestine, the comparison between the control group and the two experimental diets indicated no relevant differences in the arrangement of the lamina propria-submucosa and muscular layers. In both the control and corex diet groups, the intestinal mucosa displayed a simple columnar epithelium with basal nuclei, basophilic cytoplasm, and a prominent brush border. Furthermore, no histological changes associated with inflammatory processes or variations in villi height were observed among the fish from both the control and corex groups. However, it is important to note that fish fed the experimental diets, especially those with 25% corex inclusion, exhibited an increased number and average diameter of goblet cells along the intestinal epithelium (Figure 1A). On the other hand, the analysis of the histochemical properties of goblet cells revealed that the dietary administration of corex induced modifications in the composition of glycoproteins of mucins produced by these cells. Specifically, there was an increase in the staining intensity of neutral mucins, as well as mucins rich in carboxylated and sulphated glycoconjugates (Figure 1B, C, and D). Additionally, there was an increased affinity for the lectins WGA (Wheat Germ Agglutinin) and SBA (Soybean A.) in the mucinous content of goblet cells. However, no changes were detected concerning the lectins SNA (*Sambucus nigra* A.), DBA (*Dolichus biflorus* A.), ConA (Concanavalin-A), and UEA-I (*Ulex europaeus* A.-I) (Table 1). Finally, the study of cell proliferation revealed that the PCNA index was statistically significantly lower in the corex 25% group compared to the control and corex 12.5% groups.

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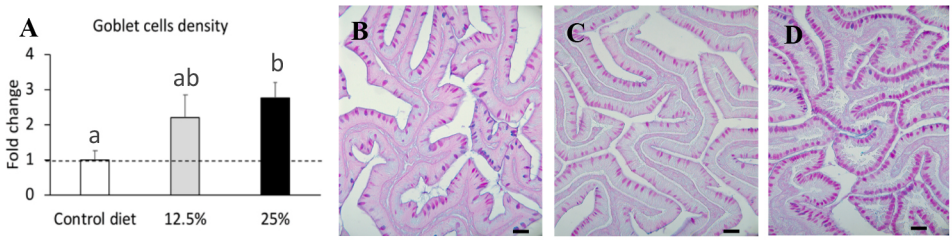

Figure 1. (**A**) Goblet cell density in the intestine of *S. dumerili* fed a control or corex diets. Different letters represent significant differences among dietary treatments (one-way ANOVA; $p < 0.05$). (**B** to **D**) Histochemical properties intestinal goblet cells from fish fed the control (**B**) and the corex (**C**; 12.5% and **D**; 25%) diets. Staining: Alcian Blue pH= 2.5/PAS. Scale bar = 50 µm.

Table 1.- Histochemical characteristics and lectin affinity of mucins produced by goblet cells from *S. dumerili* fed the control and corex 12.5% and 25% diets.

	Control	**12.5%**	**25%**
ConA (α-Manose)	0	0	0
WGA (N-acetyl-β-glucosamine)	0-3	2-3	2-4
SNA (Sialic acid-α-2,6-galactose)	0-1	0-1	0-1
SBA (α/β-N-acetylgalactosamine)	1-2	1-3	2-4
UEA-I (Fucose-α-1,2-galactose)	0	0	0
DBA (N-acetyl-α-galactosamine)	0-2	1-2	2

Semi-quantitative assessment scoring based on color intensity scores: 0, negative (non detected); 1, weak; 2, moderate; 3, intense; 4, very intense.

Discussion

The study found that the experimental diets had no significant impact on the organization of intestinal layers in *S. dumerili*. There were also no significant differences in villi height between the control and corex groups, indicating that the structural integrity of the intestines was not affected. Additionally, there were no indications of histological alterations associated with inflammatory processes in any experimental groups. However, the experimental diets, specially those containing 25% corex, did influence the abundance and size of goblet cells in the intestine. This was accompanied by modifications in the glycosylation patterns of intestinal mucins produced by these cells that would benefit fish by providing an effective immune barrier against potentially gut pathogens, indicating a potential positive impact on gut health. Furthermore, the study of PCNA index, revealed a positive potential influence of the diet with corex on cell division in the intestine. This indicates that this diet may support the homeostatic balance between epithelial cell proliferation and apoptosis, contributing to the overall health of *S. dumerili*. In conclusion, the results suggest that the experimental diets, particularly those with 25% corex inclusion, may promote better gut health and enhance the immune function, ultimately increasing the overall health and performance of *S. dumerili*. These findings highlight the potential benefits of incorporating corex into the diets of *S. dumerili* for better gut health and overall well-being.

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FISH COUNTING SYSTEM USING DEEP LEARNING WITH SYNTHETIC DATA SETS

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Introduction

Fish counting makes it possible to estimate the appropriate amount of feeding and the total volume of product in harvest timing from the fish cage. The number of fish is currently estimated by dividing the total weight of juvenile fish by the average weight of sampled ones when the fish farmer buys them. With this method, the gap between the estimated and actual fish count can be up to 50%.

At first, detecting fish is needed for counting fish in a fish cage. Deep learning meth- ods have proven to work well for such detection tasks. The challenge for training such a model for our objective is collecting enough datasets with significant variations by capturing enough videos underwater. Because water clarity changes when the season, weather, and other environmental factors change, we need to collect enough variations to train the visually based counting method that works in any conditions robustly.

Methods and Results

Our proposed method uses synthetic data generated by the realistic bio-inspired fish simulation and physically based underwater simulation, which is called Foids [1,2]. The simulation considers the density of chlorophyll and sediment, light scattering, intensity, and casting orientation varying depending on the day and time, and the shape and size of the net pen. The Foids algorithm enables us to obtain the training data of the fish school movies on the arbitrarily chosen day, time, and weather (See Fig. A). Furthermore, it is possible to set the number of fish and arbitrarily obtain the 3D configurations of fish. Then, it is a better training dataset than the videos taken in real situations. This paper introduces the fish counting application based on the Foids, in which YOLOv4 [3] (Fig. B) is used for fish detection (See Fig. C). Figure D exhibits the pictures obtained from the field works (upper) and the ones provided by the simulation (lower) for each species. In the simulation, cameras are set on the same points as in the field works carried out in the actual net pen. The result of the fish counting using our method is shown in Fig. E. Our method with deep learning methods, using the synthetic data created by using Foids, reduces the time 98% from the time taken manually, and the difference between them is approximately 3%. We will further develop the method providing the synthetic data set and apply it to estimate the fish size from the videos taken in the fish cage.

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EFFECTS OF CO-CULTURE OF EUROPEAN SEA BASS *Dicentrarchus labrax* AND *Ulva* sp. IN A RECIRCULATING AQUACULTURE SYSTEM ON THE BLOOD PARAMETERS AND STRESS BEHAVIOURAL RESPONSE: THE CASE OF HYPOXIA TEST

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Introduction

Seaweeds are considered a source of biologically active substances for fish nutrition. Macroalgae can be utilized for their bioactive compounds especially chlorophyll, carotenoids, vitamins E and C, fucoxanthin, enzymes, mycosporine-like amino acids, polysaccharides and polyphenols that possess antioxidant activities (Vega et al., 2020). The algal genus *Ulva* is often used in Integrated Multi-Trophic Aquaculture (IMTA) and has the potential to both bioremediate aquaculture water and beneficiate the reared organisms, as a source of anxiolytic substances to combat the stress caused by fish farming conditions (Calheiros et al., 2019). In this study, European sea bass (*Dicentrarchus labrax*) was co-cultured with *Ulva* sp. in an indoor RAS for 12 weeks, and exposed to a hypoxia test in order to assess the effects of *Ulva* on blood parameters and the behavioral stress responses (swimming behaviour, movements, immobilization) of sea bass.

Materials and Methods

An indoor RAS consisted of 2 units, the first (*Ulva*-RAS) was used for the co-culture of seaweed *Ulva* sp. and sea bass and the second as a control (Control-RAS) for rearing sea bass without seaweed. In each unit, six separate tanks were divided into two levels. *Ulva* was placed in the upper level of the *Ulva*-RAS. Sea bass was reared in the lower level of both units. Seawater was pumped into each of the upper-level tanks and then it flowed to the lower-level fish-rearing tanks. The effluent from this level was recirculated to the upper-level tanks after passing through the gravel bed biofilter. Rearing conditions were similar in both RAS units in terms of water flow rate, salinity, oxygen, photoperiod and light intensity. Fifty fish were placed in each tank and were fed on extruded pellets, where dietary fish oil content was mostly sardine oil, at apparent satiation, twice a day, five days per week. The rearing lasted 12 weeks. *Ulva* was collected from the Saronic Gulf, sorted and cleaned with seawater, weighed after drying in open air and transferred to the upper-level tanks of *Ulva*-RAS. Seaweed densities were kept at 0,5-1 kg m^{-2}. *Ulva* was replaced weekly. At the end of the trial, a hypoxia test was performed. From each tank of Control-RAS and *Ulva*-RAS, 10 specimens were selected for blood sampling prior to the hypoxia test (CF and UF group, respectively). Twenty-four fish remained in each fish tank of Control-RAS and *Ulva*-RAS. The oxygen supply was turned off for all the fish tanks. The average initial DO level was 5,7 mg/L and the final was 0,7 mg/L. The experiment for each tank was completed when half of its fish (12), were immobilised at the bottom of the tank. The hypoxia test was video-recorded and the time of behavioural stress responses was noted. Median Immobilization Time (MIT_{50}) was estimated as the average time interval during which 50% of the initial population was immobilized in each tank after exposure to hypoxia test. At the end of the experiment, 10 fish from each tank for both Control-RAS and *Ulva*-RAS, were selected for blood sampling (SCF and SUF group, respectively). Blood samples were collected from the caudal vein and stored in heparinized tubes. Plasma was used for the determination of cholesterol (CHOL), low- and high-density lipoproteins (LDL and HDL), glucose (GLU), albumin (ALB), alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH), triglycerides (TG), phosphorus (P), cortisol (CORT) and superoxide dismutase (SOD), with commercial kits. Fat content of sea bass was also determined at the end of the rearing period by Soxhlet extraction.

Results

At the end of the sea bass rearing, UF showed a significantly higher fat content than CF. During hypoxia stress test, the time required for 50% (MIT_{50}) of the fish to sink and become immobilized at the bottom of the tank was significantly higher in SUF compared to SCF. Under normoxia, UF exhibited significantly decreased levels of ALB, TG, CHOL and HDL and increased levels of LDH compared to CF. The concentrations of GOT, ALP and P were similar between UF and CF. Finally, plasma SOD was lower in UF than CF. After the hypoxia test, sea bass of SCF group exhibited significantly increased LDH and GPT levels, while plasma SOD remained at the same levels between SCF and CF groups. Conversely, SOD levels were significantly higher in SUF compared to the normoxia group UF, while GPT and LDH remained at the same levels. The reduction of TG and the rise of LDL followed a similar pattern in both SUF and SCF. Nevertheless, CHOL and HDL decreased only in the SCF group. ALB was not affected by the hypoxia test. ALP was increased in SUF compared to SCF, indicating a slower response to acute hypoxia. Plasma P levels were also increased, being higher in SUF than in SCF. The levels of GLU and CORT were at the same basal levels during normoxia for both UF and CF, and were elevated as a result of the stress imposed by the hypoxia test without being significantly different between SCF and SUF.

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Discussion

Present results showed that *Ulva* induced a reductive stress in sea bass during long-term co-culture, leading to decreased plasma SOD levels and increased LDH levels, associated with mitochondrial dysfunction and obesity. The elevation of SOD levels in SUF group under hypoxia test indicates an activation of antioxidant mechanism that enabled SUF to withstand the hypoxia stress for a longer time period than SCF, along with the prior lipid accumulation during normoxia. Magnoni et al. (2017) reported a decreased lipid peroxidation and an improved aerobic respiration, permitting a more efficient ATP production during hypoxia in fish fed the *Ulva*-diet. Scavenging of •OH in UF by *Ulva* may explain the changes of plasma parameters, indicating decreased OXPHOS and increased glycolysis, compared to CF in normoxia, which were similar to those observed in SCF after the hypoxia stress imposed to CF. On the contrary, the increased OXPHOS and the decreased glycolysis in SUF compared to SCF under hypoxic conditions point out increased cellular H_2O_2 levels and O_2 signaling, probably associated to the prior reductive stress of UF induced by *Ulva* in normoxia. It seems that *Ulva* acted as pro-oxidant in normoxia and as antioxidant in hypoxia on sea bass. These abilities could be attributed to ulvan compounds (García-Márquez et al., 2023) that were available to sea bass through the fragmentation of *Ulva*. It is concluded that *Ulva* in IMTA can ameliorate fish tolerance to environmental stressors like hypoxia that often occurs in intensive aquaculture.

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EFFECTS OF DIETS INCLUDING SPIRULINA-ENRICHED BLACK SOLDIER FLY (*Hermetia illucens*) PREPUPAE MEAL ON GROWTH, WELFARE, AND QUALITY OF GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii*) AND EUROPEAN SEABASS (*Dicentrarchus labrax*) REARED IN AQUAPONIC SYSTEMS

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Introduction

Modern aquaculture is moving towards more sustainable aquafeeds, and several alternatives have been proposed for replacing the conventional and less sustainable marine-derived ingredients such as fish meal and oil. On this regard, insects have gained increasing attention due to many beneficial properties. Particularly, the black soldier fly (*Hermetia illucens*) prepupae meal (HM) shows a relative high protein content (ranging from 32-53 %), a well-balanced amino acid profile, and an appreciable content of bioactive molecules (*i.e.*, lauric acid, chitin, and antimicrobial peptides) with immunostimulatory and anti-inflammatory effects. The main drawback is associated to the HM fatty acid profile, lacking in long-chain polyunsaturated fatty acids (PUFA). However, it has been widely demonstrated that the black soldier fly larvae can positively modulate their fatty acid profile if reared on growth substrates enriched with dried microbial biomass. Particularly, spirulina (*Arthrospira platensis*) can represent a valid solution to improve the nutritional properties of HM, being a great source of long-chain PUFA and pigments with antioxidant properties.

In this context, the present study was aimed to test and compare, for the first time, the effects of two experimental diets in which conventional marine-derived ingredients were replaced by 3% or 20% of spirulina-enriched full-fat HM on growth, welfare and quality of giant freshwater prawn (*Macrobrachium rosenbergii*) post larvae and European seabass (*Dicentrarchus labrax*) juveniles reared in aquaponic systems. In both of these species of commercial interest, the use of HM in aquafeed formulations has been scarcely explored and never tested using aquaponic systems.

Materials and Methods

Three test diets were formulated for each species to be grossly isoproteic, isolipidic and isoenergetic. Starting from the control diet (HM0), the test diets were characterized by 3 or 20 % (w:w) of conventional marine-derived ingredients replacement with spirulina-enriched HM (HM3 and HM20, respectively). Considering giant freshwater prawn, 1971 post larvae (initial body weight: 0.10 ± 0.01 g) were divided into 9 aquaponic systems (three systems per experimental group), with 15 lettuce (*Lactuca sativa*) seedlings (density = 10 plants/m^2) each. The feeding trial duration was 60 days, during which the prawns were hand-fed the experimental diets, provided at a daily feeding rate of 10% body weight. As regards European seabass, 270 juveniles (initial body weight: 19.3 ± 0.1 g) were divided into 9 aquaponic systems (three systems per experimental group), with 100 saffron (*Crocus sativus*) seedlings (density = 65 plants/m^2) each. During the acclimation period, salinity was gradually decreased from 30 to 5‰ to match the salinity of aquaponic systems. The feeding trial lasted 90 days during which fish were daily hand-fed the experimental diets at 2% body weight.

At the end of the trials, for both species, zootechnical parameters were measured and the lipid content, fatty acid profile as well as oxidative status (conjugated dienes and thiobarbituric acid reactive substances) of the edible portions were determined. For giant freshwater prawn post-larvae, hepatopancreas samples were collected for: (i) histological analyses (focused on the middle portion of each hepatopancreas tubule that includes the mature stage of B and R cells) to determine the B and R cells relative abundance, abundance of lipid droplets in R cells, tubule diameter, and height of the epithelium; (ii) molecular analyses (real-time PCR) to analyse the expression of genes involved in molting regulation (*jheh*), chitin and protein digestion (*chit3* and *catL*, respectively), stress and immune response (*hsp90* and *α2m*, respectively). For European seabass juveniles, histological analyses were conducted in liver to determine the hepatic lipid and glycogen deposition and in distal intestine to verify eventual alterations or signs of inflammation in the distal intestine. The expression of genes involved in immune response (*tlr1*, *myd88*, *nfkb*, *il1b*, *il10*, *tnfα*) was also assessed in the distal intestine.

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Results and Discussion

The experimental diets used in the present study properly sustained survival and growth performance and positively affected the fatty acid profile of the edible portions that did not show significant differences in terms of n3 PUFA among the experimental groups, in both species. The dietary lipid content of each experimental diet intended for giant freshwater prawns led to a massive presence of lipid droplets in R cells, in all the experimental groups, allowing a proper storage of energy sources to sustain the molting processes. However, the dietary amount of saturated and monounsaturated fatty acids that increased with the increasing dietary HM inclusions resulted in an increase in B cells abundance (also supported by the *catL* upregulation) in the HM3 and HM20 groups, able to produce and recycle fat emulsifiers to counteract their hydrophobic features that slow down the digestive processes. Similarly, as regards the European seabass, the percentage of fat fraction in liver of HM20 group was significantly higher than in the other treatments, representing one more evidence about the role of the HM fat fraction in determining a high hepatic lipid accumulation.

For both the species analysed, the inclusion of spirulina-enriched HM in the diets allowed the transfer of important antioxidant molecules (tocopherols and carotenoids) to the edible portions which possibly played a crucial role in preserving muscle-quality traits, preventing the lipid oxidation.

Finally, in giant freshwater prawn, the hepatopancreas health status was preserved in all the experimental groups, as evidenced by the histological analyses and the expression of immune and stress markers that did not show significant differences among the experimental groups. Similarly, in European seabass juveniles, histological analyses coupled with the expression of genes involved in immune response did not reveal structural alterations and signs of inflammation at the intestinal level, confirming the beneficial role on gut health of bioactive molecules typical of HM or derived from the enriching procedure of insects' growth substrate with spirulina.

Results obtained in the present study highlighted the suitability of enriched full-fat HM as a proper sustainable aquafeed ingredient for both giant freshwater prawn post-larvae and European seabass juveniles rearing. In addition, both these feeding trials represent an example of how the culture of two species of great commercial interest can be implemented with more sustainable aquafeeds and farming techniques such as aquaponic systems, promoting the valorisation of the circular economy and the zero-waste concepts in aquaculture.

This work was supported by Programma di Sviluppo Rurale 2014/2020 della Regione Marche: "INSHORE" - APPROCCI INNOVATIVI PER UNA ORTICOLTURA CIRCOLARE E SOSTENIBILE" Misura 16.1 Azione 2

BEATING THE HEART FAILURE ODDS: ELECTROCARDIOGRAPHY AS A SCREENING TOOL FOR DETECTING HEART DISEASES IN FARMED SALMONIDS

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Background

Aquaculture in the Nordic countries is dominated by salmonid (*Salmonidae spp*) species such as rainbow trout and Atlantic salmon. However, high mortality of fish before slaughter is a serious threat to the financial and sustainability of the fish farming industry, and also comprises a significant fish health and welfare challenge. Fish mortalities often occur during stressful handling events like grading, parasite treatment, and transportation, but the specific reasons behind these deaths are unclear. However, a growing body of evidence indicates that cardiac rupture is the cause of death in many fish. Indeed, salmonid fishes can develop a range of cardiovascular diseases that affect both the heart and blood vessels. For instance, farmed salmonids are reported to have a high prevalence of coronary arteriosclerosis[1], which may constrain blood flow to the heart, making it less resilient to handle stress. The implications of impaired cardiac function due to arteriosclerosis are supported by data from laboratory studies showing that rainbow trout (*Oncorhynchus mykiss*) with experimentally occluded coronaries display reduced cardiac and aerobic metabolic capacities as well as warming and hypoxia tolerance[2]. Unfortunately, there are currently no effective screening tools for monitoring cardiovascular health to early detect heart diseases in salmonids. In human medicine, electrocardiogram (ECG) analyses are widely used for screening and diagnosing various cardiac pathologies, but this has so far not been widely applied in fish. The present study was designed to comprehensively evaluate the suitability of ECG recordings as a screening tool for heart disease in salmonid fish. Specifically, we mapped abnormalities in the ECG following experimentally induced myocardial ischemia in rainbow trout with surgically ligated coronaries.

Material and methods

Fish were anesthetized in freshwater (10°C) containing buffered MS-222 and custom-made ECG electrodes were implanted subcutaneously. One experimental group (coronary ligated) had the main coronary artery occluded permanently by using a silk suture to induce myocardial ischemia, while a second group (sham operated) was treated identically except that the coronary artery was only exposed but not ligated. Experiments were performed on both anaesthetized and unanesthetized fish. Provocation maneuvers were also used to experimentally elevate the fish's heart rate (*i.e.*, atropine injection and chasing stress) in ordered to increase the sensitivity for detecting any cardiac abnormalities. After the experiments, all fish were euthanized and the heart was removed and histologically examined to link cardiac electrophysiological properties with heart morphology and pathology.

Results and Discussion

At necropsy, the coronary ligated fish had comparatively pale hearts relative to the vivid red hearts of sham operated fish. While all sham operated fish survived, there was only a 55% survival rate among the coronary ligated fish at 10 days post-surgery. Four out of five deceased fish showed signs of blood accumulation in the pericardial sac of the heart (hemopericardium) at necropsy, and this was likely due to atrial/ventricular rupture. Indeed, one of the coronary ligated fish displayed signs of ventricular aneurysm, which may be an underlying cause of cardiac rupture. Coronary ligation significantly affected ECG characteristics in both anesthetized and non-anesthetized fish. Abnormalities in the QRS morphology, which represents ventricle depolarization, included a loss of QRS voltage and prolonged QRS duration as a consequence of myocardial ischemia. The loss of QRS voltage suggests extensive myocardial injury leading to a loss of viable myocardium mass [3], while the prolonged QRS complex duration has been linked to an increased risk of ventricular arrhythmias in humans. In addition, the coronary ligated fish showed atrioventricular (AV) conduction delays, which manifested as 1st and 2nd-degree AV blocks. The 2nd degree AV block typically occured at high heart rates (Fig. 1).

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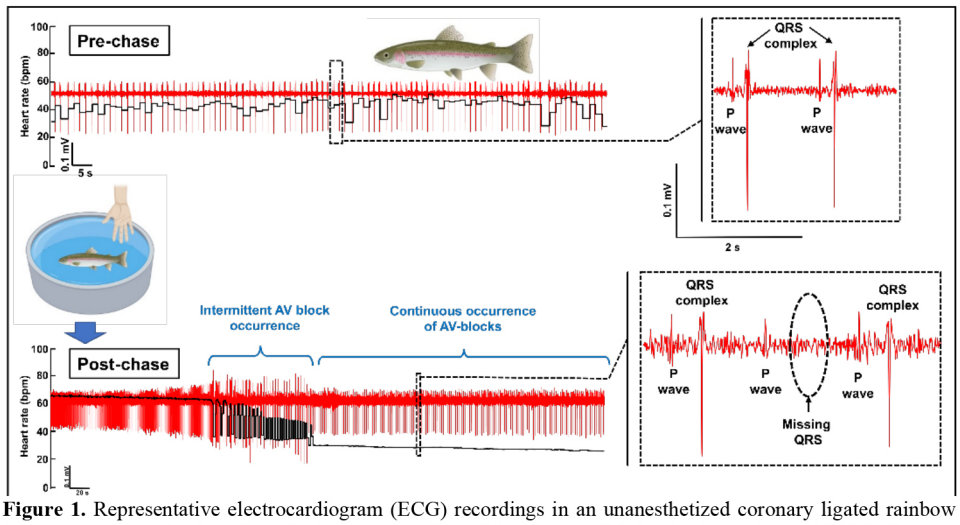

Figure 1. Representative electrocardiogram (ECG) recordings in an unanesthetized coronary ligated rainbow trout pre-, and post-exhaustive chasing stress, achieved by chasing fish by hand in a tank at 10°C. The raw ECG trace (red) with the overlayed instantaneous heart rate (black) is shown. The hatched boxes show a close-up view of the ECG with individual P-waves depicting atrial depolarization and QRS-complexes (depicting ventricular depolarization). Before chasing, every P-wave was followed by a QRS-complex and the heart rate was around 40 beats/min. After chasing, heart rate initially increased, but within minutes a second-degree atrioventricular (AV) block developed as indicated by QRS-complexes occasionally missing between P-waves, which caused the instantaneous heart rate to dramatically decline. (Modified from Wallbom, et al., 2023)[4].

Conclusion

We show that ECG analyses of both anaesthetized and unanaesthetised fish have the potential to be used for screening and diagnosis cardiac diseases in salmonid fish. Our intention is now to apply this technique under farming conditions as a quick and non-lethal screening tool to predict and reduce the risk of mortality from heart disease in farmed salmonids.

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EFFECTS OF GAMMARID AND POLYCHATE MEAL AS ATTRACTANT IN PLANT-BASED DIETS ON GROWTH AND HEALTH STATUS OF WHITE LEG SHRIMP *Litopenaeus vannamei*

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Introduction

Shrimp research has recently focused on the development of practical feeds that use plant proteins as substitutes for animal protein sources (Amaya et. al., 2007; Sabry-Neto et al. 2017; Guo et. al., 2019). According to Davis et al. (2004) and personal communication with shrimp industry partners (Euroshrimp: The Shrimp Network), the use of a purely plant protein feed may be limited by a variety of factors. These include amino acid profile, lower mineral content, low/limiting poly-unsaturated fatty acid content, the presence of antinutritional factors and lower palatability (Amaya et al., 2007). Feed attractants are being considered as a means of improving diet palatability and intake of feed in shrimp fed high percentages of plant material (Browdy et al., 2006). The efficacy of krill meal as attractant has been already proven in shrimp diets however, much research is needed for alternatives and sustainabily produced attractants. In this study, we investigated the potential of meal from gammarids and polychaetes, low trophic organisms and can be bred from agricultutal by-products and processing residues, as attractants in plant-based diets for white leg shrimp (Malzahn, A.M. et al., 2023; Ribes-Navarro et al., 2022). A controlled feeding experiment was conducted to evaluate the effects of the inclusion of the gammarids, *Gammarus locusta*, *Gammarus pulex* the polychaete *Nereis virens*, and Krill *Euphausia superba* as attractants in white leg shrimp diet. Growth performance, feed intake, survival rate, health and immune responses were evaluated.

Material and Methods

A one-month controlled feeding experiment with Whiteleg shrimp (*L. vannamei*) was conducted with 40 shrimps per tank in quadruplicate. Shrimp with an initial weight of 0.39 ± 0.02 gr were hand-fed four times daily according to the recommended feed rate, expressed as percent of body weight per day (Wyk et al., 1999) in a recirculation aquaculture system (RAS).

Six experimental diets were formulated: a commercial feed (FMD) as positive control with 10-20 % fishmeal content; a negative control (PD) containing no ingredients from marine sources and consisting of 100 % vegetable proteins and no added attractant. In addition, four test diets were produced from PD and with the addition of the 2% attractant (krill meal, gammarid meal (*G. locusta* and *G. pulex*) and polychaete meal *(N. virens)*).

Growth performance, survival rate, feed intake were monitored. At the end of the experiment haemolymph and hepatopancreas samples were taken to determine metabolic parameters (glucose, acylglycerides and total heamolymph protein), immunological capacity (phenoloxidase activity), stress resistance (antioxidant capacity) and nutritional value (fatty acid profile).

Results and Discussion

Preliminary results showed that shrimp fed the *N. virens* diet showed significantly higher weight gain than both gammarid diets. No significant differences were observed between the other diets. Moreover, survival rate did not significantly differ between treatments (Figure. 1). To date, no conclusions are possible since the full data set is not yet available.

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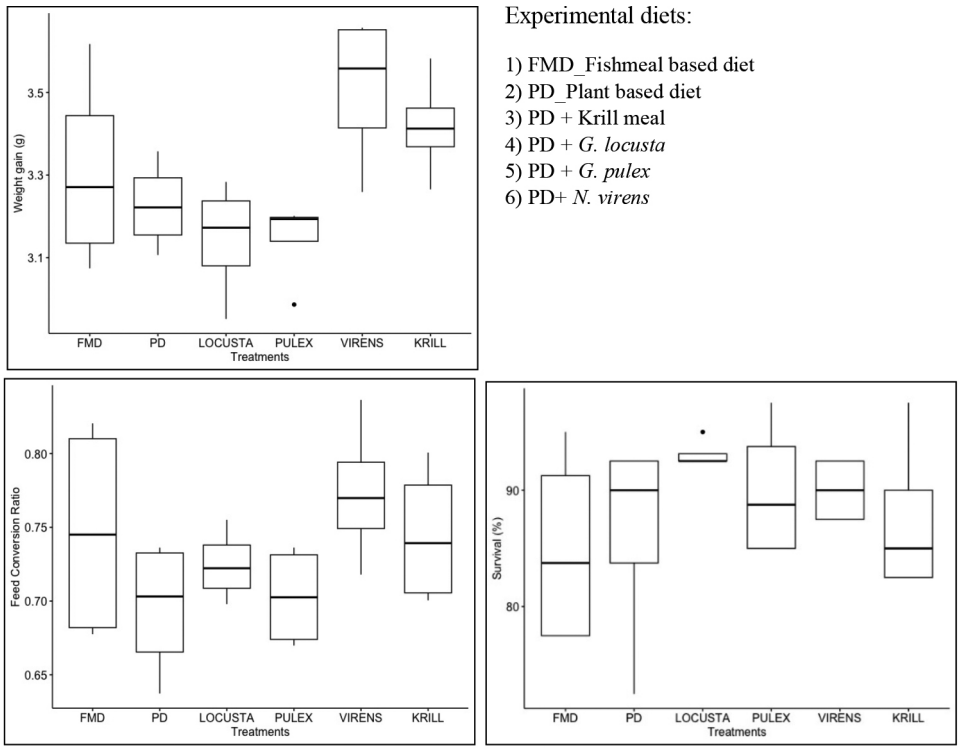

Figure 1. Weight gain, feed conversion ratio and survival rate of whiteleg shrimp fed the experimental diets.

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DEEP LEARNING-BASED METHOD FOR FISH BEHAVIOURAL CHANGE QUANTIFICATION

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Introduction

In this abstract, results are presented from the study of the behavioural change of Atlantic salmon (*Salmo Salar*) in sea cages when exposed to dynamically changing environments. Several field trials have been conducted in an industrial-scale fish farm to observe and study the behavioural responses of fish towards different influence factors, especially to the structures (obstacles) of various shapes, sizes, and colours. Ping360 sonars (BlueRobotics - Ping360 Scanning Image Sonar) were used to collect relevant fish behaviour data in industrial-scale sea cages. A deep learning-based method was applied to the 360-degree sonar data and used to identify fish swimming patterns around the structures and quantify the minimum distance the fish kept from the structures. Changes in the behaviour of the fish towards structures with different appearance were studied.

This work was financed by the Research Council of Norway through the project: CHANGE – An underwater robotics concept for dynamically changing environments [1] and RACE – Fish Machine Interaction [2].

Materials and methods

The structure was produced in six different versions that varied in shape, size, and colour, and equipped with a stereo camera and two Ping360 sonars: one on the top and one on the bottom, as shown in Figure 1. The Ping360 sonars were set with a range of 5 meters. The structure was placed in a water depth of 8 m in each of the tests conducted in June 2021, October 2021 and September 2022 in an industrial-scale fish farm of SINTEF ACE [3].

The sonar data provide images of a 360-degree view of its surroundings, with the areas of fish having greater intensity. The circular swimming behaviour of fish around structures makes them appear as rings in sonar images. After creating a training dataset via manually labelling fish swimming patterns on a few hundred randomly selected sonar images, a deep learning semantic segmentation UNet++ model [4] was trained to identify fish swimming patterns around structures. The distances between fish and structures can then be estimated by averaging the distance from each pixel on the edge of the parts identified as fish swimming patterns to the structure centre, as shown by the red curves in Figure 2.

Results

The deep learning-based method has been applied to the sonar image of fish accumulated around structures over 1-, 5-, and 10-minute time periods, Figure 2 shows examples of the accumulated fish presence around the cylindrical structures over a 5-minute period and the corresponding fish behaviour quantitation results. When the structures were big cylinders, fish with an average size of 1 kg seemed to stay approx. 1.5 m from the yellow structure and 0.8 m from the white structure. Similar fish-to-structure distances were obtained when the structures were big cubes. When interacting with small cylinders, the same fish maintained approx. 0.9 m and 0.6 m from the yellow structure and the white structure, respectively. Based on our sonar data, fish always stayed closer to the white structures than to the yellow structures if the structures were of the same shape and size.

In September 2022, the fish were of an average size of 1 kg and a population size of 172,563 individuals and stayed approx. 1.5 m away from a yellow cube structure. In June and October 2021, average fish size was 2.5 kg and 5 kg, while population size was 195,832 and 99,243, respectively. The distance to the same yellow cube structure then increased to approx. 2 m and 2.5 m, respectively. In all three cases, the biggest-sized fish had the longest distance to the structure, while the smallest-sized fish had the closest distance to the same structure. This indicates that there seemed to be a relationship between fish size and the distance the fish maintain from the structure.

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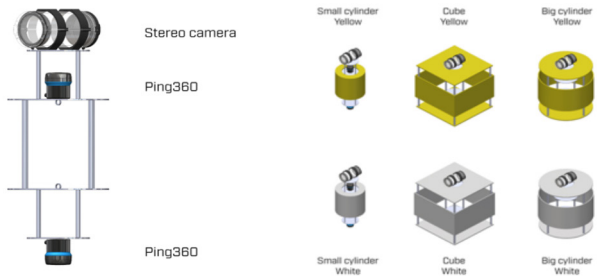

Figure 1: *The structure was equipped with a stereo camera and a sonar on the top and a sonar on the bottom, and produced with six different appearances that varied in shape, size, and colour. (Illustration by Mats Mulelid)*

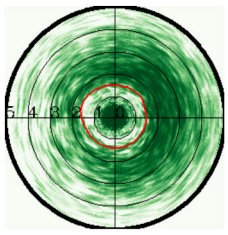

Figure 2a: *Fish swimming pattern recognition around a big cylinder yellow structure. Fish seem to keep a distance of approx. 1.5 m to the structure.*

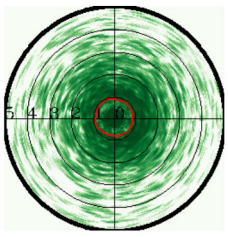

Figure 2b: *Fish swimming pattern recognition around a big cylinder white structure. Fish seem to keep a distance of approx. 0.8 m to the structure.*

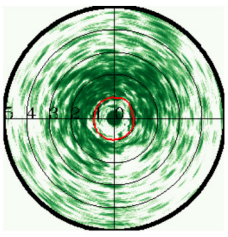

Figure 2c: *Fish swimming pattern recognition around a small cylinder yellow structure. Fish seem to keep a distance of approx. 0.9 m to the structure.*

Conclusion and future work

The colour of structures seems to be affecting fish behaviour, and fish keep shorter distances from white structures compared to the yellow ones. Our results present no evidence that structure shape has an impact on fish, but structure size may have an effect as fish were closer to smaller structures than to the big ones. In addition, fish stayed farther away from the same structure as they got bigger, suggesting that the size of fish themselves can also a factor influencing their behaviour. More studies are needed to confirm these findings and explore further relationships between fish size and fish-to-structure distance.

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REAL-TIME SIMULATION AND MONITORING OF FISH DISTRIBUTIONS IN AQUACULTURE NET CAGE

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Introduction

In this abstract, methods and technical solutions for real-time simulation and monitoring of farmed salmon (*Salmo salar*) in flexible net cage are introduced. Machine learning-based estimation methods are implemented to combine an individual-based fish model with field measurement data and quantify behavioural changes of the fish when exposed to dynamically changing environments. Fish density distributions in the cage are determined by the simulation results and assimilated echo-sounder data. Fish distributions around a submerged structure such as an underwater vehicle can also be simulated according to the 360-degree sonar data (BlueRobotics - Ping360 Scanning Image Sonar). Field trials and demonstrations have been conducted in an industrial-scale fish farm for instrument testing and verifications of the integrated simulation and monitoring system.

This work was financed by the Research Council of Norway through the project: CHANGE [1].

Materials and methods

A flexible net cage and fish were modelled in the simulation framework, FhSim, which allows a high degree of flexibility to combine different mathematical models, numerical solvers, sensors/observers and relevant estimation techniques for time-domain representation of a complex system [2]. The fish model in FhSim is individual based [3], able to simulate full-scale fish populations (e.g., 200,000 individuals in one cage) in real time. The spatial and temporal fish behavioural responses towards the cage, feed, temperature, light, water currents, waves and other individuals are considered. However, there are several influence factors (e.g., oxygen level and consumption) that are not yet included in the model, and it is notoriously difficult to measure all the environmental conditions and complex interactions in the field.

Therefore, several simulations have been conducted with randomised environmental and behavioural parameters (more than 10,000 samples). These simulation data were used to train machine learning-based surrogate models [4] which were then able to find the most possible behavioural parameters for the resulting fish distributions. As shown in Figure 1, the machine learning-based estimation methods are incorporated into the simulation model to continuously update the behavioural parameters according to measured fish distribution data. The integrated simulation and monitoring system will not rely on accurate environmental inputs that might be difficult to obtain, but can still use the environmental and structural monitoring data when available.

Results

A real-time data collection and communication system has been tested in an industrial-scale fish farm (as shown in Figure 2). The measured fish densities from a multi-beam echo sounder were assimilated with the simulation model for real-time monitoring of vertical fish distributions. The simulation results were shown to coincide with the echo-sounder data and provide more information about the fish, such as the influence of the current and cage deformation on fish distributions and changes in the fish swimming speeds. An extended setup of several echo sounders (both single-beam and multi-beam) in the cage will be tested, as well as the integrated simulation and monitoring system.

Machine learning-based estimation methods have also been implemented to identify and quantify fish distributions around an underwater vehicle or a generalised object, where the 360-degree sonar data were used to parameterize the behavioural changes. These could be extended for real-time fish detection and characterization of the possible interferences to the fish. In combination with the estimated fish distributions in the cage, a bio-interactive control routine can be implemented to ensure fish welfare and increased efficiency in relevant underwater operations (e.g., autonomous underwater data acquisition).

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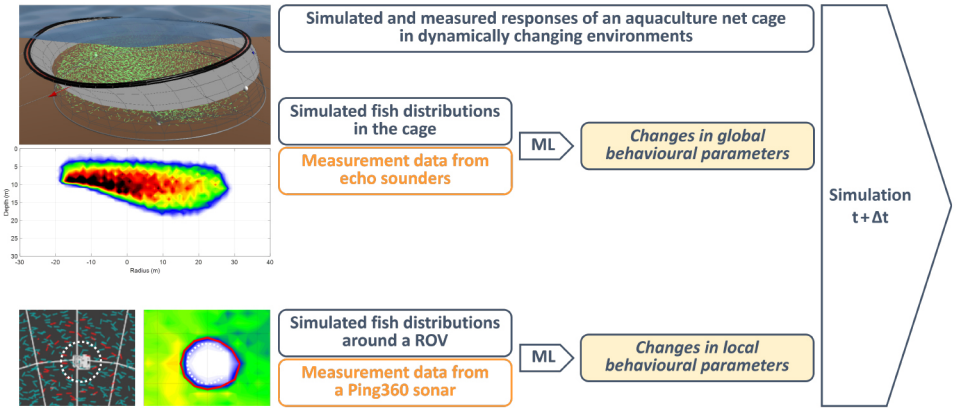

Figure 1: *Machine learning (ML)-based estimation methods incorporated into the simulation framework (FhSim) to quantify behaviour change of the fish, where the global behavioural parameters refer to those are affecting all the simulated fish, and the local behavioural parameters are related to the responses towards an object within a certain area of the cage.*

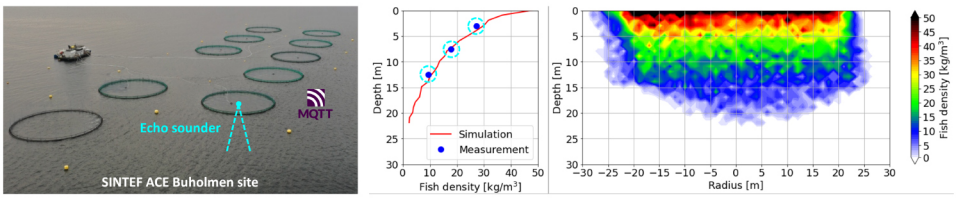

Figure 2: *Demonstrated real-time monitoring of vertical fish distributions in an industrial-scale fish farm, where MQTT is a standard messaging protocol used for data transfer (through 4G network).*

Conclusions and future work

An integrated simulation and monitoring system is being developed and shown to be suitable for real-time applications in an industrial-scale fish farm. The tested instrument setup is preliminary and will be extended for actual use. The machine learning-based estimation methods incorporated into the simulation framework form a basis for hybrid analysis and modelling [5] and relevant digital twin implementations. A holistic digital twin solution for aquaculture structures and farmed fish will improve the ability to monitor, control and document aquaculture productions and facilitate knowledge-based decision making, thereby contribute to the realisation of precision fish farming.

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METAGENOMIC SEQUENCING FOR IDENTIFICATION OF OFF-FLAVOUR PRODUCING MICROORGANISMS IN SWEDISH RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

Off-flavours are of great concerns in various systems such as aquaculture, drinking water and food production. Geosmin (GSM) and 2-methylisoborneol (MIB) are the most widespread off-flavour compounds in recirculating aquaculture systems (RAS), known for their earthy/muddy smell and extremely low detection threshold for humans. GSM and MIB are secondary metabolites of microbial origin and the main off-flavour producers are *Actinobacteria*, *Myxococcota*, and *Cyanobacteria* (Lukassen et al., 2017). Typically, two distinct routes of GSM and MIB biosynthesis occur in prokaryotes: the 2-C-Methyl-D-erythritol 4-phosphate (MEP) pathway and the mevalonate (MVA) pathway (Lange et al., 2000). The latter pathway also has an auxiliary leucine-dependent isoprenoid pathway. In this study, we investigated microbial communities of 50 samples from five RAS in three Swedish fish farms by metagenomic analysis.

Materials and methods

Two of the sampled RAS were aquaponic systems combining production of rainbow trout with tomatoes or vegetable cultivation, two produced eel, and one produced sturgeon. Three RAS used moving biofilm carriers for nitrification, one used a stationary trickling filter, and one relied on nitrification in gravel bed. Four RAS had drum filters for particles separation and one used sedimentation tanks. Samples of microbial communities were collected from various locations within the system including biofilters, sedimentation tanks, gravel bed, and wall growth. Following DNA extraction, the samples were analysed using shotgun metagenomic sequencing. The raw data were filtered and trimmed by using Fastp (v0.20.0). Then seven batches of metagenomics data were co-assembled into contigs using MEGAHIT (v1.1.3), and the scaffolds longer than 2000 bp were selected for binning with MetaBAT (v2.12.1) to obtain metagenome-assembled genomes (MAGs). CheckM (v1.2.1) was used to evaluate the completeness and redundancy of MAGs. The coverage of MAGs (%) in each sample was estimated using CoverM (v0.6.1). Taxonomic classifications of MAGs were conducted using GTDB-Tk (v2.1.0), and phylogenetic analysis was performed by using PhyloPhlAn(v3.0.3).

Results and discussion

A total of 76 potential off-flavour producers, affiliated with 10 bacterial phyla (*Acidobacteriota*, *Actinobacteriota*, *Myxococcota*, *Proteobacteria*, *Nitrospirota*, *Planctomycetota*, *Bacteroidota*, *Chloroflexota*, *Gemmatimonadota*, *Verrucomicrobiota*) and 1 archaeal phylum (*Iainarchaeota*), were identified from 1378 MAGs based on functional genes annotation. After conducting a blast (basic local alignment search tool) search for functional genes responsible for the MEP and MVA pathways, we identified 38 MAGs with complete MEP pathway, 33 MAGs with complete MVA pathway, and 5 MAGs with both complete MEP and MVA pathways (Fig. 1). The GSM and MIB synthase genes (geoA and MIBS) were found in all the 76 MAGs, indicating a high diversity of previously unknown off-flavor producers in RAS. In addition, a large number of bacteria (203 MAGs, affiliated with 16 bacterial phyla) with almost complete MEP or MVA pathways but lacking the final genes of geoA and MIBS were also identified (Fig. 2), suggesting that these bacteria may interact with potential off-flavor producers in RAS by providing precursors or intermediates.

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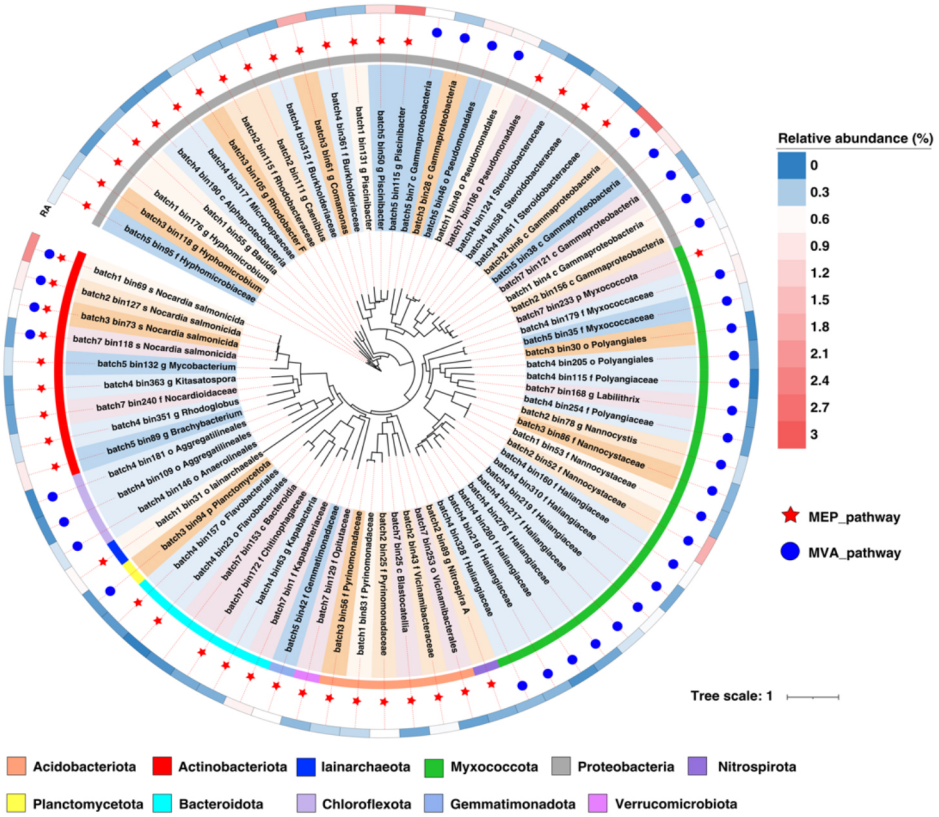

Fig. 1 Phylogenetic tree of 76 MAGs as potential off-flavour producers reconstructed from 50 samples. Relative abundance is shown in the outer circle heatmap.

Phylum	batch1	batch2	batch3	batch4	batch5	batch6	batch7	Sum
Acidobacteriota			2				3	5
Actinobacteriota	5	8	5	16	3	1	3	41
Armatimonadota				3			1	4
Bacteroidota	4	7	6	29	7	9	16	78
Bdellovibrionota							2	2
Chloroflexota		1		5			2	8
Desulfobacterota_B				1				1
Fibrobacterota							1	1
Firmicutes_A						2		2
Gemmatimonadota				3	1			4
Myxococcota	1			2			4	7
Patescibacteria		1						1
Planctomycetota	1	1		1			2	5
Proteobacteria	4	5	1	12	3	1	8	34
Spirochaetota						3		3
Verrucomicrobiota	2	1		1			3	7
Sum	17	24	14	73	14	16	45	203

Fig. 2 Summary of 203 MAGs with almost complete MEP or MVA pathways but lacking the final genes of geoA and MIBS.

FISH INDIVIDUAL IDENTIFICATION USING DIFFERENT CNN BASED ARCHITECTURES

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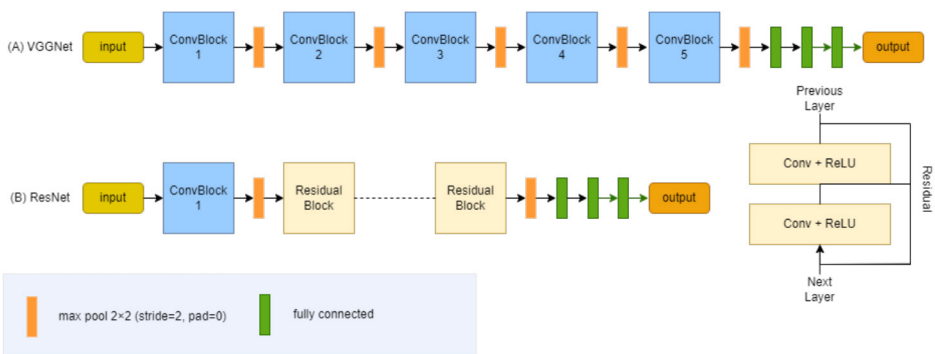
Introduction

Nowadays, biometric systems have numerous applications in different fields. In recent years various applications of modern computer vision systems have been applied for different tasks like object detection, segmentation, and classification for analysing different fish vertebrates and aquatic animals. There are a lot of applications available for using artificial intelligence automated manners in different research fields of aquaculture studies, like fish welfare, disease detection, and fish behaviour. Fish species identification is the major tool for biologists' studies to identify and trace these types of species over a period of time. Since machine learning can provide an automated manner to identify each individual fish, these methods can be much easier to use especially for real condition identification tasks. New machine learning approaches, such as different deep neural network architectures, could provide essential and more trustable tools for researching and developing the fisheries industry. Thus, in this article, we present a comparison of different convolutional neural neteorks (CNN) architectures in the field of individual image-based fish identification. The results of different techniques are discussed. Finally, we provided conclusions from applying different CNN methods of fish identification.

Materials and methods

In recent years numerous artificial neural network architectures have been introduced for different tasks like image classification and recognition. CNN have been used extensively in different image classification tasks like fish species identification. In this research, we used three different CNN architectures for performance review for fish individual identification tasks for Atlantic Salmon.Atlantic salmon identification – The dataset consists of 30 individual Atlantic salmon. The average fish weight was 251±21 g, and the length was 29.5±2.5 cm. The age of the fish was five months. All fish were tagged with PIT (passive integrated transponder) tags. The tagged fish were cultivated in a 2m^3 recirculation freshwater tank for six months. Every two months, 4-8 images per fish were taken for each individual (session). Two to four images for each fish from each session were used to create the reference database for identification. All sessions were mixed together to identify the fish at different growth stages. The other images for each individual were used to evaluate the identification task.

We used simple CNN, VGG 16 and ResNet-50 for fish identification tasks. Stacked colour images of all individual fishes were used as the CNNs input for training and testing. A simple CNN can provide an accurate model over simple visual data; However, the pattern of salmon is more complex and change over time, so we need to use deeper networks to train a more accurate model for fish identification. Training different models with ResNet-50 with 23 million trainable parameters and VGG-16 with over 138 million trainable parameters show that using a deeper network can effectively handle the fish identification task for long-term identification. Below you can see the two deep networks used for fish identification.



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Results

Fish identification using CNN: A simple CNN architecture with four hidden layers applied over the collected data, and the accuracy rate for identifying the 30 individual fish was 93.4% which corresponds to the high accuracy of individual identification.

Fish identification using VGGNet: The accuracy rate for identifying the 30 individual fish was 95.1%, which shows a better performance than simple CNN for fish identification.

Fish identification using ResNet-50: Finally, the accuracy rate for identifying the 30 individual fish using ResNet-50 using triplet loss function was 99.3%, which shows the best efficiency among the used CNN-based structures.

Conclusion

Identifying individual fish species and other aquatic species by tagging them is one of the most important tools for following up on their footprint in the aquatic habitat to estimate their health, growth assessment, and welfare for the fishing industry. This study showed the ability to use different CNN architectures on the visual data for individual fish identification. The three CNN architectures were evaluated on the salmon dataset. The best metrics result reported for the fish identification by applying ResNet-50 using triplet loss. A high accuracy rate of about 99% showed that it shows the effectiveness of applying this ResNet-50 architecture for fish identification tasks.

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DETERMINATION OF THE ENVIRONMENTAL IMPACTS OF INTEGRATED MULTITROPHIC AQUAPONIC SYSTEMS THROUGH LIFE CYCLE ASSESSMENT APPROACH

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Introduction

Aquaculture is increasingly considered as the main source for meeting the world's growing demand for fish products (FAO, 2022). However, in parallel with the growth of the sector, concern about its sustainability has also grown. In this regard, the SIMTAP project "self-sufficient integrated multi-trophic aquaponics systems' (EU PRIMA 2018) aims to implement and test a multi-trophic aquaponic system to reduce: (i) fish meal and fish oil use for feed (ii) water and energy consumption, (iii) emission of nutrient compounds. These are the main factors identified as environmental hotspots in aquaculture systems (Aubin et al., 2009; Abdou et al., 2017). In particular, the proposed system allows for the recovery wastewater from aquaculture and its use as a nutrient source for hydroponics, achieving a run-off reduction and nutrient recovery effect as well as the co-production of other alternative food and/or feed. In addition, the project aims to analyze its performance in terms of sustainability and, for the environmental pillar, Life Cycle Assessment (LCA) approach is considered the most suitable methodology to apply. In this study, using the LCA method, the environmental performance of SIMTAP systems developed in Italy were analysed and compared with that of a traditional aquaculture inland farm.

Materials and method

The concept behind the analysed SIMTAP system is to utilise wastewater from the rearing of Gilthead Sea bream (*Sparus aurata*) and European Sea bass (*Dicentrarchus labrax*) for both a hydroponic halophytic system (for Salicornia, *Salicornia europaea* and Beta Maritima, *Beta vulgaris subsp. maritima*), which exploits the dissolved nutrients in the water, and for the breeding of DFFO (polychaetes) that feed on the solid waste from the fish, in order to recycle the the nutrient in the water loop.

The functional unit (FU) selected for the LCA (i.e. the reference unit of the study to which all inputs and outputs are referred) was 100 kcal derived from all the outputs of the systems analysed (fish, Salicornia, Beta maritima, polychaetes). This FU was chosen because it expresses the functionality of the food (McLaren et al., 2021) while at the same time allowing the different outputs of the SIMTAP system to be taken into account. We applied *cradle to farm gate* perspective for the definition of the system boundaries, including the production of the infrastructure and equipment (e.g. pumps, tanks, filters), the production of inputs (e.g. electricity, diesel, liquid oxygen), feeds production and supply and the net nutrient emission due to the metabolism of the fish. To facilitate the comparison of results with a commercial reference system, the scenario inventory was built by upscaling the experimental data and results of the pilot plant to a production farm of 10 tonnes of fish/year. To this purpose, literature data, estimates and questionnaires submitted to experts were also used. In particular, two scenarios were constructed that differ in terms of fish feeding: (I) commercial feed (CF) scenario in which fish are fed with a commercial feed; (II) alternative feed (AF) scenario: in which fish are fed with an alternative feed consisting of locally produced mussels, clams and polychaetes.

The commercial reference farm, used as a benchmark, is a traditional Italian inland farm with a production of about 400 tonnes/year of Sea bream and Sea bass. This farm consists of several ponds, where fish are reared from a size of 3g up to 400-500g. The system is high energy demanding, both for pumping water, blowers and the liquid oxygen consumed.

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Results

For the main impact categories analysed in the aquaculture sector (global warming potential, eutrophication, cumulative energy demand, acidification) the two SIMTAP scenarios always have a lower impact than the commercial farm. In more details, the global warming potential is 0.230, 0.247 and 0.266 kg CO_2 eq./100 kcal respectively for CF scenario, AF scenario and commercial farm. The cumulative energy demand is reduced by 25% in both SIMTAP scenarios, while the most promising results are in the eutrophication impact category. This category depends mostly on the release of nitrogen and phosphorous compounds into the environment due to fish metabolism. In SIMTAP systems, thanks to the coupling of the hydroponic system (which absorbs the dissolved part of the nutrients) and polychaetes (DFFO that feed on fish faeces, removing the solid part of the waste), eutrophication is significantly reduced (more than 50% reduction). However, the main hotspots of the analysis, as in the commercial farm, remain the high electricity consumption due to pumping water, oxygen consumption and feed supply. Furthermore, since a large surface area is required for the hydroponic system, the impact of the infrastructure required for the system is also not negligible.

Conclusions

Improving the sustainability of aquaculture is a need in the current European context. As demonstrated in this study, following the example of the SIMTAP system, it is possible to improve the environmental performance of aquaculture systems with new diets characterised by the use of locally produced raw materials, the coupling of a hydroponic system to a RAS and the reuse of nutrients. Furthermore, in a context where energy consumption is the main driver, maximisation of the use of renewable energy sources (e.g. solar energy) can lead to significant improvements. It is important to emphasise that the analysis is concerned with theoretical scenarios, so the analysis of the uncertainty of data and results will be a further important step. Finally, in addition to environmental performance, economic and social sustainability should also be assessed for a more comprehensive evaluation of the innovative systems.

Acknowledgements

This study was conducted within the framework of PRIMA S2 2018 project SIMTAP. SIMTAP (https://www.simtap.eu/) is part of the PRIMA Programme supported by the European Union.

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THE BIORAS_SHRIMP PROJECT: IMPROVEMENT AND INNOVATION OF A BIO-SECURE RECIRCULATING AQUACULTURE SYSTEM FOR SHRIMP AND ADDITIONAL BIOMASS CIRCULAR PRODUCTION

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Introduction

The development of sustainable, productive, climate-neutral and resilient farming systems is, nowadays, an obliged way to provide consumers with affordable, safe, traceable, healthy and sustainable food, while minimizing pressure on ecosystems. The culture of aquatic organisms is still the fastest growing sector in food production and now provide almost half of all sea food consumed globally. Crustaceans, with a total production of 11,2 million tonnes in 2020 (SOFIA, FAO 2022), represent 13 % of all aquaculture products, with the white leg tiger shrimp (*Litopenaeus vannamei*) contributing for nearly 52 %. Shrimp farming has been historically practiced in Asia and in the Americas. During the last decade the interest to shrimp culture has arisen also in Europe (Euroshrimp.net, 2020), due to the increasing demand for freshly harvested, sustainably produced shrimps and to the application of highly intensive systems, such as the clear water recirculating aquaculture system (RAS) and the biofloc technology (BFT). Both RAS and BFT are considered eco-friendly and have low or zero-water exchange rates, which improves the efficiency of water use and reduces the risk of introducing pathogens. However, both can be improved and fully integrated in a land-based multi-trophic aquaculture system that allows to produce high quality seafood, additional biomass, and valuable environmental services, ensuring sustainability and circularity.

The BIORAS_SHRIMP project objectives

The main goal of the BIORAS_SHRIMP project (www.bioras-shrimp.eu) is to develop, improve and innovate a bio-secure land-based shrimp culture model to enhance productivity, minimize waste and recover energy and nutrient for additional biomass production, in the view of a circular economy process. In particular, an innovative recirculating aquaculture system (RAS and hybrid RAS-BFT) with improved technology and husbandry efficiency, for shrimp intensive culture, will be developed and implemented in Malta, Norway and Italy. Furthermore, systems for the recovery of waste, co-products and side-products generated by the aquaculture activity, will be developed and tested. Finally, sludge and residual nutrients in the effluent will be used to generate additional valuable biomass and bioactive compounds.

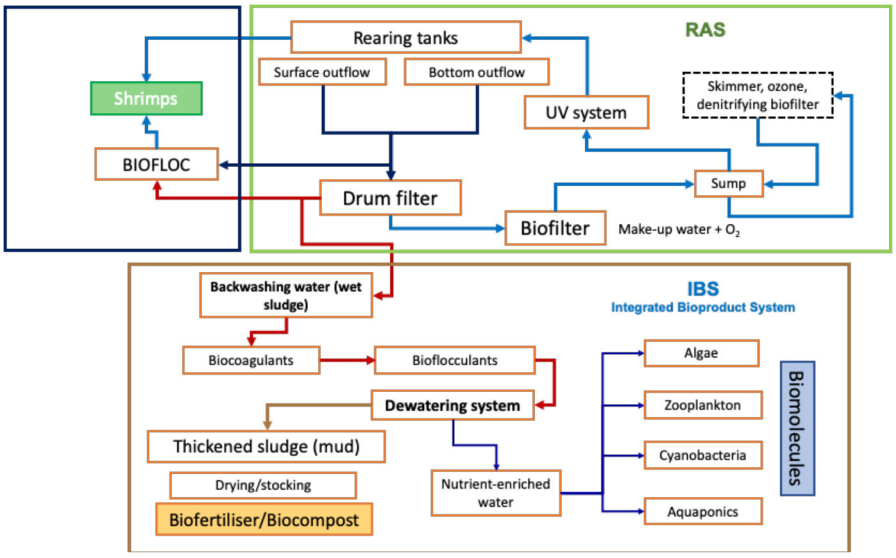

Figure 1: Conceptual layout of the BIORAS_SHRIMP system

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Research approach and methodology

The application of the bio-system principles (food first, sustainable yields, cascading approach, circularity, and diversity) is at the backbone of the R&D and methodology proposed. A conceptual layout of the full system to be developed, implemented, and tested is shown in figure 1. As clearly indicated, the rearing units (RAS and Hybrid RAS-BFT) are structurally and functionally connected to the effluent treatment system, in a full circular process able to produce, via separate streams, different valuable end-bioproducts (indicated in the colored boxes): 1) high quality shrimps for human consumption; 2) a bio-fertilizer obtained through the conditioning and thickening processes of the extracted solids; 3) a set of bioactive compounds derived from the aquatic biomasses (algae, plants, cyanobacteria), appropriately cultured in the nutrient-enriched residual water.

Industrial innovation for shrimp culture

All outcomes have high potential industrial and commercial impact being innovative products and processes immediately applicable to support a sustainable ecological and digital transition in aquaculture.

Stakeholders' engagement

Our network involves scientists, professionals, and students to develop relevant knowledge, technologies and innovations, skills, abilities and experience to be shared with a wide range of stakeholders, including farmers, researchers, policymakers, Consumer Associations, and other end-users, to facilitate growth and development of the circular aquaculture in participating countries.

Capacity building opportunities

A specific work package is dedicated to the organization of a training course based on the results achieved during the project lifetime. The course will describe the general management of the shrimp culture RAS, including the effluent treatment system, additional biomass production and by-products, co-products and side-products potential utilisation and valorisation.

The course will be open for attendance by farmers, technicians, PhD students, representatives of Aquaculture Producers and NGOs.

Acknowledgments

The BIORAS_SHRIMP Consortium Partners have received funds from the National Granting Agencies of Malta (Malta Council for Science and Technology), Italy (Ministry of Universities and Research), and Norway (Norges Forskningsråd), under the BlueBio ERA-Net Cofund initiative (EU Horizon 2020 grant agreement n.817992)

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SPERMATOGONIAL PROLIFERATION AND APOPTOSIS IN PREPUBERTAL MEAGRE *Argryrosomus regius* TREATED WITH RECOMBINANT FOLLICLE STIMULATING HORMONE, AND COMPARISON WITH ADULTS

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Introduction

The meagre *Argyrosomun regius* (Asso, 1801) is a promising aquaculture species and advancing puberty using recombinant gonadotropins could shorten the generation time for selective breeding programs (Zupa et al., 2023). The aim of this study was to assess the effects of recombinant follicle stimulating hormone (rFsh) administration on spermatogonial proliferation and apoptosis in prepubertal meagre reared in indoor tanks through a comparison with adult fish reared in sea cages.

Material and Methods

Prepubertal meagre males (18-months old) reared in indoor tanks at IRTA (La Ràpita, Spain) underwent a six-weeks treatment with increasing doses of rFsh (week 0: 6 μg/kg; week 1: 9 μg/kg; week 2 to week 6: 12 μg/kg); control prepubertal males were injected weekly with 1 mL of saline solution. Prepubertal fish samplings took place before the treatment (week 0; control fish, N = 6) and after 6 weeks of treatment (week 6; control fish, N = 9 and rFsh-treated fish, N = 4). Adult males (6-years old) belonging to a commercial stock reared in sea cages in the Gulf of Taranto (Ionian Sea, Italy) were sacrificed during early (March-April 2021; N = 7) and advanced (June 2021; N = 4) phases of spermatogenesis. Testis samples were fixed in Bouin's solution and embedded in paraffin wax. Deparaffinized sections were stained with hematoxylin-eosin; proliferating spermatogonia were identified through the immunohistochemical detection of the proliferating cell nuclear antigen (PCNA); apoptotic germ cells were identified through the TUNEL method.

Results and Discussion

The rFsh-treated fish had larger testes compared to both control groups, had larger seminiferous tubules that contained all stages of spermatogenesis and had more abundant luminal spermatozoa (Fig. 1a, b). The testes of adult fish sampled in March-April were in active spermatogenesis with all germ cell types in the germinal epithelium and luminal spermatozoa (Fig. 1c); while in June, all adults were fully mature, showing residual spermatogenetic activity in a thin germinal epithelium and plenty of luminal spermatozoa (Fig. 1d). Anti-PCNA immunostaining was observed in the nuclei of single spermatogonia, spermatogonia in cysts and primary spermatocytes (Fig. 1e), but only single spermatogonia were considered for quantitative analysis. The TUNEL reaction labelled spermatogonia and spermatocytes (Fig. 1f). Fish treated with rFsh showed a significant decrease of proliferating and apoptotic single spermatogonia. In adults, spermatogonial proliferation was significantly higher during the early phase of spermatogenesis compared with the advanced phase in June, and apoptosis significantly increased from the early to the advanced phase of spermatogenesis (Table 1).

The treatment with rFsh stimulated spermatogenesis advancement in prepubertal meagre and induced a significant reduction in spermatogonial proliferation and apoptosis. In adult fish, germ cell apoptosis was low during the early spermatogenesis phase and increased during the advanced spermatogenesis phase. This observation confirms that apoptosis plays a major role in regulating germ cells/Sertoli cells ratio and in preventing aberrant germ cell development during spermatogenesis in adult fish (Prisco et al., 2003; Zupa et al., 2013). Moreover, the present data support our previous hypothesis that in prepubertal meagre apoptosis is involved in the inhibition of spermatogonial survival and progress towards meiosis (Zupa et al., 2023).

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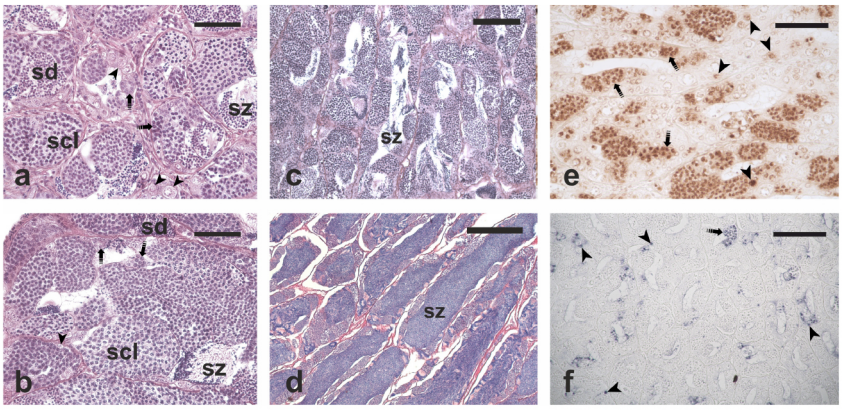

Fig. 1. Micrographs of meagre testis sections. a) control prepubertal fish; b) rFsh-treated prepubertal fish; c) adult fish in early phase of gametogenesis; d) adult fish in advanced gametogenesis phase; (e) anti-PCNA positive germ cells with nuclei stained in brown; (f) TUNEL-positive germ cells appearing as dark blue dots. Magnification bars = 50 µm in (a, b, e), 100 µm in (c, f), 300 µm in (d). Arrowhead = single spermatogonium; dashed arrow = spermatogonial cyst; scI = primary spermatocyte cyst; sd = spermatid cyst; sz = spermatozoa.

Table 1. Density of proliferating single spermatogonia and apoptotic germ cells in prepubertal and adult meagre.

	Mean (± sd) density of anti-PCNA proliferating single spermatogonia (n/mm^2 germinal epithelium)	Mean (± sd) surface occupied by apoptotic germ cells ($\mu m^2/mm^2$ germinal epithelium)
Prepubertal, control week 0	98.2 ± 47.2[a]	58799.8 ± 12903.7[a]
Prepubertal, control week 6	121.3±52.9[a]	23451.4±1554.8[b]
Prepubertal, rFsh-treated week 6	12.4±4.8[b]	1700.8±297.3[c]
Adult, early spermatogenesis (March-April)	487.7±291.0[a]	11531.7±7536.6[a]
Adult, advanced spermatogenesis (June)	22.7±12.8[b]	32147.3±18796.2[b]

Different letters indicate significant differences among prepubertal fish groups or between adult fish sampled in early and advanced phases of gametogenesis (ANOVA; $P < 0.05$).

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ADDENDUM

UTILIZING REGASIFICATION ENTHALPY OF LIQUEFIED GASES FOR EFFECTIVE COOLING AND FREEZING IN AQUACULTURE SYSTEMS

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In aquaculture various deep-cold liquefied gases are used for different purposes: oxygen (LO2 or LOX) for oxygenation [1, 2], nitrogen (LN2 or LIN) for cooling [3], petroleum gas (LPG) [4] and natural gas (LNG) [5, 6] for energy supply and cooling, hydrogen (LH2) for water treatment [7] or – potentially in the future – also for energy supply.

All these liquefied gsases are stored at deep temperature (-150 to -250 °C) and enhanced pressure (up to 20 bar) in liquid state. For the different applications the gases are generally regasified (i.e. vaporized), usually by using ambient air or (sea) water in open rack vaporizers. The used media are cooled, while this (exergetic) cold potential is wasted to the respective environment in most of the application. Though there are approaches to use that potential [5, 6] for cooling, none of those approaches refers to the enormous possibilities of using the very low temperatures and appreciable regasification (evaporation) enthalpies of the liquefied gases (~ 200…400 kJ/kg, depending on pressure level).

In a number of projects – mainly initiated and managed by the small company REGASCOLD GmbH – different heat exchange technologies and equipment were designed, improved and tested for several commercial application sectors [8, 9]. Special focus was given to aquaculture systems, i.e. indoor (RAS) as well as open sea farming. As cooling media (i.e. secondary or refrigerant media), e.g., different thermofluids, propane, water and carbon dioxide were investigated, water and carbon dioxide also in the form of the respective solid state – ice. Special (heat) exchangers were designed and tested, whereas a twisted coil type and a double tube (tube-in-tube) were developed and commercially produced for the industrial applications.

Especially for applications in RAS and indoor aquaculture, a joint project addressed the needs of freeze drying (up to -50 °C, preferably with thermofluid as cooling agent) and water cooling or indoor and room climatization (5 … 15 °C, preferably with water or water ice as cooling agent). The investigations are transferred into a special RAS modernizing and energy efficiency project, which is up to be implemented.

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THE SEA-URCHIN *Sphaerechinus granularis* (Lamarck, 1816) CULTURE

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Introduction

In response to the increasing demand for sea urchin gonads (known as roe or uni) in both Asian and European markets, coupled with concerns about the overexploitation of wild sea urchin populations, this initial study aims to address the necessity for cost-effective protocols within echinoculture. The primary objective of this research was to assess the gonadosomatic index (GI) in *Sphaerechinus granularis* that have been raised in captivity over a five-month period and compare it with that of their counterparts captured from the wild. Additionally, we examined two different methods for inducing spawning: injection of potassium chloride (KCl) and agitation.

Material and Methods

For this preliminary study, *S. granularis* with test size superior of 50 mm were collected from local wild populations by snorkeling in the subtidal at east Madeira Island (Quinta-do-Lorde; 32º74’11.25’’N; 16º70’96.36’’W). The broodstock was reared for a five-month period in 200 L tanks with running ambient seawater at water exchange rate of 45 % per hour. Urchins were fed with *Zea mays*, at 0.7 % of the biomass present in the rearing tanks three times a week, and before each feeding the uneaten food and feces were siphoned. After the five-month period gonadosomatic index (GI), was compared between conditioned sea-urchins (n=20) and wild caught conspecifics (n=15). Additionally, two spawning induction methods were evaluated: KCl injection, and agitation, by assessing the spawning response within 30 minutes, and survival up to seven days.

Results

Results indicate that five-month conditioned sea-urchins GI average was 5.30 ± 2.14, as opposed to wild caught *S. granularis* presented a GI average of 3.34 ± 1.51. In the spawn induction techniques evaluation, the two methods were able to trigger spawning. Mortality was higher with KCl injection method with a mortality rate over 90%. The agitation method showed a significantly lower mortality rate of 10% of the induced urchins, that highlights the potencial of this spawning inducing method as an alternative to intracelomic KCl injection without impairing broodstock survival.

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MARINE AQUACULTURE IN SEMI-ENCLOSED REGIONS WILL BE DISPROPORTIONATELY IMPACTED BY CLIMATE CHANGE

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Introduction

Aquaculture currently accounts for the majority of seafood produced for human consumption (FAO, 2022). However, the production security of marine aquaculture is still largely dependent on ambient environmental conditions. Climate change is a major driver of shifting environmental conditions which, in turn, drives a global redistribution of marine species as they shift to remain within their ideal conditions (Pinsky et al., 2013; Fredston et al., 2021). This impacts the way in which humans utilise marine resources and the aquaculture industry, where farmed species are unable to track ideal climates, will need to adapt. Environmental changes may lead to production losses in regions where species already live at the edge of their environmental thresholds. Conversely, temperate regions may benefit from an increase in growth rates and a localised diversification of species (Pauly, 1980; Kitchel and Pinsky, 2023). The current and projected development of the industry highlights a growing need to assess future aquaculture suitability in a changing climate.

In this study, we projected the extent to which global marine aquaculture species and regions will experience dissimilar climates in future. To achieve this, we used the climate dissimilarity metric which drives the climate analog approach, identifying regions with similar environments to a current region across time and space (Mahony et al., 2017). Using climate dissimilarity to estimate the difference between present-day conditions and contrasting future climate scenarios can identify countries or regions best suited for marine aquaculture in future, estimating how species may move in response to changing conditions. Here, we determined the climate dissimilarity for 333 marine aquaculture species aggregated into five taxonomic groups. Present-day environments were defined for each taxonomic group based on biologically and aquaculturally-relevant environmental variables, and future projections were made at the end of the century for three climate change scenarios: an optimistic scenario (SSP1-1.9), a moderate scenario (SSP3-7.0), and a worst-case scenario (SSP5-8.5).

Research Outcomes

Under an optimistic climate scenario, regions experiencing climate dissimilarity are concentrated in the Northern Hemisphere, with Arctic regions having the highest proportion of marine aquaculture species facing climate dissimilarity. This highlights the rapid rate of environmental change already being observed in the Arctic (Rantanen et al., 2022). In total, 111 Exclusive Economic Zones (EEZs) were projected to face no dissimilarity, including important aquaculture nations such as Indonesia and the Philippines. Conversely, projections for the moderate and worst-case climate scenarios find that regions experiencing extreme climate dissimilarity are largely within semi-enclosed regions, including the Baltic Sea, the Black Sea, and the Red Sea. Additionally, equatorial regions are projected to experience moderate to extreme dissimilar climates, with a high proportion of species facing climate dissimilarity. Under the moderate climate scenario, four EEZs face no dissimilarity, while three EEZs face no dissimilarity under the worst-case scenario. However, these EEZs are all small island nations or territories that currently have no commercial marine aquaculture. Regions where species already live at the edges of their environmental thresholds may face losses in aquaculture production with climate dissimilarity. Conversely, opportunities for aquaculture development may arise for high-latitude regions, including those with moderate or greater dissimilarity, as climate change aids in poleward species movement.

Our findings aim to aid governments and industry in establishing adaptive management strategies to mitigate the impacts of climate change, to facilitate the continued growth and sustainability of the aquaculture industry.

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ASSESSMENT OF *Salicornia ramosissima* RESIDUE VALORIZATION AS A WHEAT FLOUR SUBSTITUTE IN FEEDS FOR EUROPEAN SEABASS AND WHITELEG SHRIMP

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Introduction

Halophytes like *Salicornia ramosissima* are valuable sources of biomass that can be grown in regions where traditional crops may not thrive. The green tips of *S. ramosissima* are utilized as a human food product, whereas the rest of the plant is treated as residue. To establish a more sustainable and profitable production cycle, the residue must be valorized into by-products, which can be used in aquafeed formulations, reducing reliance on traditional feed sources, and promoting more sustainable practices within the aquaculture industry. The current study aims to assess the feasibility of using *S. ramosissima* aerial by-products, as a substitute for wheat meal, in aquafeed formulations for juvenile stages of European seabass, *Dicentrarchus labrax*, and whiteleg shrimp, *Penaeus vannamei*, evaluating survival (%), relative growth rate (RGR, % day^{-1}), feed conversion ratio (FCR) and economical conversion ratio (ECR, □ spent in feed per Kg of biomass gain).

Methods

Four experimental diets were tested on European seabass in triplicates: a commercial-like diet (CTRL), comprising 16.3% wheat meal, and three experimental diets featuring lignified *S. ramosissima* at inclusion levels of 2.5%, 5% and 10%, replacing wheat meal as an alternative ingredient. European sea bass juveniles (mean wet weight 7.3 g) were kept at 22 ± 0.5 ºC and fed *ad libitum* for 62 days.

Five experimental diets were tested in quintuplicates on whiteleg shrimp: a commercial-like diet (CTRL), comprising 25% wheat meal, two experimental diets containing lignified *S. ramosissima* stems (S) and two containing lignified *S. ramosissima* leaves and seeds (L), both at 5% and 10% inclusion levels, replacing wheat meal as an alternative ingredient. Whiteleg shrimp juveniles (mean wet weight 6.1 g) were kept at 28 ± 0.5 ºC and fed *ad libitum* for 55 days.

Voluntary feed intake was registered daily. At the end of the trials, fish and shrimp were weighted and counted for survival, RGR, FCR and ECR determination.

Results and discussion

No significant differences in survival, RGR and FCR were observed among treatments for European seabass (Table 1). These results suggest that lignified *S. ramosissima* can be used to replace wheat meal in aquafeed formulations for juvenile European seabass in inclusion levels up to 10%, with no detrimental effects to growth performances, survival and FCR. It was possible to observe that in scenarios where *S. ramosissima* was included at a rate of 5%, this by-product could be valued at up to 80% of the value of wheat flour while still maintaining a slightly lower ECR value than the CTRL diet. In the case of a 10% inclusion of *S. ramosissima*, it was observed that this inclusion could be limited to only 50% of the value of wheat flour in order to maintain the desired ECR levels. In this context, it is possible to consider the valorization of *S. ramosissima* aerial by-products. Furthermore, the use of these by-products could reduce the environmental footprint of European seabass production due to the proximity of both industries, in the Mediterranean region.

On the other hand, whiteleg shrimp fed diets containing *S. ramosissima* showed significantly higher FCR values, despite having similar RGR and survival values to the those fed the CTRL diet (Table 2). These results indicate that Salicornia by-products might not be a viable alternative to wheat meal in diets for juvenile whiteleg shrimp, as these increases in FCR, in a production scenario, would represent increments of around 20% in feed associated costs, even if considering a reduction in diet price by including Salicornia by-products retailed below wheat meal market value.

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Table 1. Survival, relative growth rate (RGR), feed conversation ratio (FCR) and economical conversion ratio (ECR) of European seabass juveniles fed the experimental diets containing *S. ramosissima* for 62 days. ECR is presented in three different scenarios: *S. ramossima* biomass price per kg being 0.00 €, 0.145 € (50% of wheat meal cost) and 0.232 € (80% of wheat meal cost). Results expressed as mean ± standard deviation (n = 3 experimental units).

	European seabass: *S. ramosissima* - whole plant			
	CTRL	2.5 %	5 %	10 %
Survival (%)	94.60 ± 4.1	97.10 ± 1.6	97.90 ± 1.2	95.80 ± 1.6
RGR (% day^{-1})	2.93 ± 0.01	2.93 ± 0.05	2.93 ± 0.03	2.93 ± 0.03
FCR	1.00 ± 0.03	0.99 ± 0.01	0.99 ± 0.02	1.01 ± 0.00
ECR (Salicornia 0.000 € Kg^{-1})	1.164	1.147	1.140	1.152
ECR (Salicornia 0.145 € Kg^{-1})	1.164	1.151	1.147	1.166
ECR (Salicornia 0.232 € Kg^{-1})	1.164	1.153	1.151	1.175

Table 2. Survival, relative growth rate (RGR), feed conversation ratio (FCR) and economical conversion ratio (ECR) of whiteleg shrimp juveniles fed the experimental diets containing *S. ramosissima* for 55 days. Economical conversion ratio (ECR) in one scenario: *S. ramossima* biomass price being 0% of the cost of wheat meal. Results expressed as mean ± standard deviation (n = 5 experimental units). Different superscript letters indicate significant statistical differences between treatments in a one-way ANOVA, followed by a Tukey-HSD multiple comparison test (P>0.05).

	Whiteleg shrimp: S. ramosissima stems - S; leaves+seeds - L				
	CTRL	S 5 %	S 10 %	L 5 %	L 10 %
Survival	96.70 ± 1.5	97.10 ± 2.4	98.20 ± 2.6	96.00 ± 2.7	97.80 ± 1.5
RGR (% day^{-1})	2.00 ± 0.00	2.00 ± 0.10	2.00 ± 0.10	2.00 ± 0.00	2.00 ± 0.00
FCR	3.10 ± 0.20^{a}	3.60 ± 0.20^{b}	3.70 ± 0.10^{b}	3.60 ± 0.20^{b}	4.00 ± 0.10^{c}
ECR (Salicornia 0.000 € Kg^{-1})	3.031	3.486	3.549	3.474	3.802

Conclusion

Results obtained in this study suggest that *S. ramosissima* aerial by-products can be used to replace wheat meal in aquafeeds formulations for European seabass juveniles. This scenario would allow Salicornia farmers to valorize a residue, that potentially could be marketed at wheat meal retail price, while also contributing to the implementation of a circular economy paradigm in halophyte farming and the aquaculture industry. Based on the European seabass production in 2021, approximately 300,000 tons, it was feasible to estimate that around 15,000 tons of Salicornia lignified biomass could potentially be utilized as an ingredient in aquafeed diets for this production.

Nonetheless, it appears that using *S. ramosissima* by-products as a substitute for wheat meal in diets for whiteleg shrimp juveniles may not be a practical solution. Observations revealed an increase in both FCR and ECR when shrimp were fed diets containing Salicornia, potentially leading to substantial additional feed-related costs for producers.

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