BLUE FOOD

GREEN SOLUTIONS

AQUA 2024

August 26-30, 2024

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Welcome To Copenhagen for ***the*** global aquaculture event!

BLUE FOOD, GREEN SOLUTIONS is the overarching theme of AQUA 2024. Here we bring together scientists, industry leaders and entrepreneurs, governmental bodies, and regulators from all over the world with the common passion for aquaculture. The AQUA events are co-organised by the European Aquaculture Society (EAS) and the World Aquaculture Society (WAS) and are held every six years.

Blue food from aquaculture contributes to Sustainable food systems at a local and global level. The potential for aquaculture to be a driving force in for sustainable food systems and the green shift is huge. But this depend on science-based solutions and innovations actually being implemented at scale. AQUA 2024 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 for blue food and aquaculture industry.

This year theme of seafood and aquaculture as green solutions will be reflected in the 60 scientific sessions over four days ranging from genomics to socioeconomics, covering the full scope of European and World aquaculture scientific disciplines and species. AQUA 2024 will also feature an international trade exhibition with 240 booths, 12 special sessions, industry forums and workshops, as well as updates on EU research. The event will highlight the latest aquaculture research and innovation to underpin continued growth of this exciting food production sector.

A regular feature of EAS events is the Industry Forum and the Innovation Forum and these will take place during AQUA 2024. The Industry Forum will be held all day on Tuesday, August 27th and will address the main event theme, with key questions about the status and future of the sector with regards, to adaptation to climate change, mitigation of its effects, circular approaches and other externalities. The Innovation Forum will be held all day on Wednesday, August 28th on “Exploring Inter-Regional Collaboration & Innovation Transfer vehicles for Aquaculture.” This year Innovation Forum is being co-organised by EAS, EATiP (European Aquaculture Technology and Innovation Platform) and the European Commission. The Forum will explore inter-regional collaboration for innovation transfer through the lens of EU policy and initiatives, but also at the global level.

This year we are expecting more than 2500 attendees from close to 100 countries. The scientific conference will include more than 60 sessions covering all aspects of aquaculture research worldwide. All submitted scientific abstracts have been reviewed by the session chairs and integrated into an impressive programme by EAS representatives Luisa Valente (CIIMAR, Portugal) and Kjell Maroni (FHF, Norway) together with WAS representatives Lorenzo Juarez (World Bank consultant, USA) and Anne Cooper (ICES, Denmark) as AQUA 2024 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 AQUA 2024 possible, as for my colleagues on the Boards of the EAS and WAS and the EAS home office. A big thanks also for the support of our local partners, the Danish Export Association Fish Tech, ICES, DTU AQUA, the University of Copenhagen, EUROFISH and the Copenhagen Convention Bureau.

We are also extremely grateful for the support of our event sponsors, with GOLD SPONSOR BIOMAR, SILVER SPONSORS BIORIGIN, US SOY, UNIVERSITY OF STAVANGER and the BLUE PALNET ACADEMY/STIIM AQUACULTURE CLUSTER and with SESSION SPONSORS AQUASOJA.

I hope you will enjoy the event, the people, and the science. I’m excited about the aquaculture program we have for you, and I look forward to seeing you all in Copenhagen!

Bente Torstensen

Welcome to AQUA 2024, in Copenhagen
WAS/EAS premier aquaculture event

Sustainable aquaculture produces healthy and nutritious fish and seafood, is the most efficient protein production industry, generating less environmental pollution and a lower carbon footprint, and can potentially use less water and space using state-of-the-art technologies. Aquaculture will play a large role in alleviating food security problems. With over 400 species cultured worldwide, the potential for production expansion is enormous. This is relevant as a demand of at least 40 million tons per year of additional production is expected by 2050.

Like other protein production industries, aquaculture faces a series of problems and we must use our best efforts to divulge, transfer and implement the best knowledge-based solutions we have. With the theme **BLUE FOOD, GREEN SOLUTIONS,** this year´s **AQUA 2024** event in Copenhagen, a joint effort by the European Aquaculture Society (EAS) and the World Aquaculture Society (WAS), will bring together over 2,500 stakeholders, including industry leaders, entrepreneurs, investors, scientists and government officials from almost 100 different countries. The Steering and Local Organising Committees, together with the Program Co-chairs, have made certain that the 60 scientific sessions, dedicated industry and innovation forums, and over 200 booths at the trade exhibition, provide a unique experience for our members and participants, where information on the latest aquaculture research, systems and innovation are availabe.

The World Aquaculture Society, as the most relevant aquaculture association worldwide, facilitates the generation and dissemination of knowledge among its Members and the society in general, through conferences, workshops, and effective use of social networks. We now offer free WAS Membership to students worldwide, have an open-access Journal that is very well regarded by the scientific and industry communities and have also made the World Aquaculture Magazine open-to-the-public. I invite you to talk to our Board Members at the meeting and engage with our Chapter Presidents, as we´d like to hear how we can improve our efforts to help develop sustainable aquaculture worldwide.

I would also like to thank our event sponsors at AQUA 2024, including Gold Sponsor BIOMAR, Silver Spondors BIORIGIN, US SOY, UNIVERSITY OF STAVANGER and the BLUE PLANET ACADEMY/STIIM AQUACULTURE CLUSTER, as well as our Session Sponsor AQUASOJA, for their support. Similarly, thank you to our local collaborators the Danish Export Association Fish Tech, ICES, DTU AQUA, the University of Copenhagen, EUROFISH and the Copenhagen Convention Bureau

I hope you will enjoy the event, the knowledge exchange, the interactions and networking. I´m looking forward to meeting you at AQUA 2024. Cheers.

Dr. Humberto Villarreal

President, World Aquaculture Society

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AQUA 2024

ABSTRACTS

THE LIKELYHOOD FOR MOBILE LICE TO LEAVE A HOST DEPENDS ON SPECIES, TEMPERTURE AND DAYLENGTH

Gunnvør á Norði*, Birgitta Andreasen, Tróndur Kragesteen, Signar P. Dam, Kirstin Eliasen

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The most common infection pathway of salmon lice is through the planktonic copepodid stage, but there is an increasing concern that the mobile stages of salmon lice leave/fall off the salmon during crowding operations, potentially re-infecting treated fish or fish in adjacent cages within the same or nearby farms. Knowledge on how many lice fall off during the crowding operation as well as behavioural traits of the various mobile development stages and gender will help to identify the severity of the potential problem.

In this study the number of lice that fell off fish during crowding was quantified at the thermal delousing of 9 individual net pens. In addition, a large dataset from sea lice monitoring of farmed salmon was analysed for behavioural traits regarding lice that fell off. In the Faroese national sea lice monitoring program registrations of lice that fall off during sedation of the fish is imposed. Data from more than 30000 counted net pens from mid-2016 to February 2024 was statistically analysed.

In the dataset sea lice were registered as species (*Lepeophtheirus salmonis* or Caligus *elongatus*) and for salmon lice the development stage was also registered (adult females, large mobile which include preadult stage 2 and adult males, and preadult 1). Temperature and date were also registered and used to analyse potential variations in detachment due to temperature and daylength.

C. elongatus were much more prone to detach from the fish than salmon lice and both species showed seasonal variations in the percentage of lice that detached from the fish (Figure 1).

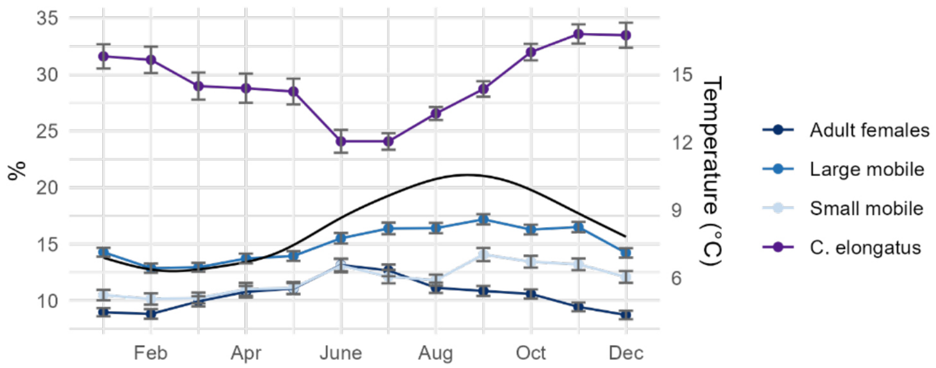

Figure 1 Percentage of lice that fell off during sedation of the fish prior to sea lice monitoring (mean ± SE) and average temperature (black line, secondary y-axis).

EFFECTS OF LIVE AND INACTIVATED BACTERIAL FEED ADDITIVES ON THE GROWTH PERFORMANCE AND MUCOSAL HEALTH OF MIRROR CARP *Cyprinus carpio* UNDER A LOW FEEDING REGIME

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Dietary administration of probiotics can modulate the gut microbiota and improve host fish intestinal morphology, digestion, and immune response. Inactivated probiotics, or paraprobiotics, are gaining interest due to their better stability during feed production and longer product shelf life. This study aimed to investigate the effects of potential autochthonous probiotics, in live and paraprobiotic forms, on the growth, feed utilisation, and intestinal health of mirror carp.

Bacterial isolates (n = 150) derived from the intestinal mucosa and digesta of carp (n = 5) were screened *in vitro* for haemolytic activity, pathogen antagonism and extracellular enzyme activity. One of the best performing isolates from the *in vitro* screening, identified as a strain of *Bacillus subtilis*, was top-dressed onto the basal diet to produce the live probiotic diet (BSpro; equivalent to log 6 CFU/g). Another experimental diet was prepared using heat-inactivated version of *B. subtilis* (Bspara; equivalent to log 6 CFU/g). Two commercially available inactivated *Lactobacillus* products (Lactob1 and Lactob2; both equivalent to log 6 CFU/g) were used to prepare two further experimental diets. Sterile PBS was used as the vehicle for top dressing and an equal volume of sterile PBS was top-dressed onto the control diet (CON). A 35-day feeding trial was conducted in the Tropical Unit Aquarium of the University of Plymouth. Using triplicate tanks per treatment, 18 fish per tank (5.59 ± 0.08 g) were distributed randomly into 13 L tanks. Fish were fed at 2-3.5% biomass per day, to assess fish zootechnical performance under suboptimal conditions. At the end of the trial, three fish per tank were humanely euthanised and samples were taken for analysis.

No statistically significant differences of zootechnical performance or intestinal morphometrics were observed between groups (data not shown). However, skin goblet cell coverage and density were significantly higher in carp fed with the paraprobiotics (Table 1). These results indicate a potential for modulation of skin mucus production. Further analyses are being conducted to elucidate the effects of the experimental diets on the molecular microbial ecology, host gene expression and digestive enzyme activity.

Table 1 Skin histology of mirror carp fed with experimental diets.

	CON	**BSpro**	**BSpara**	**Lactob1**	**Lactob2**
% Goblet cell coverage (n/400 µm)	2.59 ± 1.66^{a}	9.38 ± 6.21^{ab}	14.59 ± 12.02^{b}	15.09 ± 6.90^{b}	11.80 ± 5.11^{b}
Goblet cell density (n/400 µm)	4.86 ± 1.89^{a}	13.78 ± 5.95^{ab}	14.11 ± 6.80^{ab}	25.94 ± 12.01^{b}	18.06 ± 9.26^{b}

Note. Values are expressed as mean ± SD. Different letters in the same row indicate significant difference (*p <0.05*). CON, control; BSpro, live *B. subtilis* supplemented diet; BSpara, inactivated *B. subtilis* supplemented diet; Lactob1 and Lactob2, inactivated *Lactobacillus* spp. supplemented diets

BIOMARKER RESPONSES IN NILE TILAPIA, *Oreochromis niloticus*, AS A TOOL TO ASSESS AQUATIC POLLUTION IN EGYPT

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With a global farmed production of more than 6 million tons annually, the tropical cichlid Nile tilapia (*Oreochromis niloticus*) is one of the most important farmed fish species worldwide, with Egypt being the leading producer on the African continent. Monitoring of water pollution by chemical analysis alone cannot completely assess the effects of the pollutants on aquatic species because of the high number of unknown chemicals and the mixture presence of these chemicals. In contrast, the use of pollutant-responsive biomarkers in fish can be an effective approach to monitor water pollution, as this provides a more comprehensive and realistic assessment of the exposure and response of aquatic organisms to chemical stressors.

Molecular biomarkers are used as a tool to quantitatively measure a sub- organismal change, including apoptosis, DNA damage, micronucleus formation and gene expression changes, within organisms in response to external chemical stress. In this context, fish larvae may offer a suitable model for studying the effects of water pollutants.

Our aim is to develop an array of gene expression biomarkers in Nile tilapia larvae that can detect environmentally relevant concentration of water contaminants of different mechanisms of action (Figure 1)

A total of 1101 genes (226 up-regulated and 875 down-regulated) were identified by gene expression profiling using RNA-Seq (figure 2)

The results of GO and KEGG enrichment analyses indicated a disruption of the immune system, particularly the induction of apoptosis, inflammation, oxidative stress, and autoimmunity in addition to biological processes involved in detoxification. Also, there was a significant inhibition of neuronal developmental pathways displayed with some of these contaminants (Figure 3)

Figure 1: experimental design

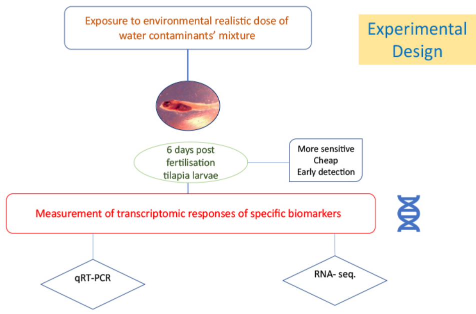


Figure 2: Bar plot illustrating the number of differentially expressed genes in each treatment compared to its Group control.

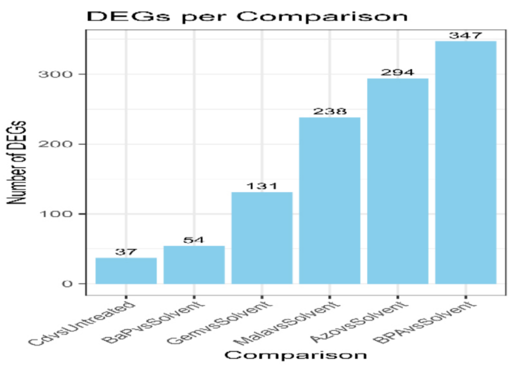
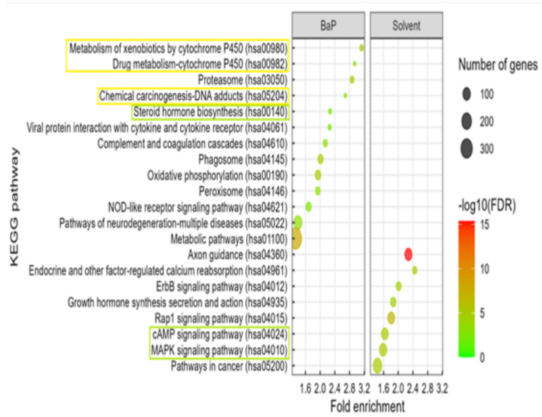


Figure 3: Scatter plot of differentially expressed genes enriched in KEGG pathways



RIO KINASE 1 GENE IN TILAPIA (*Oreochromis niloticus*): ITS CLONING AND CHARACTERIZATION

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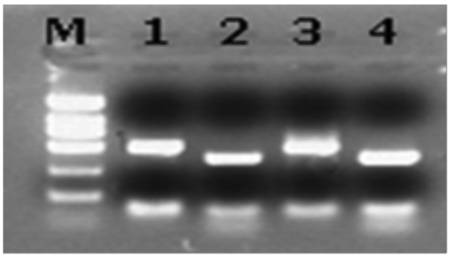
A new member of RIO kinase 1 protein family (RIOK-1) was identified in Tilapia *Oreochromis niloticus* immunized by killed *Flavobacterium columnarae*. Suppressive subtractive hybridization (SSH) was utilized to construct a cDNA library and quantitative RT-PCR analysis used to examine *Oreochromis niloticus* Riok1 gene expression. The complete sequence of the RIOK-1 cDNA is composed of 2226 bps with 10 open reading frames, the predicted gene product is 742 amino acids with molecular weight of 190.41 kDa.

Analysis using quantitative RT-PCR revealed that the gene encoding RIOK-1 was broadly and strongly expressed in tissues of the stimulated fish, as up-regulated gene suggesting that this member of RIOK-1 genes is probably involved in the general immune response against the pathogenic bacteria.



ligation analysis efficiency (Lane 1, 3: Tester; lane 2, 4: β-actin and M:

Tissue specific expression revealed by RT-PCR in *Oreochromis niloticus*; 1=βactin, 2=immunized fish, 3= unimmunized fish.

```
GTLVRRFSR*ASFTIFFLSFLSIGASSLCCCSSSSSTSSSSSSSSSSSSCEEEQLSSRRA
GTV*TPERSFFSPVSVWYRTLSFCPLMADSSSCVFIRSTSRS**LTSVRVRGM*AFLNTS
SSTPS*LDRSSDVLSAAITIALSRYWSMFWLVMDGSVMKSKSSLTVITATPCFTKNSLML
LQSFRRNSRA*G*SCSTDCDTSMM*ASPL*YSMLNSLRSAWTSRAS*NIFLMFCMTCR*S
SRAFDSESEVFFSRGAGTLSFPMNPISRHGF*GGEVLGSGFPPSVA**VLISFSAHVSPS
RGSLQYCSGYTSEAWRGLGTNFPQSIFPQLAWLGSDCHRHLTTVRRHSKDSRVRAEFDTG
LTTASAKLACLQVDD*RGRGRGTLVRRFSR*ASFTIFFLSFLSIGASSLCCCSSSSSTSS
SSSSSSSSSSCEEEQLSSRRAGTV*TPERSFFSPVSVWYRTLSFCPLMADSSSCVFIRST
SRS**LTSVRVRGM*AFLNTSSSTPS*LDRSSDVLSAAITIALSRYWSMFWLVMDGSVMK
SKSSLTVITATPCFTKNSLMLLQSFRRNSRA*G*SCSTDCDTSMM*ASPL*YSMLNSLRS
AWTSRAS*NIFLMFCMTCR*SSRAFDSESEVFFSRGAGTLSFPMNPISRHGF*GGEVLGS
GFPPSVA**VLISFSAHVSPSRGSLQYCSGYTSEAWRGLGTNFPQSIFPQLAWLGSDCHR
HLTTVRRHSKDSRVRAEFDTGL
```

Deduced amino acid sequences of ON RIOK-1

CARP EDEMA VIRUS INFECTION INDUCES CORTISOL RELEASE AND IMMUNOMODULATION IN COMMON CARP

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Gill diseases have a significant impact on fish health and a very negative impact on aquaculture, mainly due to the multifunctional properties of the gills in fish physiology. Carp edema virus (CEV) is a large DNA poxvirus that primarily infects the gills of common carp (Cyprinus carpio L.), causing a highly contagious and fatal disease known as Koi Sleepy Disease (KSD). Our previous studies have shown that, when experimentally infected with genogroup IIa CEV, different strains of carp exhibit high (Amur wild carp - AS) or low (koi carp) resistance to this virus. The increased susceptibility of koi leads to severe impairment of gill function.

In the present study, blood parameters, viral load and expression of selected immune-related genes were determined in the gills of both carp strains. The experiments were carried out at two temperatures: 12 and 18 °C. In the case of the koi carp, we also introduced a salt rescue model based on the addition of 0.5% NaCl to the tank water, which prevents mortality of the fish.

Nanoscale qPCR analysis of 40 genes showed that CEV induced a significant antiviral response in the gills of all infected fish groups compared to uninfected controls. We also found that the viral load was higher at 18°C than at 12°C in all fish groups studied, and at both temperatures the highest viral load was present in koi carp compared to the koi salt rescue group and AS carp. Interestingly, CEV-infected koi carp had higher glucose and cortisol levels and lower plasma sodium levels than the koi salt-rescue group and AS carp at both temperatures. This clearly indicates that CEV infection in a susceptible strain correlates with high stress parameters that can trigger immune modulation. Further analysis showed that disease severity and higher stress response in koi were indeed associated with immunomodulation, as reflected by a down-regulation of T-cell responses.

In conclusion, our data indicate a clear, temperature-dependent relationship between CEV-induced gill disease, stress and immunomodulation in susceptible koi carp.

Acknowledgements:
This research was supported by the German Research Foundation (DFG project number 426513195) and the National Science Centre of Poland (NCN project number UMO-2018/31/F/NZ6/02311).

TESTING INTRA-VITAM DIAGNOSTICS FOR GILL DISEASE IN COMMON CARP – A CASE STUDY FOR CARP EDEMA VIRUS INFECTION AND EPITHELIAL OR ENVIRONMENTAL SAMPLING BASED METHODS

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Recently, non-lethal or environmental sampling has been recognised as a tool for monitoring the presence of pathogens in aquaculture. Measurement of immune responses in the epithelium and the presence of pathogens in the epithelium and water appear 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, and can induce a plethora of different immune responses. For example, carp edema virus (CEV) infection is indicative of immunosuppression of adaptive responses and often occurs with co-infections with ectoparasites such as Ichthyobodo necator and the bacterium Flavobacterium branchiophilum, which drive the proinflammatory responses and pathology, making diagnosis and treatment difficult. As carp cannot always be sacrificed for sampling during the production cycle, we tested the applicability and robustness of epithelial immune response monitoring methods and environmental DNA-based methods for the detection of pathogens associated with KDS.

To test the selected 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 a Qiagen DNA mini kit. Immune responses were measured using a Fluidigm array and correlated with pathogen load and pathological changes.

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 concentration of CEV increased rapidly from day 4, when the first clinical signs appeared. Furthermore, the DNA of all pathogens could be detected in the water for at least 8 days after removal of the infected fish. Gill biopsies and swabs allowed the detection of immune responses previously measured during CEV infections: increased antiviral and proinflammatory responses and decreased levels of adaptive immunity markers.

Conclusion: 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 e.g. a 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. Gill swabs appeared to be as reliable as gill biopsies or post-mortem samples for detecting immune responses characteristic of KDS. Additional immune markers need to be evaluated to better distinguish between other types of infection.

Funding
Funded by the European Union under the Horizon Europe Programme, Grant Agreement No. 101084204 (Cure4Aqua). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

DANGEROUS AS TILAPIA LAKE VIRUS - WHAT CAN WE LEARN FROM STUDYING THE VIRUS HOST RANGE AND NATURAL DISEASE RESISTANCE OF SELECTED NILE TILAPIA STRAINS?

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The occurrence of viral diseases that cause very high mortality can disrupt aquaculture production. This has recently occurred 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. TiLV has reached global distribution, while the host range remains to be defined to better assess the threat posed by the virus. 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. Further investigations were carried out in canonical and non-canonical hosts to determine if resistance to the TILV is possible.

The susceptibility of several cell lines was assessed at different temperatures to find the optimal conditions for virus replication. The susceptibility of juvenile rainbow trout, brown trout and Atlantic salmon larvae to infection with TiLV was studied in infection experiments based on cohabitation with infected tilapia or intraperitoneal injection of the virus at elevated water temperatures of 20°C and 25°C. In addition, two Nile tilapia strains from Nilotic regions (Lake Mansala (MAN) and Lake Turkana (ELM)) and one from an unknown region (DRE) were used in infection experiments at 25°C. Immune responses were measured using a Fluidigm array and correlated with viral load and pathological changes.

TiLV was able to infect salmonid cells *in vitro* over a wide temperature range from 15°C to 25°C. 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 of rainbow trout, brown trout and Atlantic salmon. Similarly, injection of the virus resulted in high permissiveness in all three Nile tilapia strains. However, when we used infection by cohabitation, we found that the ELM strain was resistant to the disease, showed no clinical signs of infection and had almost 100% survival. 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.

To summarise: The bad news is that TiLV has some pathogenic potential in salmonids, which could theoretically be enhanced by climate change and anthropogenic activities. The good news is that TiLV-resistant Nile tilapia strains can be used as a cost-effective ad hoc solution to the TiLV challenge. However, it is important to note that fish of the resistant strain become persistent carriers of the virus and can potentially further transmit the virus. Therefore, the resistant strain should be used as part of an integrated approach that includes biosecurity, diagnostic and vaccination measures as appropriate. Further studies should determine which factors in the mucosal barrier lead to resistance in canonical and non-canonical hosts.

PRACTICES OF BIOSECURITY MEASURES IN SELECTED FISH FARMS IN EKITI STATE, NIGERIA

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There is a dearth of information on biosecurity practices in major fish farms in Ekiti State. With the rising activities of fish farming in the State, it is imperative to know the biosecurity measures been practiced by farmers to protect their farms. The study identified, evaluated and compared different biosecurity measures in eighty (80) randomly selected farms across eight (08) Local Government Areas (LGA) of the State at ten (10) farms per LGA.

Primary data were generated through validated questionnaires, while reliable secondary data were sourced from both the State and Federal Ministries of Agriculture. Data were analyzed using frequency, percentage counts and pie charts. 50% of the farm sites were fenced, either with blocks or wire gauze, while 30% had security Dogs. Only 5% place disinfectants as dip at farm entrance. Less than 10% disallowed outsiders from entering into the main facilities of the farms, while 5% engaged in the use of voodoo (African magic) to secure their farms. 40% of the farmers engaged the services of security men. The study revealed that unchecked human traffic in and out of farms led to high mortality of fish, with occasional disappearance of farm tools. High percentage of hired security men did not translate to better protection of farms compared to the use of security dogs and voodoo. Fencing and installation of anti-birds nets are necessary. It is recommended that outsiders should be disallowed from entering into the main facilities of the farms while the use of non-human security measures should be encouraged.

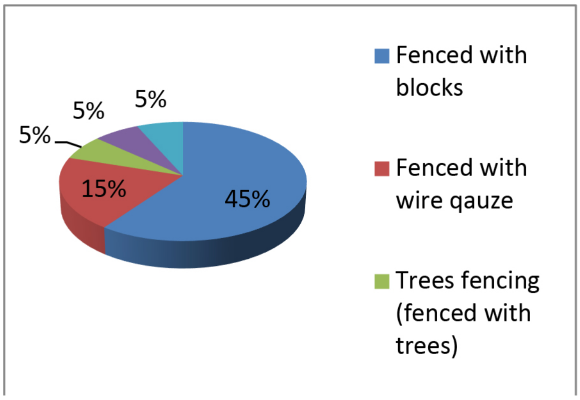

Figure 1: Farm fencing

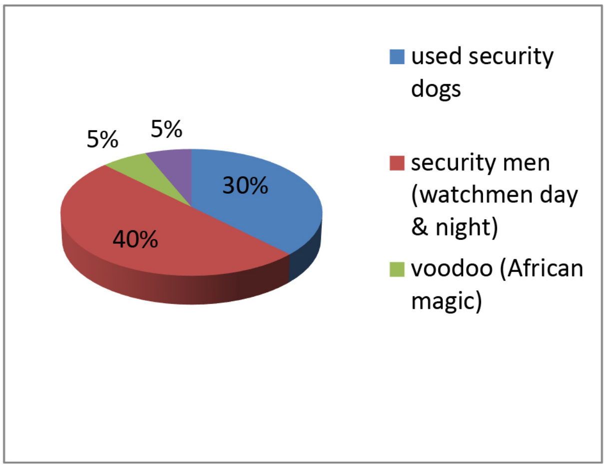

Figure 2: Use of Security Apparatus

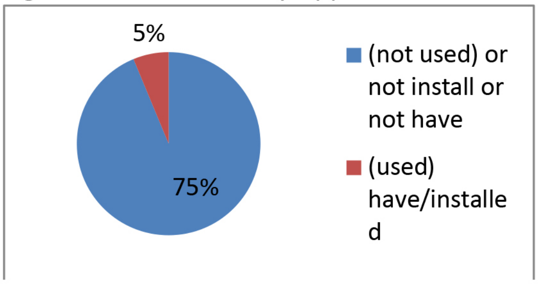

Figure 3: Use of Disinfectants as dip at Entrance

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

Adeosun Festus Idowu[1] Adeosun, Olamide Modinot2; Oyekanmmi Funmilayo Bosede3; and Olemoh, Oluwadamilola Esther[1]

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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.

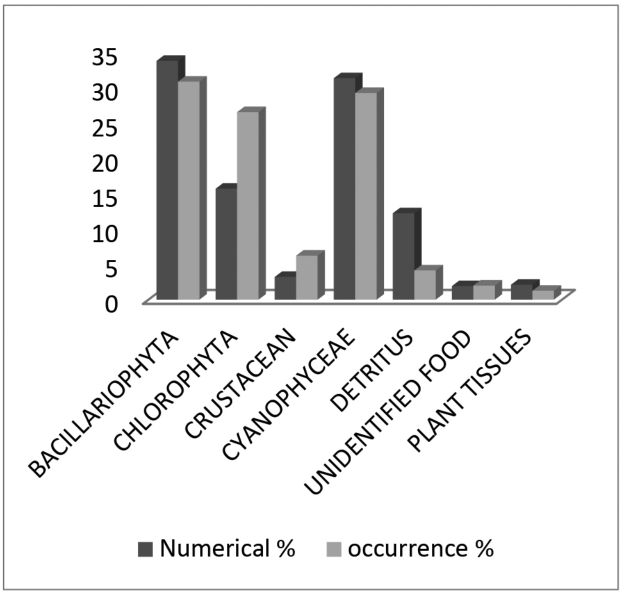
Materials and Methods

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.



DIETARY FENUGREEK (*Trigonellafoenum-graecum*) SEED AMELIORATES THE GROWTH PERFORMANCE, HAEMATOLOGY AND ANTIOXIDANTS PERTURBATIONS IN JUVENILE AFRICAN CARP (*Labeo coubie* Ruppell, 1832) EXPOSED TO DELTAMETHRIN

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Study was conducted to investigate the effects of dietary fenugreek seed meal (FSM) on the growth performance, haematology and antioxidant profile of juvenile African carp (*Labeocoubie*) fish exposed to Deltamethrin. 450 fish were allotted into any of 5 groups 1000 L capacity tanks (consisting of three replicate per group). Each group was fed any of five experimental diets amended with 0% (group A, positive control), 0% (group B, negative control), and diets C, D and E (5%, 10% and 15% FSM respectively), for 56 days. Fish cultured in Deltamethrin contaminated water and fed the basal diet (group B) exhibited significant ($P < 0.05$) reduction in weight gain, specific growth rate and a significant ($P < 0.05$) increase in the feed conversion ratio compared fish fed the same diet but cultured in water free from Deltamethrin. There were significant increases in the erythrocytes and leucocytes numbers in fish fed the basal diet and cultured in Deltamethrin contaminated water compared with fish fed the same diet but cultured in water free from Deltametrin. Deltametrin exposure caused significant increases in glutathione reductase and malondialdehyde concentration and a significant reduction the superoxide dismutase, catalase and glutathione transferase activity. Dietary fenugreek seed meal at 5% 10% and 15% inclusion elicited significant reduction in the glutathione transferase and malondialdehyde concentration in fish cultured in Deltamethrin contaminated water.

CAN BLUE BIO PRODUCTION BE STIMULATED BY THE USE OF ECOSYSTEM APPROACH ?

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Introduction

With increasing activities at sea, the ecosystem approach is seen as a valuable tool for entrepreneurs to consider the impact of their activities on the entire marine environment. According to the Blue Mission Banos Roadmap 2030, one of the initial actions needed to increase sustainable blue biomass production is to establish a framework (local, national, or regional) for the quantification and monetization of Ecosystem Services. The Ecosystem Approach ladder is an overarching instrument that could support this increase in sustainable blue biomass production by evaluating and promoting blue eco-nomy activities that are in balance with marine habitats and ecosystems and align with current and future business models. The Ecosystem Approach ladder assesses a com-pany's ecosystem approach and provides insights into what is or is not going well in terms of their impact on the ecosystem. This should result in a nuanced picture of the company's performance and targeted actions to improve their performance. Besides creating positive impacts by blue bio companies, this performance ladder could be used to incentivize non-blue bio companies, such as construction companies, to engage with blue bio companies. This would positively impact ecosystem services by incorporating a blue bio activity, providing an ecosystem service, into the primary activity of the non-blue bio company (e.g., biodiversity enhancement by macroalgae cultivation between offshore windmills). Within the BlueBioCluster project, the aim is to develop tools for supporting businesses in integrating ecosystem services into novel ecology-driven busi-ness models and to induce cross-sector collaborations along the value chain. This should increase blue bio production by expanding production areas through multiuse and providing a larger economic return for their activities due to their ecosystem services.

Materials and methods

Data collection was carried out by the University of Tartu across the project partners' regions, and a survey on the ecosystem services of the blue bioeconomy across the EU and beyond was conducted by SAMS. The survey assessed the knowledge gaps and needs for using ecosystem services assessments to develop sustainable blue bio value chains. Together with input from three projects supported by the Blue Cluster (SUMES, MEsP, and WABESCO), which address the gaps in tools and data, a regional workshop was held to establish further steps to implement the ecosystem approach.

Results and Discussion

There are tools available that can help value ecosystem services, but few ecosystem service valuation strategies or methods are currently being used. An overarching sup-porting framework to incentivize the ecosystem approach in companies is still missing. A lack of awareness is one of the reasons for this deficiency, and specific workshops should be organized to increase awareness and understanding of the Ecosystem Approach's possibilities. These workshops will also help identify the key players who can establish regulatory support for the incorporation of the Ecosystem Approach.

References

*Submariner network (2023). "Blue mission banos roadmap 2030: steps for effective deployment of the mission ocean". Germany. 22 pp

BIOSECURITY IN TILAPIA CULTURE IN ANGOLA

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Introduction
Tilapia production is a vital industry in Angola that may contribute to economic development, food security, and employment in the country. Thanks to government efforts, there has been a noticeable increase in production over the years. However, as production continues to rise, the emergence of diseases poses a threat to the sustainability of the industry. This work presents various aspects related to biosecurity practices in tilapia culture in Angola. The authors note that while serious tilapia diseases have not been reported in the country so far, there is a lack of appropriate measures to prevent and control potential outbreaks.

Angolan Production System
Tilapia is the most commonly farmed species in Angola, followed by the African Catfish. According to data from INE-RAPP 2019/2020, tilapia culture is primarily focused on large-scale commercialization in the business sector. However, some authors suggest that governmental initiatives have also supported production units across the country, including small-scale rural fish farming practiced by a few community farmers. In these cases, low-level technology is utilized due to a lack of training and experience among farmers (Silva 2015; FAO 2018). Tilapia culture in Angola is conducted in various facilities such as ponds, cages, concrete tanks, and polyethylene tanks, either individually or in combination, with water sourced from nearby rivers or reservoirs. Earth ponds are the most common facilities for tilapia culture in Angola, allowing for semi-intensive or intensive systems based on the financial capacity of the owner (Dombaxe *et al.,* 2015; Silva 2015; Onde & Samuel 2018).

Sanitary Management and disease reports
To ensure sanitary conditions in production, farmers must adhere to good management practices, including disinfection of equipment and personnel, regular monitoring of water quality parameters, predator control, and pathogen analysis in production facilities. Despite reports of disease clinical signs and mortality rates in both wild and farmed fish, there is a lack of comprehensive studies on pathogens in tilapia culture in Angola, leading to a lack of formal biosecurity protocols tailored to the local production environment.

Identified challenges in biosecurity
Challenges facing the Angolan tilapia industry include the introduction of tilapia from Brazil and Egypt without proper hazard identification, the absence of specific biosecurity programs on farms, limited knowledge of local pathogens, inadequate water quality monitoring, presence of predators and pests, the need for skilled labor and training, and institutional support for aquaculture governance. To ensure sustainable growth, profitability, and efficiency in the sector, it is crucial to conduct studies on pathogens in tilapia culture in Angola and implement biosecurity programs for safe and healthy food production.

Conclusion
This communication may be the first step taken in order to warming the Angolan tilapia industry to prevent and control disease occurrence and keep the sector growth sustainable, profitable and efficient, thus, it is recommended studies on pathogens in tilapiculture in Angola to permit the creation of efficient biosecurity programs and healthy and secure food production.

(Continued on next page)

References

Anuário Estatístico do Ministério das Pescas de Angola, 2016. https://www.ine.gov.ao/Arquivos/arquivosCarregados//Carregados/Publicacao_637586852612407176.pdf

Bondad-Reantaso, M. G. 2019. Risk Analysis in Aquaculture. https://www.researchgate.net/publication/336285422

Dombaxe, D.A. M. *et al.,* 2015. Angola e os Desafios de uma Aquicultura moderna. Angola_article_spatial_planning.pdf (fao.org)

Food and Agriculture Organization of the United Nation, 2007 The Republic of Angola. Microsoft Word - AngolaFCP- sent to WWW_270508.doc (fao.org).

Food and Agriculture Organization of the United Nations, 2014 National Aquaculture sector overview Angola. https://www.fao.org › countrysector › naso_angola

Instituto Nacional de Estatística. 2019-2020. RAPP - Resultados dos Relatórios das explorações Agropecuárias e Aquícolas empresariais. ANG_RAAP_POR_2019_2020.pdf

Onde, N. A. & Samuel N. 2018. Angolan Aquaculture. p3 Angola.pdf

Silva, J. E. 2015. PLANNING AND MANAGEMENT FOR SUSTAINABLE DEVELOPMENT OF INLAND AQUACULTURE IN ANGOLA https://www.researchgate.net/publication/228589503

AQUACULTURE IN ANGOLA

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Introduction
Aquaculture in Angola is a relatively new activity compared to other longstanding producer countries, being only 24 years old. This industry was initiated as part of the governmental programme of economic diversification and national food production plan to address issues such as hunger, unemployment, and poverty, as well as reduce reliance on imports. In Angola, aquaculture is primarily practiced in freshwater environments, typically in small-scale communal ponds where tilapia, catfish species, and European eel are cultivated either individually or in combination for local consumption. However, there has been a recent emergence of large-scale commercial aquaculture with investments and support from countries like Brazil, China, and Israel. Since its inception, aquaculture in Angola has shown consistent growth in terms of production, trade, and academic research, with expectations for continued expansion in the coming years. This work provides insights into aquaculture production in Angola as part of a study conducted by the authors focusing on the detection and identification of microorganisms and parasites in tilapia production, aiming to ensure the health, food security, and sustainability of the industry.

Angolan Production System characterization
Tilapia (Oreochromis *niloticus*) introduced from Brazil and Israel is the most farmed species in Angola, followed by African catfish. According to data from INE-RAPP 2019/2020, aquaculture activity in Angola used to be restricted to the business sector focused on large-scale commercialization, practiced by entrepreneurs and cooperatives, however, according to FAO, 2018 there is a number of rural independent farmers. In these cases, low-level technology is employed since farmers lack fundamental training and experience (Silva 2015; FAO 2018). Aquaculture in Angola is carried out in ponds, cages, concrete tanks, and polyethylene tanks. In some facilities, different systems and methods are present, including monoculture and polyculture, and water is pumped from nearby rivers or reservoirs. Earth ponds are the predominant tilapia culture facilities in Angola, allowing the adoption of both semi-intensive and intensive systems. The fish produced is sold at the local market (Dombaxe *et al.,* 2015; Silva 2015; Onde & Samuel 2018; FAO 2024).

The industry faces several challenges, such as a lack of feed production capacity, funding, specific biosecurity programs, the need for husbandry training, health management, lack of institutional assistance, and a lack of studies regarding different areas in the production chain (Bondad-Reantaso, 2019; FAO, 2024).

Aquaculture growth potential from a demand side perspective by 2030
According to FAO 2024, given the 14.17 kg baseline per capita fish and seafood consumption, 636 268 tonnes of fish and seafood will be needed to satisfy the demand of Angola's 44 912 thousand total population in 2030, which is 162 683 tonnes higher than its 473 584 tonnes of baseline fish and seafood demand in 2020 when the population was 33 428 thousand.

If Angola would like to increase its per capita fish & seafood consumption back to the 2014 level (24.45 kg), then 624 505 tonnes of extra fish & seafood supply would be needed to satisfy the extra demand generated by the population growth and higher per capita consumption. Angola's aquaculture production increased from 1 339 tonnes in 2017 to 2 808 tonnes in 2021. Following this trend linearly, the country's aquaculture production would reach 5 543 tonnes in 2030. The 3 481 tonnes of extra supply compared to the baseline would nevertheless be insufficient to cover the 162 683 tonnes of extra fish and seafood demand driven by population growth only (with a deficit of 159 202 tonnes), let alone the 624 505 tonnes of extra fish and seafood demand driven by the population growth and higher per capita consumption (with a shortage of 621 024 tonnes). Angola's aquaculture production would need to reach 164 745 tonnes in 2030 (79.9 times growth; 54.97 percent annually between 2020 and 2030) in order to generate enough extra supply to cover the 162 683 tonnes extra demand driven by population growth only. The production would need to reach 626 567 tonnes (303.9 times; 77.12 percent annually) in order to cover the 624 505 tonnes of extra demand driven by both the population growth and higher per capita consumption.

(Continued on next page)

Conclusion

Aquaculture in Angola is a growing industry with significant potential for expansion due to the demand, climate, and favorable territorial qualities for aquaculture production. Therefore, studies of various kinds are necessary to guide the sustainable development of the industry. Research and development play a crucial role in promoting comprehensive studies across all aspects of Angolan aquaculture.

References

Anuário Estatístico do Ministério das Pescas de Angola, 2016. https://www.ine.gov.ao/Arquivos/arquivosCarregados//Carregados/Publicacao_637586852612407176.pdf

Bondad-Reantaso, M. G. 2019. Risk Analysis in Aquaculture. https://www.researchgate.net/publication/336285422

Dombaxe, D.A. M. *et al.,* 2015. Angola e os Desafios de uma Aquicultura moderna. Angola_article_spatial_planning.pdf (fao.org)

Food and Agriculture Organization of the United Nation, 2007 The Republic of Angola. Microsoft Word - AngolaFCP- sent to WWW_270508.doc (fao.org).

Food and Agriculture Organization of the United Nations, 2014 National Aquaculture sector overview Angola. https://www.fao.org › countrysector › naso_angola

Food and Agriculture Organization of the United Nation, 2024. Aquaculture growth potential in Angola. https://openknowledge.fao.org/server/api/core/bitstreams/0f972d9e-7d9d-418a-af8f-004329190edf/content

Instituto Nacional de Estatística. 2019-2020. RAPP - Resultados dos Relatórios das explorações Agropecuárias e Aquícolas empresariais. ANG_RAAP_POR_2019_2020.pdf

Onde, N. A. & Samuel N. 2018. Angolan Aquaculture. p3 Angola.pdf

Silva, J. E. 2015. PLANNING AND MANAGEMENT FOR SUSTAINABLE DEVELOPMENT OF INLAND AQUACULTURE IN ANGOLA https://www.researchgate.net/publication/228589503

PROGRESSION OF WHITE SPOT SYNDROME VIRUS (WSSV) INFECTION IN MUD CRAB *Scylla serrata:* VIRAL LOAD DYNAMICS AND INFECTION OUTCOMES ASSAYED USING QUANTITATIVE PCR

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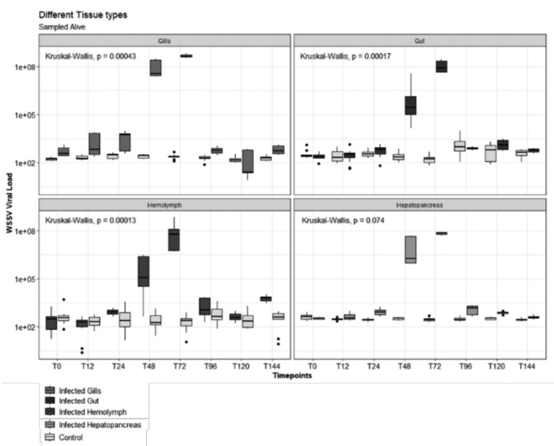
The mud crab *Scylla serrata* is a high-value aquaculture species across the Indo-West Pacific. Like many crustacean species, mud crabs are also known to be susceptible to white spot syndrome virus (WSSV), the causative agent of white spot disease, which remains to be a threat to crustacean aquaculture. However, there is limited information on the progression of WSSV infection in mud crabs. In this study, we characterized tissue-specific viral load dynamics from mud crab (gills, hepatopancreas, gut and hemolymph). Mud crabs were intramuscularly injected with WSSV inoculum (infected) or phosphate-buffered saline (PBS, control), and maintained in aerated tanks until 144 hours post injection. Dead and live crabs were sampled from the two treatments at selected timepoints (hours post infection, hpi). Markedly different viral load curves were observed among infected animals with different disease outcomes at the time of sampling ('dead', 'alive').

While all infected animals exhibited viral loads which increased at 48-hpi and peaked at 72-hpi, the 'alive' group exhibited a drastic reduction in viral load

at 96-hpi which continued until 144-hpi. For the 'dead' group, viral load remained elevated until 144-hpi. Further, similar viral load curves were observed across the different tissue types, for both 'dead' and 'alive' groups. These results suggest that: (1) for crabs sampled dead, the progression of viral infection which remained high until 144-hpi was likely cause of mortality; (2) crabs that were sampled alive from 48-hpi to 144-hpi were able to clear the viral infection, with viral loads comparable to non-infected animals and exhibit potential resistance to WSSV disease. The methods employed here for WSSV detection and quantification for mud crabs can contribute to developing further interventions and recommendations for infection control, particularly for mud crab aquaculture.



CONTROLLING SCOLIOSIS IN STURGEONS: THE ROLE OF NUTRITIONAL INTERVENTIONS

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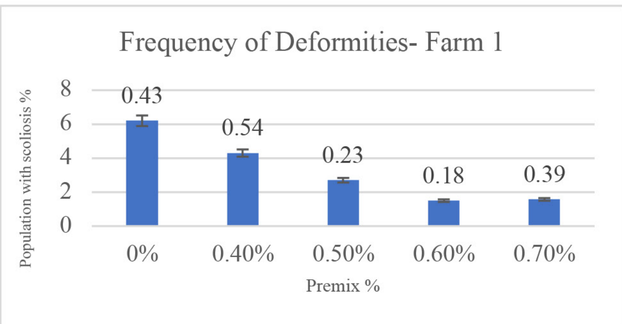
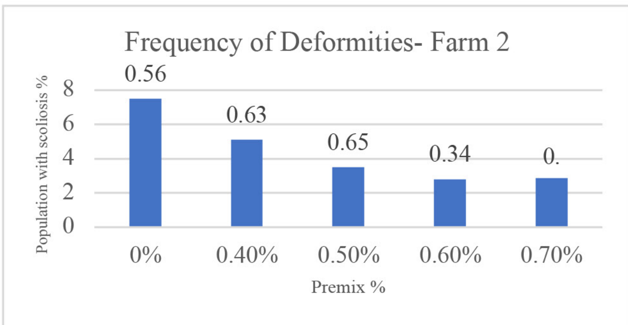
Introduction: Sturgeon fish are crucial to the caviar industry, but scoliosis, characterized by an abnormal spinal curvature, can negatively impact caviar yield and market value. This condition arises from various factors, including genetics, nutritional deficiencies, environmental stressors, and infections. Research has shown that balanced diets can significantly reduce the occurrence of skeletal deformities. This study aims to optimize key nutrients in the Beluga sturgeon diet to maintain skeletal health and control deformities.

Materials and Methods: A premix containing Methylsulfonylmethane, chondroitin sulfate, glucosamine, hyaluronic acid, vitamins C (AQUAVIT® C Stable - Adisseo), K, D3 (Microvit®- Adisseo), organic zinc, manganese, and calcium was formulated to address the scoliosis in sturgeons. Four doses of this premix (0.4%, 0.5%, 0.6%, and 0.7%) were added to the sturgeon basal feed prepared by BFM® (Beyza21 Feed Mill) and compared to the control group (0% of the premix). The three-kilogram great sturgeons (*Huso huso*) in two different farms were fed with the treatments for 12 months. Key metrics, Feed Conversion Ratio (FCR), Daily Growth Rate (DGR), and Survival Rate were measured and the frequency and severity of deformities were reported. Radiographs assessed scoliosis, defined as a lateral spine deviation greater than 10 degrees. Data were analyzed with SPSS 21 using one-way ANOVA and Tukey's post hoc test.

Results: The study found that various premix doses effectively reduced spinal deformities in sturgeon without impacting survival rates. In Farm 1, deformity rates fell from 6.2% (control group) to 1.5% with 0.6% of the premix. However, 0.7% did not improve outcomes further ($P > 0.05$). In Farm 2, the rate decreased from 7.5% (control) to 2.8% with 0.6% of the premix. Radiological exams confirmed that premixes lessened both the frequency and severity of deformities. The 0.6% premix also yielded the best growth rates and Feed Conversion Ratios (FCR) with significant difference in compare to the control group ($P<0.05$), likely due to the positive effects of vitamin C and other vitamins on appetite and digestion. No significant difference was observed between 0.6% and 0.7% doses of the premix in growth performance ($P>0.05$). Overall, the 0.6% of the premix with specifically optimized levels of the ingredients proved the most effective and cost-efficient in controlling scoliosis in sturgeons compared to the control group ($P< 0.05$).





DIGITAL PHENOTYPING AND QUANTITATIVE GENETICS OF FEED INTAKE AND EFFICIENCY IN ATLANTIC SALMON

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Atlantic salmon is the prime aquaculture species in Norway with high global demand and export value. Most of the salmon production cost goes to feed and thus improving feed efficiency (FE) is crucial to reduce this cost and environmental impact. However genetic studies on feed efficiency are limited, primarily due to difficulty in phenotyping individual feed intake. This study aims to overcome this limitation by (i) quickly and accurately phenotyping individual feed intake under commercial conditions; (ii) training deep learning model for efficient object (bead) detection and (iii) analyzing the genetic architecture of FE traits including individual feed intake (FI), average daily weight gain (ADG), Residual feed intake (RFI), and feed conversion ratio (FCR).

Atlantic salmon belonging to the 2021-year class of MOWI genetics, Norway underwent digital phenotyping experiments. The snapshots of individual FI were recorded using X-ray imaging and a deep learning model (YoloV5) for bead detection was trained to estimate the FI accurately from images. The model performed exceptionally well with an R^2 of 0.99, low RMSE and a slope of ~ 1 across training, validation and test sets.

FE-related traits showed varied potential for selective breeding with intermediate to high heritability estimates for FI (h^2 = 0.20 ±0.05 - 0.50 ±0.06) and ADG (h^2 = 0.47 ±0.07 - 0.54 ±0.06), and low to moderate for FCR (h^2 = 0.08 ±0.04 - 0.23 ±0.06) and RFI (h^2 = 0.10 ±0.05 - 0.17 ±0.11). We are currently diving deep into GWAS and genomic prediction to better understand the genomics of FE, and more results will be ready for the conference.

Table 1:Fit statistics for beads detection model

Set	No. of fish	R^2	RMSE (bead count)	Slope
Train	60	0.999	4.23	1.02
Valid	20	0.998	9.34	1.04
Test	10	0.996	5.14	1.02

Table 2: Descriptive summary and genomic estimates of FE traits

Time	No. of fish	DWG (g)			FI (g)			RFI (g)			FCR		
		Mean	SD	h^2	Mean	SD	h^2	Mean	SD	h^2	Mean	SD	h^2
1	684	1.56	0.35	0.48 ±0.07	1.0	0.27	0.20 ± 0.05	0.0	0.18	0.10 ±0.05	0.65	0.13	0.09 ±0.04
2	680	2.66	0.55	0.44 ±0.06	1.85	0.55	0.35 ± 0.06	0.0	0.40	0.14 ±0.07	0.70	0.16	0.08 ±0.04
3	680	2.76	0.62	0.55 ±0.06	2.52	0.78	0.50 ± 0.06	0.0	0.47	0.17 ±0.11	0.90	0.17	0.23 ±0.06

IN VITRO ANTIBACTERIAL ACTIVITY AGAINST FISH PATHOGENS OF SELECT MEDICINAL PLANTS FROM BANGLADESH

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Disease outbreak is one of the major challenges of the rapidly growing aquaculture sector. Plant derivatives, especially with a long history of ethnobotanical use, can be a potential alternative to fight diseases. Bangladesh is one of the major aquaculture producers and has rich plant diversity. The present study was aimed to screen potential plants with antibacterial activity against target fish pathogens.

A total of fifty-five plants were collected from Bangladesh and extracted using ethanol, acetone, hexane, and water. Screening of antibacterial activity of all plant extracts and determination of minimum inhibition concentration of active plant extracts was conducted using the broth microdilution method against the target fish pathogens: *Aeromonas hydrophila, Pseudomonas fluorescens, Pseudomonas aeruginosa, Flavobacterium columnare,* and *Streptococcus iniae*. The initial concentration of tested extracts was 1 mg/ml.

The average extraction yields varied with the solvent used; water demonstrated the highest yield (on average 25 mg/mL, range: 10-47mg/ml) and hexane the lowest (average 4 mg/mL, range: 2-10mg/ml). Acetone, ethanol, and hexane extracts typically exhibited greater growth inhibition activity than water extracts. Hexane extracts displayed no inhibition against A. *hydrophila, P. aeruginosa,* and *P. fluorescens,* whereas, 100% and 84%, of hexane extracts inhibited *Streptococcus iniae and F. columnare* respectively. *Aeromonas hydrophila, Pseudomonas aeruginosa,* and *Pseudomonas flourescens* exhibited more resistance against tested plants, while *Streptococcus iniae* and *Flavobacterium columnare* were more susceptible. *Terminalia arjuna, Terminalia bellirica, Mikania micrantha, Peperomia pellucid, Lawsonia inermis, Phyllanthus emblica, Eryngium foetidum, Amaranthus spinosus, Barringtonia acutangula, Clitoria ternatea, Alstonia scholaris* and *Sphagneticola trilobata* displayed broad-spectrum antibacterial activities against the tested pathogens. MIC_{50} value of active extracts varied from 1 to 444 µg/ml.; 53% of the active extracts had MIC_{50} value less than 25 µg/mL against *Flavobacterium columnare*. *Terminalia bellirica* fruit active extracts had MIC_{50} ranging from of 4-51 µg/mL, with broad-spectrum activity against *Pseudomonas fluorescens, Pseudomonas aeruginosa, Flavobacteriaum columnare* and *Streptococcus iniae*.

The preliminary screening results provide a foundation for further studies that aim to identify potential compounds with antibacterial activity applicable in aquaculture to control bacterial infections.

EVALUATION OF THE HEALTH STATUS AND HISTOPATHOLOGY OF GILLS AND LIVER OF RAINBOW TROUT (*Oncorhynchus mykiss*) REARED IN A RECIRCULATING AQUACULTURE SYSTEM WITH A TiO_2-BASED PHOTO-ELECTROCATALYSIS FILTERING TECHNIQUE

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The Fish-PhotoCAT project, (PRIMA2019), aims to evaluate the efficiency of a nanotube TiO2-based photo-electrocatalytic (PEC) system on a recirculating aquaculture system (RAS) and its impact on the growth and health of fish reared at different densities and stages of development. This study refers to the low-density trial, where rainbow trout (*Oncorhynchus mykiss*) weighing 90 g were raised for 30 days at a density of 15 kg/m^3 in a RAS. Six 500 L tanks were equipped with the standard water filtration set-up: three tanks of the control groups (CTR), and three of the treated groups (T), where a PEC system was installed together with the classic UV lamp. Water physical and chemical parameters were monitored throughout the trial. At the end of the trial, fish were collected, and gills and liver were sampled for histological analyses as alterations of the structure of these tissues can serve as reliable indicators of the water quality. Furthermore, gene expression analyses of three cytokines (IL-1β, IL-10, and TNF-α) and HSP70 were performed on the gills, and the melanomacrophage centers (MMCs) evaluation was done in the liver, as their number and area are good indicators of environmental conditions. Finally, the presence of TiO_2 nanotubes was investigated both in the water by ICP analyses, and in tissues by transmission electron microscopy (TEM). Authorization code: OPBA_20_2020.

No significant differences were found regarding NH_3 and nitrite concentrations between the experimental groups. The mean concentration of nitrates, however, was significantly higher in the CTR (122.211 mg/L *vs*. 108.510 mg/L; $p<0.001$), likely due to the parallel ammonia oxidation to molecular nitrogen performed by the PEC. CTR and T fish exhibited similar specific growth rates and condition factors. No histological differences were found in the structure of the gills between treatments: primary lamellae appeared arranged in double rows and secondary lamellae lined by squamous epithelial cells (Fig. 1A, lamellae, asterisk). The expression of IL-1□, IL-10, TNF- □, and HSP70, showed no differences between groups, confirming the histological observations. Liver histological analyses revealed no structural differences between experimental groups (Fig.1B, MMC, arrows). The relative number of MMCs was not statistically different between treatments even if numerically, it was higher in the CTR. The relative area of the MMCs was significantly higher in the CTR (Fig.1C, $p<0.01$), indicating an activation of the immune response in this group. This difference may be justified by the lower levels of nitrates found in the PEC tanks. Lastly, no TiO2 nanotubes were detected in the water or in the tissues (Fig.1D), implying the safety of this innovative PEC system. These are encouraging results that indicate a beneficial effect of the TiO_2-based PEC reactor in a RAS, even if further investigation is needed.

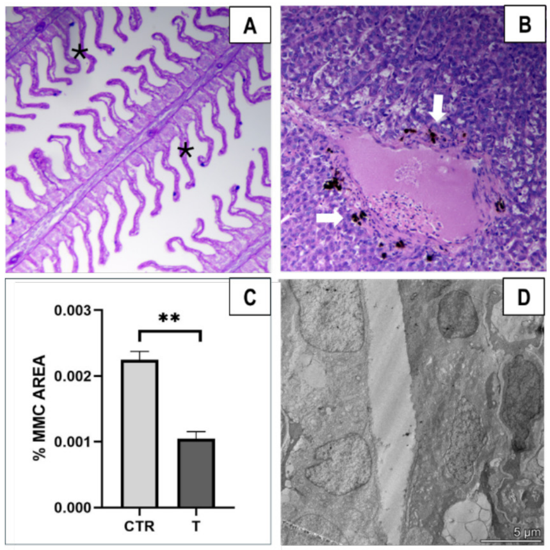

Fig.1. representative images of gills (A, histological section, 100x; D, TEM;) and liver (B, histological section, 200x); quantification of the MMCs area in the liver (C).

UNRAVELLING THE GENETIC ARCHITECTURE OF AMOEBIC GILL DISEASE AND GILL LESIONS IN ATLANTIC SALMON

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Gill diseases pose a significant concern to the salmon industry; in Norway for instance, they are ranked among the leading causes of mortality in salmon farming. Genome-wide association studies (GWAS) can help to unravel the genetic architecture of susceptibility to gill diseases and help to guide mitigation efforts. Amoebic gill disease (AGD) is one of the major gill diseases affecting salmon. Furthermore, during a production cycle, fish gills get exposed to different kinds of pathogens which may cause lesions on the gills. In this study, we conducted a GWAS of AGD and gill lesions on two distantly related cohorts of Atlantic salmon: Norwegian and Canadian.

The Norwegian cohort consists of four (sub) populations that were gill-scored during outbreaks of AGD from 2016 to 2018, while the Canadian cohort consisted of six (sub) populations that were gill-scored at harvest from 2017 and 2018. The total number of fish in the Norwegian and Canadian cohorts is 8506 and 12823, respectively. All cohorts are from the breeding nucleus of Mowi Norway and Canada West and were all genotyped with 55K SNP chip. After quality control, 50456 SNPs remained for the Norwegian cohort, while 50144 SNPs remained for the Canadian cohort. GWAS was conducted with GCTA (--mlma option). In addition to the polygenic effect, the model for AGD had year as a fixed effect, while that for gill lesions had sex and cage nested within year as fixed effects. Genome-wide significance level was defined as 0.05/no of markers.

While only chromosome 12 was implicated in the risk of AGD (Figure 1), both chromosomes 2 and 12 were implicated in the risk of gill lesions (Figure 2), which suggests that chromosome 12 has a pleiotropic effect on AGD resistance and overall gill health. The proportion of genetic variance explained by the lead SNP associated with the risk of AGD was 7%, while the proportion of genetic variance explained by the lead SNP for risk of gill lesions on Chromosomes 2 and 12 is 3 and 10%, respectively.

Our results provide novel insight into the genetic architecture of AGD and overall gill health in Atlantic salmon.

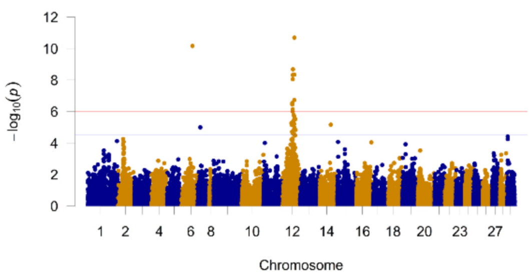

Figure 1: Manhattan plot of GWAS of AGD

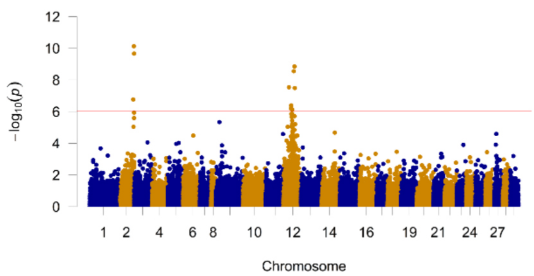

Figure 2: Manhattan plot of GWAS of Gill lesions

GENOME-BASED CHARACTERIZATION OF VIRULENCE AND ANTIBIOTIC RESISTANCE IN *Aeromonas hydrophila* ISOLATED FROM RAINBOW TROUT

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Aeromonas hydrophila, a Gram-negative bacteria common in freshwater, poses a risk to both rainbow trout and humans. Known for causing human infections, it presents a potential zoonotic threat. This bacterium's presence in rainbow trout, a commonly farmed fish, raises concerns about the possibility of transmission from fish to humans, especially given its frequent involvement in aquaculture outbreaks. These strains possess virulence factors like toxins and demonstrate an alarming rise in antibiotic resistance. Understanding the characteristics of *A. hydrophila* from rainbow trout is crucial for mitigating threats to fish health and potentially reducing zoonotic risks.This study aimed to characterize the virulence and antimicrobial resistance potential of two *A. hydrophila* strains (A-12 and A-13) recovered from rainbow trout exhibiting discoloration, lethargy, and exophthalmia.

Samples were taken from the kidney and cultured on Tryptic Soy Agar (TSA) agar 28°C for 24-48h. The isolate was identified using 16S rRNA gene sequencing. Next-generation genome sequencing of the A-12 and A-13 strains were performed on an Illumina NovaSeq 6000 platform. The high-quality reads of the A-12 and A-13 were assembled into contigs by de novo assembly using the Unicycler assembler 0.4.8. Genome-based species delineation of the strains was done with Type Strain Genome Server (https://tygs.dsmz.de/). Virulence factors and antibiotic resistance genes, were identified using: Virulence Factor Database (VFDB) and Comprehensive Antibiotic Resistance Database (CARD). The A-12 and A-13 strains have been found to have a 100% similarity with *Aeromonas hydrophila* in GenBank, based on their 16S rRNA sequences. According to genome-based species delineation, the A-12 and A-13 isolates were found as an *A. hydrophila*.

Analysis of the A-12 and A-13 strain genomes revealed 17 putative antimicrobial resistance (AMR) genes (Table 1). Additionally, these strains encode 224 putative virulence genes associated with various mechanisms, including anti-phagocytosis, serum resistance, biofilm formation, efflux pumps, glycosylation systems, hemolytic activity, immune evasion, and motility**.**

Future studies will investigate the zoonotic potential of *Aeromonas hydrophila* strains A-12 and A-13 in fish and human models to understand their possible risk of transmission to humans.

Table 1. Putative Antimicrobial Resistance Genes Identified in the Genomes of *A. hydrophila* Strains A-12 and A-13

AMR Genes	Drug Class
sul1,sul2	Sulfonamide
qacEdelta1	Disinfecting agents and antiseptics
aadA2, APH(6)-Id, APH(3")-Ib, APH(3')-Ia	Aminoglycoside
dfrA12	Diaminopyrimidine
imiH, cphA2	Carbapenem
QnrS2, rsmA	Fluoroquinolone
tet(D), tet(M)	Tetracycline
catII	Phenicol
CepS	Cephalosporin
EF-Tu	Elfamycin

EFFECT OF FEED CARBON-NITROGEN RATIO AND CARBOHYDRATE TYPE ON FISH PERFORMANCE OF POLYCULTURE PONDS

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Carbon-nitrogen ratio plays an important role in enhancement of natural food and subsequent fish production in ponds while carbohydrate type (starch vs NSP level) has an impact on the nutrient digestibility of fishes. This study investigated the effect of feed carbon-nitrogen ratio (C:N) and carbohydrate type (CT), on the fish production in carp-tilapia polyculture ponds stocked with 36 fish of each three carps (rohu, catla and silver carp) and 12 tilapia. Three dietary C:N ratios (low 7.6; medium 9.5; high 12.8) and two carbohydrate types (High starch - Low NSP S_HNSP_L; Low starch – High NSP S_LNSP_H) were tested in a 3x2 factorial random design.

Survival was not affected by the experimental factors. Survival of all species was good, except for catla (21%). This was possibly due to high interspecies competition for feed among catla, tilapia and silver carp. Tilapia, though low in number, clearly monopolized the feed resource supplied, shown by its high growth rate (5.5 g.d^{-1}) averaged over all treatments (figure 1). This indicates that even low inclusion of tilapia can hamper carp production.

The dietary C:N ratio did not affect total fish production while carbohydrate type influenced fish production showing improved biomass gain with diets containing S_LNSP_H carbohydrate ($P<0.05$). Interaction effect of CT x species showed that growth rate of tilapia improved with S_LNSP_H containing diets (figure 1). Feed conversion ratio was significantly lower with S_LNSP_H diets (1.9) than with S_HNSP_L diets (2.5). However, the body protein content of all fish species was lower with S_LNSP_H diets (156 g.kg^{-1}) than with S_HNSP_L diets (161 g.kg^{-1}). Average fat content of all fish species increased with increasing C:N ratio from low (77 g.kg^{-1}) to medium (80 g.kg^{-1}) to high (90 g.kg^{-1}). Increasing the C:N ratio and NSP content in the diet considerably lowers the feed cost, while maintaining or increasing fish production in ponds.

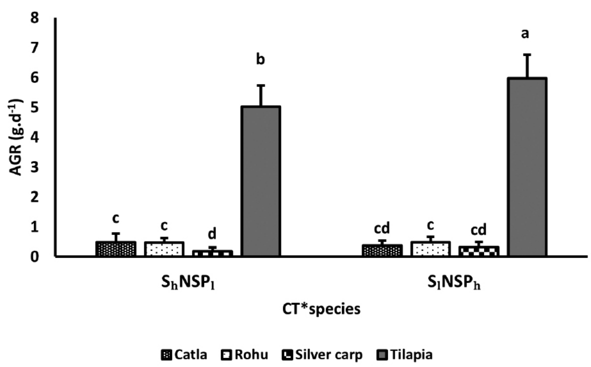

Figure 1 : effect of CT × species on growth rate of fishes

MAPPING THE GROWTH: AQUACULTURE PRODUCTIONS OF SALMONOIDS IN THE EUROPEAN UNION OVER 70 YEARS

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This study presents a comprehensive analysis of the evolution of aquaculture in the European Union and United Kingdom spanning from 1950 to 2020, focusing exclusively on salmonoids. Leveraging data from FAO databases, the analysis encompasses all the farmed species in 27 countries, including both European Union members and the United Kingdom. Through rigorous univariate and multivariate statistical techniques, including regression models, heatmaps, and Non-Metric Multidimensional Scaling (NMDS), key production trends, cultivated species, country-wise outputs, and economic impacts are investigated. The findings reveal significant growth, culminating in a peak production of about 400,000 tons in 2001. Subsequently, over the successive 20 years, production has entered a stationary phase.

The dominance of Atlantic salmon and rainbow is underscored, with production volumes revealing a concentration on these commercially viable species. Atlantic salmon and rainbow trout account in average for 98% of total annual production. Minor species productions are negligible. Notably, there is a discernible move from rainbow trout to Atlantic salmon which is gaining prominence. Visualization of specie succession with heatmaps aid in understanding production patterns. Economic analysis further emphasizes the profitability of intensive aquaculture species, particularly Atlantic salmon, while also shedding light on regional disparities in production and economic contributions. Western European countries, notably UK, emerge as major producers.

Overall, this analysis provides valuable insights into the dynamics of European aquaculture, focusing specifically on commercial salmonoids, and highlights key species, production trends, economic drivers, and the role of diversity in fostering resilience and growth.

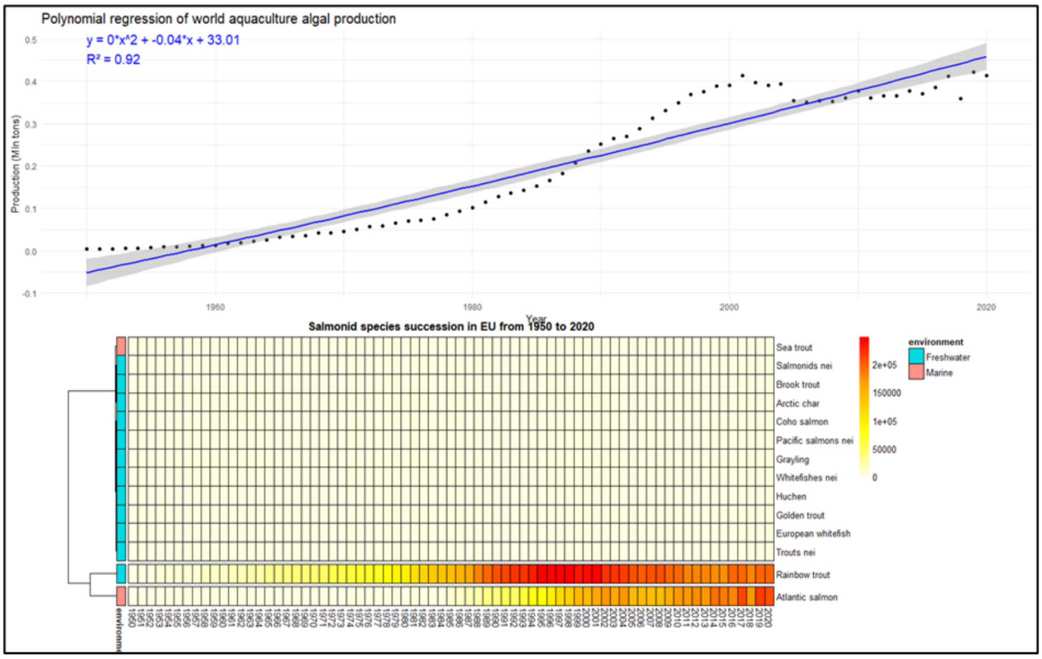

Figure 1: Aquaculture production and species succession of Salmonoids in EU from 1950-2020

EMBRACING COPEPS FOR SUSTAINABLE ACQUATIC FARMING IN GHANA

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Africa`s aquatic farming faces significant challenges with nutritional sustainability, larval survival, and the ecological impact of traditional practices. Nonetheless, copepods may offer a promising opportunity in addressing these issues. The objectives were to explore copepod and the potential benefits of embracing copepodology.

The study investigates copepods nutritional value, focusing on their role as natural and nutrient-rich live feeds. It assesses the ecological impact of incorporating copepodology into aquaculture systems, emphasizing the potential to maintain biodiversity and ecological balance by mimicking natural food webs. Finally, it measures whether copepodology offers a sustainable alternative to traditional wild-caught live feeds. A multidisciplinary approach, combining nutritional analysis, larval rearing experiments, and ecological assessment was used. Data was collected from farms, considering diversity in practice, target species, and environmental conditions.

The results suggest that through controlled cultivation, copepod-based diets contribute to enhanced larval survival, promoting faster growth and reducing mortality rates during critical early developmental stages. Copepod cultivation in controlled environments offers a sustainable alternative to traditional wild-caught live feeds, minimizing the risk of introducing pathogens to aquaculture facilities.

GROWTH, SURVIVAL, FEED CONVERSION RATES AND PROXIMATE COMPOSITION OF JUVENILE GILTHEAD SEABREAM *Sparus aurata* CULTURED IN BRACKISH WATER TANKS FED TWO DIFFERENT FEED TYPES

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Sparus aurata were cultured during a 4-month period in brackish water (salinity around 14 ppt) in a culture system comprising eight fiberglass tanks, each with a water surface of 500 L. Initial mean wet weight of fish in all tanks was 33.90 ± 1.9 g/fish. The eight tanks were randomly allocated for two experimental treatments (four tanks/treatment). In the first treatment, tanks were fed a locally produced tilapia pellet feed containing 36% crude protein produced by extruder machine. Whereas the other tanks fed by marine pellet feed containing 45 CP. Under the present experimental circumstances, *Sparus aurata* fed extruded sinking tilapia pellets (36% CP), showed good productive performance in brackish water (14 ppt).

The 4-month experiment was carried out at an interior farm located 6 km from the sea. Water was sourced from a groundwater connected to eight fiberglass tanks (four tanks/treatment) at 14 ppt. A daily water exchange of 5–10% of the tank volume was drained and refilled. Fish (25 fish/tank) were fed tilapia and marine pellets three times daily at 6% of their biomass. The mean final body weight for marine pellet treatment was significantly (P <0.05) higher (126.51g) than tilapia pellet treatments (89.20 g). Specific growth rate (SGR) of 0.81and 1.0 %/day, were obtained in sinking tilapia and marine pellets, respectively. Feed conversion ratios (FCR) for treatments with the extruded marine pellets was 1.7 g feed/g gain, which was significantly (P <0.05) better than treatments with tilapia pellets (2.50 g feed/g gain). Survival rates were 100% survive for all the groups fed with extruded sinking tilapia and marine pellets, respectively (Table 1).

Among the various biochemical parameters in the present study, there were significantly different (P<0.05) in treatments with tilapia and marine pellets for seabream cultured in brackish water except for ash, fiber (Table2).

Table 1. Growth, FCR and survival rates of two type of feeds used in the experiment. Values are means and standard deviation of four replicates. Means in a column with different superscripts are significantly different (P<0.05).

Type of feed	Initial weight (g)	Final weight (g)	FCR (g/g)	SGR	Survival rate (%)
Tilapia feed (36% CP)	33.90[a]± 0.36	89.20[b] ± 0.02	2.50[b]± 0.03	0.81[b]± 0.12	100.00[a]
Marine feed (45% CP)	33.52[a]± 0.52	126.51[a] ± 0.07	1.70[a]± 0.24	1.00[a]± 0.13	100.00[a]

Table 2. Chemical compositions of seabream fillets used in the experiment. Means in a rows with different superscripts are significantly different (P<0.05).

Feed	Tilapia feed	Marine feed
Dry matter (%)	23.57[a]	24.26[a]
Protein (%)	14.62[b]	20..45[a]
Lipid (%)	1.29[b]	8.16[a]
Ash (%)	1.12[a]	1.41[a]
Fiber (%)	0.48[a]	0.70[a]

HISTOPATHOLOGY DIAGNOSIS OF ORGANS OF MULLET FISH FROM LAC NOKOUE(REPUBLIC OF BENIN) AND LAGOS LAGOON (NIGERIA) INFECTED BY METAZOAN PARASITES

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Histopathology diagnosis, revealed histopathological changes induced by myxobolus and copepods parasites on the intestines and gills of economically important fish (*Mugil cephalus* and *Liza falcipinnis)* from Lagos lagoon and Lac Nokoue in Nigeria and Republic of Benin respectively. Histopathological changes revealed that the nature of damage observed in the gills and intestines of both *M. cephalus* and *L. falcipinnis* remained the same. The histological sections, showed clusters of myxobolus spores in direct contact with the host tissues, resulted in the destruction of intestinal wall and also gradually erode the gill filaments resulting in the appearance of a cavity. The merger of two or three cavity caused by liquefaction of cartilage leads to the destruction of the gill fillaments. Histopathological observation revealed serious damage and destruction of lamellae and gill filaments due to attachment and feeding of copepods. The resultant hypertrophy of the underlying epithelial reducing the surface area for effective respiration, could lead to suffocation, particularly at high temperature. The histopathological changes induced by myxobolus and copepods parasites will eventually lead to reduced growth, low productivity and mortality resulting in economic loss.

POTENTIAL OF INTEGRATED MULTITROPHIC AQUACULTURE TO MAKE FRESHWATER PRAWN FARMING SUSTAINABLE IN BANGLADESH

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Integrated aquaculture boosts productivity, minimizes environmental impact, and bolsters global food security. In southwest Bangladesh, the common practices of integrated rice-prawn farming are economically profitable, viable, and sustainable, offering farmers year-round cash flow compared to conventional aquaculture or agriculture. However, the growing concern is that prawn farming has been criticized to release about 1.0 MT CO_2-equivalents/year, corresponding to 18.8 kg CO_2e/MT prawn. Integrated Multi-Trophic Aquaculture (IMTA) could provide a potential solution to reduce greenhouse gas (GHG) emissions in integrated rice-prawn farms in Bangladesh. In a typical IMTA system, prawns are cultivated by allocation of feed, while extractive inorganic, such as aquatic vegetation or seaweeds, use the inorganic waste, and animals, e.g., mussels and oysters, utilize the organic waste materials.

Extractive organisms, and aquatic plants in prawn farms, can sequester blue carbon and reduce GHG emissions, mitigating climate change impacts on the environment. Besides prawns, mollusks, and plants provide sustainable food and feed options, minimize food crises, and enhance nutritional support. Aquatic plants can supplement expensive protein sources to minimize the cost of meat, milk, and other animal products. Studies investigating the incorporation of indigenous species into prawn farms are necessary, to minimize biosecurity risk, and to protect and maintain native biodiversity and ecological processes. To popularize IMTA practices among prawn farmers, some initiatives like action research with farmers, training facilities, technical assistance, and credit support are required. The research and development organizations should collectively undertake essential steps for action research and development in conjunction with IMTA within the prawn farming region of Bangladesh, aiming to establish sustainability of this export-oriented farming system.

SUPPLEMENTATION OF SINGLE CELL PROTEIN, SEA BEANS MEAL AND A COMBINATION OF ALTERNATIVE PROTEIN SOURCES TO REPLACE FISHMEAL PROTEIN IN THE DIETS OF JUVENILE BLACK SEA BASS *Centropristis striata*

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The use of ocean-caught fish to produce fishmeal as a source of protein for aquaculture feed threatens ocean-caught fish stocks and biodiversity and creates economic uncertainty for aquaculture businesses. Therefore, three feeding trials were conducted to evaluate the replacement of menhaden fishmeal protein (MFP) by single cell protein (SCP) *Methylorubrum sp* meal (Trial 1), *Salicornia virginica* (sea beans), a halophyte plants meal (Trial 2) and a combination of non-conventional and conventional protein sources (Trial 3) in the diets of juvenile black sea bass *Centropristis striata*. In trial 1, 2 and 3, a total of 21 iso-nitrogenous (48% crude protein) and isolipidic (13%) test diets (eight, seven and six, respectively) were formulated and prepared replacing graded levels of MFP by supplementing SCP meal, *Salicornia* meal and a combined alternative protein sources, respectively. Fifteen juvenile fish were stocked in each 75-L tank in a recirculating aquaculture system and each test diet was fed twice a day to triplicate groups of juvenile fish for 8 to 10 weeks.

The diets proximate, amino acids and fatty acid profiles and growth performance, feed utilization and body biochemical compositions of fish were evaluated after the feeding trials. In trial 1 and 2, supplementing SCP and *Salicornia* meal up to 30% and 25%, respectively did not affect on growth performance as compared to the control diets. In trial 3, supplementing 10% SCP with a combination of conventional and non-conventional alternative protein sources showed better performance, in terms of growth and body composition as compared to other test diets. Survival was higher than 90% among the treatments with no significant differences for all three trials. Results to date suggest that black sea bass juveniles are able to utilize high levels of SCP and *Salicornia* meal without affecting fish growth. With the supplement of SCP, a combination of alternative protein sources could be used to formulate an eco-aquafeed for the juvenile black sea bass reducing the amount of fishmeal protein in the diets.

BLACK SEA BASS IN A RECIRCULATING AQUACULTURE SYSTEM: SENSITIVITY ANALYSES OF FARM INPUT COSTS AND GENETICALLY INDUCED GROWTH INCREASES AND ALTERATION OF PROTEIN SOURCES IN AQUAFEEDS

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Based on the operation of University of North Carolina Wilmington's (UNCW) pilot marine fish hatchery and recirculation aquaculture system (RAS) growout facility a spreadsheet production economics analysis was conducted for a hypothetical commercial scale RAS growout facility for black sea bass, *Centropristis striata,* in coastal North Carolina, and profitability of alternative production scenarios were explored via sensitivity analysis.

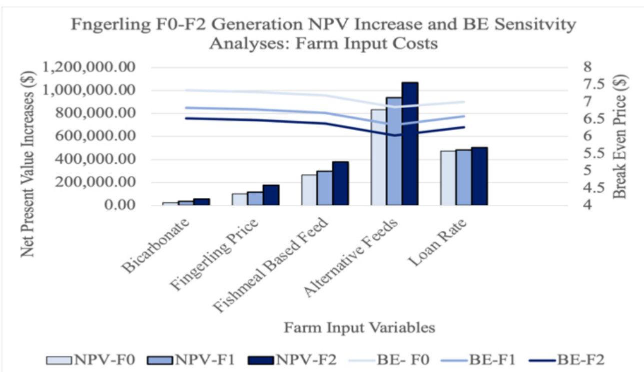
Financial performance was measured by assessing farm input costs (e.g. labor, feed, energy), duration of production cycle, time to first harvest, farm gate revenues and returns to owner per production cycle, break-even (BE) prices, discounted payback period, modified internal rate of return (MIRR), and net present value (NPV). A base biological growth model was developed through linear regression analysis using empirical growth data from black sea bass raised in RAS at UNCW and at North Carolina State University. Alternative models based on a 12.5% increase per generation in weight-at-age over two generations that might be realized through selective breeding were also investigated.

In comparison, with selective breeding the benchmark of 75% of the cohort at premium marketable size or larger is reached at 23.9 months for F0 and at 22.8 mos for the F1 generation and finally 21.7 mos for the F2 generation, at BE prices of \$8.228, \$7.376 and \$7.084, respectively. When F2 generations were fed plant-based aquafeeds, BE drops to \$6.842. The results of this study show that black sea bass can be grown using RAS methods at commercial scale and at competitive prices. Furthermore, implementing genetic selection for improved growth concurrently with plant-based aquafeeds has the potential to drastically improve the economic performance of a black sea bass RAS facility.

Table 1. Commercial base case cumulative net present value (CNPV), Modified Internal Rate of Return (MIRR), Discounted Payback Period (DPP), and breakeven price (BE) for base case generation (F0) and two generations of selective breeding (F1 and F2).

Generation	NPV	MIRR	DPP	BE
F0	$432,141.95	7.556	12	$8.236
F1	$2,253,800.08	11.664	7	$7.376
F2	$3,053,031.52	12.862	6	$7.084

Figure 1. Each farm input cost was varied by 5 and 10 % above and below the base price while the loan rates were varied by 2, 4 and 6% above and 2% below the base loan rates. The net present value (left axis) and break-even cost (right axis) are shown for F0-F2 generations.



DESIGN AND OPERATION OF OCEAN AQUACULTURE INSTALLATIONS

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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. As a result of the current situation fish farming is growing out of sheltered, near-shore areas and moved to locations more exposed and further offshore. Several innovative solutions are being tried out in offshore fish farming in the process of industrialization.

MAIN APPROACH

The numerous concepts 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. All these concepts may be operated with permanent manning or a combination of remote operation and daily visits. A more specific description of the main solutions applied in the development of ocean farming:

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

Concepts and solutions listed have several advantages and weaknesses that must be considered and evaluated. These various solution’s ability to perform depends on a long list of functional aspects. The essential parameters to be taken into consideration assessing performance are considered to be: Oxygen level in sea water, crowding and live transfer of fish, dead fish handling and ensilage processing, logistics of supplies and goods going both on and off the fish farming installation and personnel safety.

DIGITAL SOLUTIONS IN OCEAN AQUACULTURE - POSSIBILITIES AND CHALLENGES

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INTRODUCTION

Smart aqua operations are developing to be a common solution in modern aqua-culture installations. Real time monitoring of fish condition combined with surveying the integrity of fish farm itself are considered a competitive advantage. The aquaculture industry is in the middle of a major transition as vessels and fish farms are transformed into sensor hubs, generating data, and linking in an expanding interconnected web.

MAIN APPROACH

Essential elements to consider for a strategy supporting the smart transformation may be split into four main categories: 1) Management, recourses and capabilities focusing on utilizing/developing potential in organization. 2) Integrated systems, tools and connectivity addressing primarily IT infrastructure and data management. 3) Technology and efficient solutions dealing with automation, autonomy, and remote operations. And lastly 4) Energy efficient- and enhanced performance embracing energy efficiency together with sustainability enhancing concepts. Digital asset management is a term often used in this context where inspection as well as operational decisions are based on continuous data streams supplied by sensors on board.

Digitally driven condition-based maintenance result in improved reliability by continuously assessing the condition of fish farming installation together with the state of the fish in it. The objective is to identify potential issues before they become major problems and take proactive measures to prevent failures and unplanned downtime. Digital asset management will help improve efficiency and reduce operational costs. For example, by identifying equipment that needs maintenance or replacement, fish farmers can avoid costly breakdowns and reduce downtime.

The digitally driven operational monitoring is based on the process of capturing information, analyse data collected, learn plus synthesis, and finally act accordingly.

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

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

CONCLUSION

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

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

OVERALL RISK PERSPECTIVE FOR OCEAN AQUACULTURE

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INTRODUCTION

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

MAIN APPROACH

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

2.1 Asset integrity

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

2.2 Personnel safety

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

2.3 Fish welfare

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

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

2.4 Ocean health

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

(Continued on next page)

2.5 Fish control

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

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

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

CONCLUSION

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

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

MUELLER’S PEARLSIDE *Maurolicus muelleri* AS ALTERNATIVE PROTEIN RESOURCE IN FEED FOR ATLANTIC SALMON *Salmo salar*

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Mesopelagic fish represent a huge biomass that has been explored as a sustainable new source of marine protein and lipid in several large interdisciplinary projects in terms of resource mapping, capture technology, stabilization, and processing. In this project, fish meal and silage were produced in pilot scale from fresh frozen Mueller’s pearlside at Nofima’s Feed Technology Centre in Bergen, and the nutritional potential evaluated in a feeding trial with Atlantic salmon. Five diets were produced where the control diet was a standard formulated salmon feed that contained 30 % fish meal produced from blue whiting *Micromesistius poutassou*. The mesopelagic fish meal protein replaced respectively 50 % (Fm 50) and 100 % (Fm 100) of the protein in the control meal, while the mesopelagic silage replaced 10 % (FPC 10) and 20 % (FPC 20) of the protein in the control meal.

An 11-week feeding trial was carried out with salmon (initial weight: 93.6 ± 0.5 g), showing quadrupled fish body weight (final weight: 382.5 ± 9.9 g) and efficient growth in all dietary groups (average SGR: 1.85 ± 0.03 %). No significant differences in feed intake, growth or feed conversion were found in the initial 6 weeks of feeding, and in the total feeding period (ns). In the first feeding period, fish fed mesopelagic Fm tended to improve the dietary feed intake slightly (3 - 6 %) as compared to fish fed the control meal (ns). Fish fed mesopelagic Fm showed significant reductions in protein digestibility: 91.2 % (control), 89.3 % (Fm 50 %) and 87.7 % (Fm 100 %), consistent with the difference in true protein digestibility of 90.6 % (control Fm) and 84.6 % (mesopelagic Fm) measured in mink. The protein retention efficiency was high in all dietary groups, and no differences in PER, PPV or BV was detected (ns), possibly due to the initial increased feed intake in fish fed mesopelagic fish meal. Lipid digestibility was high in all dietary groups (ADC lipid: 96.0 ± 0.9 %), and no significant differences in lipid digestibility or liver fatty acid composition was found (ns). Mesopelagic ingredients (Fm, FPC) slightly increased liver lipid (ns), and significantly increased HSI and DOP in fish fed Fm 100 as compared to fish fed the control diet ($P < 0.05$). No significant dietary differences in plasma enzymes (ASAT, ALAT), lipid classes (TG, Pl, total Chol, HDL Chol), immune parameters (lysozyme), and stress markers (FRAP, TAC) were detected (ns). The slightly higher plasma FRAP levels found in fish fed the mesopelagic Fm ($P = 0.09$) was consistent with higher levels of natural tocopherols (Naturnox 15) added to stabilize the mesopelagic Fm. The effects of feed composition on intestinal tissue segments showed that mesopelagic Fm significantly reduced the signs of enterocyte steatosis in the mid-intestinal epithelium of control fish ($P < 0.05$), a condition associated with cholin deficiency, although other explanations need to be considered.

Additional analyses to study dietary impacts on the digestive functions will be presented.

In conclusion, Fm and FPC produced from M. pearlside demonstrated efficient growth, feed intake and feed conversion despite lower protein digestibility, showed high lipid digestibility and significantly improved transport of lipids from the mid-intestinal epithelium into the body as compared to control fish, thereby reducing the signs of enterocyte steatosis in A. salmon.

CALANUS *C. finmarchicus* HYDROLYSATE AND SILAGE IMPROVES FEED INTAKE, GROWTH AND HEALTH OF ATLANTIC SALMON *Salmo salar* IN THE FIRST PERIOD AFTER SEAWATER TRANSFER

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Calanus (*Calanus finmarchicus*) is a small copepodite with large lipid storage that periodically constitutes 90% of the standing stock of zooplankton in the Norwegian Sea and the Barents Sea. The PUFA rich oil has been commercially utilised for many years and has been explored as a source of marine n-3 fatty acids in feed for Atlantic salmon. In this study, calanus hydrolysate (FPH) and silage (FPC) was produced in pilot scale by Nofima and evaluated as a feed intake and growth promoter in early transfer feed for Atlantic salmon. A total of 6 diets were produced where the control diet was a standard formulated salmon diet. The pilot produced calanus ingredients were added at two levels either by top coating (FPH 2.5 %, FPH 5.0 %), or into the diets (FPC 2.5 %, FPH 5.0 %). In addition, a commercial calanus FPH produced by Zooca AS was added by top-coating (2.5 %).

A 12-week feeding trial was carried out with salmon (84 g), starting out from the first day of seawater transfer. All calanus ingredients (FPH, FPC) significantly increased feed intake and growth of salmon as compared to fish fed the control diet in the 6 first weeks of feeding ($P < 0.05$), while no differences with respect to the inclusion levels were found. Compensatory growth was observed in fish fed the control diet in the next 6 weeks of feeding and resulted in no significant differences in growth after 12 weeks of feeding (mean SGR = 1.71 ± 0.04 %). Significant increased total feed intake and FCR was found only in fish fed FPH 2.5 % ($P < 0.05$) for the complete 12 weeks feeding period ($P < 0.05$). Liver lipid and vitamin E did not show dietary differences (ns), while liver malondialdehyd (MDA) was significantly reduced in fish fed FPH 5.0 % and FPC 5.0 % ($P < 0.05$), indicative of reduced oxidative stress in fish. Transcriptional profiling of the skin showed that the response to FPH was stronger than to FPC, while the correlation of the expression profiles was high (Pearsson r = 0.69). Transcriptional responses to FPH were relatively strong in the skin and the profile of downregulated genes resembled results in previous studies associated with changes often present in wound healing study.

In conclusion, the increased feed intake and growth observed in fish fed all calanus ingredients are consistent with previous reported feed attractant and growth properties of hydrolysed fish raw materials. Reduced levels of liver MDA suggests strong antioxidant properties of calanus FPH and FPC, as also confirmed in laboratory studies. The transcriptional responses to FPH were relatively strong in skin and many of the downregulated genes showed similar profiles as in previous skin wound healing studies. The functional consequences of the transcriptional effects are unclear, although the observed changes could be of significance for transcription or regulation of the cellular composition of the skin. In conclusion, calanus FPH and FPC stimulated feed intake and growth, reduced markers of oxidative stress, and tended to improve skin health of salmon in the first challenging period after seawater transfer.

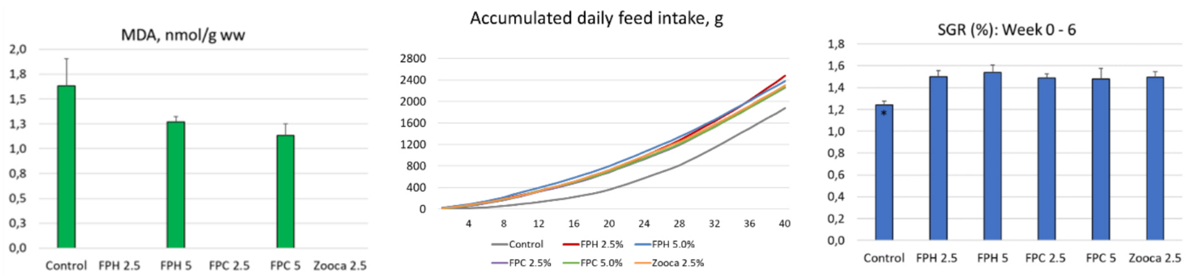

Fig.1. Accumulated feed intake (g), growth (SGR), and liver MDA in fish fed a control diet, calanus hydrolysate (FPH 2.5%, FPH 5%), calanus silage (FPC 2.5%, FPC 5%) and a commercial calanus hydrolysate (Zooca 2.5%)

IDENTIFICATION OF SUITABLE SPECIES COMBINATION FOR POLYCULTURE OF PABDA (*Ompok pabda*) IN CAGE CULTURE SYSTEM

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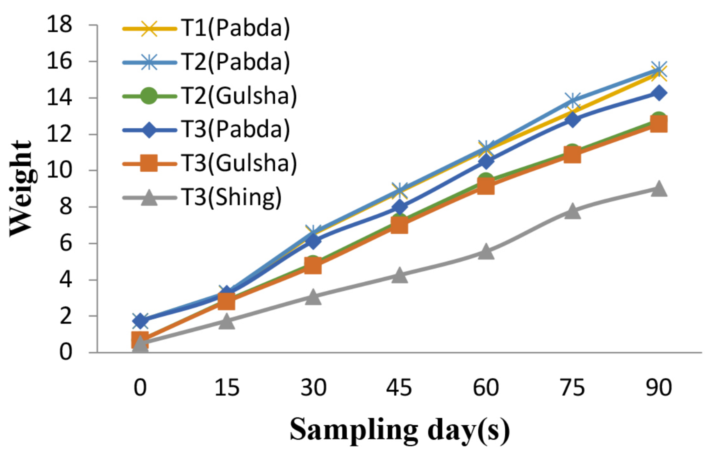
Bangladesh is one of the world's leading fish-producing country in the world. Cage aquaculture has grown rapidly during the past decades in different countries to mitigate the worldwide global demand for aquatic products. Cage culture has been successfully practiced in the open water body of Bangladesh. Pabda (*Ompok pabda)* is an indigenous commercially important fish that is highly demanding for cage culture.

The study was conducted over 90 days from July to October 2022 in an earthen pond of Patuakhali Science and Technology University to assess the species suitability for polyculture of Pabda (*Ompok pabda*) in a cage culture system where Gulsha (*Mystus cavasius*) and Stinging catfish (*Heteropnuestes fossilis*) practiced as co-species with Pabda. Stinging catfish is locally known as shing. The experiment was designed with three treatments namely T_1 (only Pabda- 100%), T_2 (Pabda -50% and Gulsha-50%), and T3 (Pabda-40%, Gulsha-30% and Stinging catfish -30%) respectively each having three replications. The stocking density was 300/m^3. Firstly they are fed with starter powder feed which was then replaced with floating feed containing 30% protein supplied twice a day. Water quality parameters were recorded every fifteen-day interval throughout the culture period.

Water quality parameters were within the suitable range for fish culture. At the end of the culture period higher weight gain of Pabda (13.81g) was observed in T_2 followed by (13.59g) in T_1 and (12.53g) in T_3 and Gulsha showed higher weight gain (12.08g) in T_2 followed by (11.87g) in T_3, lowest weight gain observed for Stinging catfish (8.54g) in T_3. The survival rate of Pabda ranges from 73.53 to 75.52 % and Gulsha from 95.78 to 95.89 %. The lowest FCR value was obtained from T_2 (2.42) followed by T_1 (2.5) and T_3 (3.0). Higher production of fish (kg/m^3) was recorded in T_2 (3.59 kg) followed by T_1 (3.48 kg) and T_3 (2.86 kg). However, a short economic analysis of the present experiment revealed that the maximum profit obtained from T_2 followed by T_3 due to the combination of three different species commanding a higher market price and than T_1. The BCR was higher in T_2 (1.65), followed by T_3 (1.36) and T_1 (1.30). The result of the study revealed that the culture of Pabda with Gulsha in the cage is more suitable than with Gulsha and Stinging catfish (both) because the growth performance and survivability of Stinging catfish were very poor. Overall Pabda in T_1 showed averagely good performance but in terms of profitability Pabda with other species (Poly culture) was more profitable than single species (monoculture) and these studies recommended Gulsha (Mystus *cavasius*) to culture with Pabda in terms of production and profitability. So, it is not recommended to culture Shing with Pabda in the cage culture system.



EFFECTS OF *Pediococcus acidilactici, Saccharomyces cerevisiae* AND YANG ON GROWTH AND THE INTESTINAL MICROBIOTA OF THE COMMON CARP (*Cyprinus carpio* L.)

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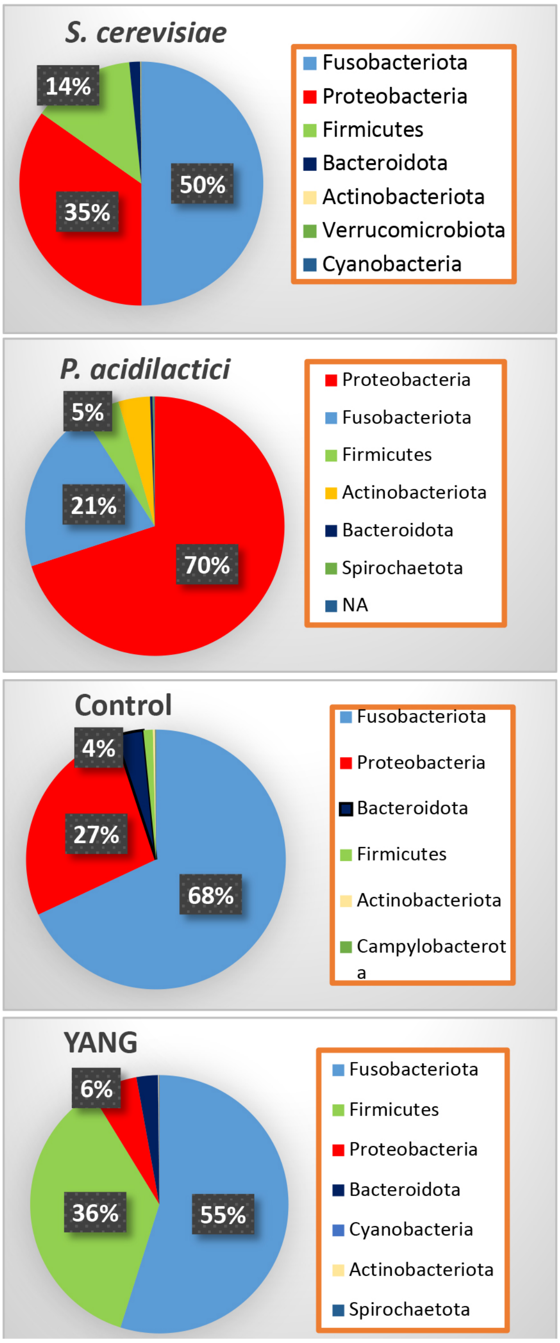
Limited studies discussed the impact of probiotics and prebiotics on fish microbiome. This research aimed to examine the effects of different feed additives on the intestinal microbiota of the common carp (*Cyprinus carpio*).

Three different types of feed additives including live lactic acid bacterium *Pediococcus acidilactici*, *Saccharomyces cerevisiae* and YANG prebiotic were tested at the concentration of 1 g/kg. Fish with average body weight of 932±163 g were fed with supplemented diet of each type for 41 days in a recirculation system containing 12 units (size=1000 liter each unit, 8 fish per unit). Water temperature, pH, TDS, dissolved oxygen, NO^{-}_{2}, NO^{-3}, and NH^{+4} concentrations were maintained to be at the optimum levels in all fish tanks. At the end of the experiment, fish feces samples were collected and the bacterial communities structure found in the intestines of common carp were analyzed through a metagenomic approach using 16S rRNA sequencing.

The results of the metagenomic analysis of carp fed with a diet supplemented with YANG showed 11 different groups of bacterial phyla detected with the top three phyla, Fusobacteria, Firmicutes, and Proteobacteria. In contrast, fecal samples from carp fed with *P. acidilactici* recorded 10 phyla, the top three phyla were Proteobacteria, Fusobacteria, and Firmicutes. Fish fed with *S. cerevisiae* recorded 9 phyla, the top three phyla were Fusobacteria, Proteobacteria, and Firmicutes. However, samples from carp fed with the control diet recorded 14 phyla, the top three phyla were Fusobacteriota, Proteobacteria, and Bacteroidota.



EFFECTS OF DIETS CONTAINING DRY EXTRACTS OF *Achillea millefolium, Mentha piperita AND Echinacea purpurea* ON GROWTH, HEMATOLOGICAL AND IMMUNOLOGICAL INDICES OF JUVENILE COMMON CARP (*Cyprinus carpio*)

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In this study, the effects of three herbal dry extracts *(Achillea millefolium, Mentha piperita* and *Echinacea purpurea)* were investigated on growth, hematological and immunological indices in juvenile common carp (*Cyprinus carpio*). Four hundred juvenile fish with initial weight of 14.30 ± 0.77g were studied in 10 treatment groups (9 treatment groups & a control) with four replicates for 60 days. Three levels (0.1, 0.5 and 1%) of dry extracts of each herb were prepared according to standard method and added to the commercial common carp feed. At the end of period twelve fish collected out of each group and the parameters were measured.

In order to the results, weight gain, specific growth rate (SGR) and complement C4 were not affected by dietary treatments ($P>0.05$). Red blood cell (RBC) counts in 0.5 and 1%-diet groups as well as Hemoglobin in three levels of all herbs was increased ($P\leq0.05$). Hematocrit in 0.5%, 1%-diet *M. piperita* and *E. purpurea* groups was shown significant increases ($P\leq0.05$).

Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in all groups except 0.1% *M. piperita* group and 0.5% *E. purpurea* were increased compare with control group ($P\leq0.05$). MCHC in 0.5% *E. purpurea* and 0.1 and 1% *M. piperita* groups showed the highest values. Levels of 0.5% *M. piperita* and 1% *E. purpurea* and *A. millefolium* make significantly increases in total leukocytes and neutrophils ($P\leq0.05$).

Significantly increases of lymphocytes and decrease of monocytes were observed in levels of 0.5% *E. purpurea* and 1% level of all herbs groups ($P\leq0.05$). Increased levels of immunoglobulin compared to control were significant only in 1% level of all herbs ($P\leq0.05$). Complement C3 was also increased 1% of *A. millefolium* and *M. piperita* groups compared to the control ($P\leq0.05$). All levels of *A. millefolium* and *M. piperita* and 1% *E. purpurea* groups caused a significant increase in lysozyme concentration compare with the control ($P\leq0.05$). The results indicated all three herb extracts in diet can improve immune responses and hematological parameters in common carp. Comparing these extracts, the *M. piperita* extract with a lower concentration is more efficient.

LIGHT, SHADING AND SUBMERGENCE AS METHODS TO CONTROL FARMED COD SEXUAL MATURATION

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Sexual maturation in fish is a major challenge in aquaculture that can negatively impact fish growth and health. In farmed cod (*Gadus morhua*), early maturation can result in discharge of fertilized eggs in the sea cages from spawning individuals and therefore to possible genetic interactions with wild cod. To avoid any additional pressure on already depleted local coastal cod stocks, a rapid and easily applied solution to control early maturation in this species is needed. Indeed, Norwegian cod aquaculture is currently developing, and its future growth depends on the capacity to solve these issues. While continuous light (LL) treatment is known to prevent or delay maturation in cod in indoor tanks or in sea cages, respectively, it does not seem to completely arrest maturation in the cages. This because the reproductive cycle in cod is controlled by photoperiod, and the suppression of melatonin secretion is tied to previously experienced daytime intensity limits. Therefore, reducing the relative intensity difference between day and night will enhance the effectiveness of additional artificial light in suppressing sexual maturation.

To develop a better protocol for controlling sexual maturation in farmed cod by light treatment we combined either net/tarpaulin shading at the surface with a strong LL (2500W) or shading using submerged sea cages to 30 m depth with LL of 600W. We tested triplicate cages of shaded surface or submerged cages with natural light (NL) or LL. For each group, 1200 cod of 200 g, reared at LL over summer, were placed in the cages in September and sampled bimonthly for growth, blood, tissue collection and ultrasound to analyze fish sexual maturation at macroscopic, microscopic, hormonal and gene expression levels. In addition, the presence of parasites was examined to document any potential effects of different environments.

Fish growth did not significantly differ between environments during the initial phase of the experiment, although individuals in submerged cages tended to be larger. However, sexual maturation was observed in individuals of all NL cages, whereas very few individuals initiated maturation under LL conditions, and none in submerged LL cages. The proportion of maturing fish varied between the sexes, with males exhibiting higher rates compared to females. The treatments influenced the presence of parasites, specifically *Caligus*, with submerged cages exhibiting a higher prevalence of parasites, particularly under LL conditions. Overall, the obtained results are promising, and we anticipate that these revised methods can promptly benefit the aquaculture industry. With standardized and optimized technology for light control and shading, aligned with biological principles, we can potentially effectively prevent fish from becoming sexually mature before reaching slaughter size.

UNLOCKING POTENTIAL BIOACTIVE PEPTIDES FROM THE COMMON OCTOPUS *Octopus vulgaris* IN RESPONSE TO A BACTERIAL CHALLENGE USING AN INTEGRATIVE PROTEOMICS AND TRANSCRIPTOMICS APPROACH

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The search for novel preventive and therapeutic strategies is paramount in response to the pressing challenge of limiting the spread of microbial infections in aquaculture. Synthetic antimicrobial agents have been widely employed but face the significant obstacle of bacterial resistance, posing threats to human health. In that sense, marine species represent a significant portion of global biodiversity and produce compounds with unique antibacterial properties, thus constituting a promising source for discovering natural bioactive compounds.

Within the Marie Skłodowska-Curie BIOPTAL project, we have focused on the cephalopod *Octopus vulgaris*, a potential reservoir of underexplored peptide diversity. Given its relevance for aquaculture and the adaptability of octopi to environmental conditions, investigating its potential bioactive compounds under challenging circumstances holds significant promise for developing novel therapeutic solutions. To this end, ten adult specimens (Mediterranean Sea) were divided into control and challenged groups for an *in vivo* bacterial challenge with *Vibrio parahaemolyticus*, a common aquaculture bacterial pathogen. Then, skin samples were collected, and transcriptomics and proteomics analyses were conducted using state-of-the-art methodologies and custom pipelines to address the complexity of these non-model organism samples (Figure 1).

By analyzing their skin proteomes and transcriptomes, we identified several differentially expressed genes/proteins potentially related to *O. vulgaris* defense mechanisms that translated into a list of peptide candidates with potential antimicrobial activities against several targets, namely Gram-positive and/or Gram-negative bacteria, fungi, viruses and cancer cells (AMPpredictor, *in-silico* machine learning pre-screening).

Overall, this integrative multi-omics approach offers insights into the octopus's defense mechanisms and its molecular adaptation to environmental stressors. This study contributes to a deeper understanding of the common octopus's adaptive strategies, particularly in combating multi-antibiotic resistant bacteria prevalent in aquaculture, offering an interesting window for the bioprospection of antimicrobial peptides. Specifically, the insights gained may help optimize culture conditions and disease prevention strategies for aquaculture-relevant species.

APPLICATIONS OF ANTIMICROBIAL PEPTIDES FROM AQUATIC INVERTEBRATES IN AQUACULTURE

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The rise of semi-intensive and intensive aquaculture has led to increased stocking densities, resulting in elevated stress levels and subsequent disease outbreaks. Initially, traditional antibiotics were used to combat these diseases. However, their overuse led to the emergence of multi-resistant bacteria, posing a significant threat to public health. To address this challenge, it is imperative to explore alternatives such as antimicrobial peptides (AMPs), which have shown a remarkable ability to target multiple sites in pathogens, reducing the risk of resistance development. Aquatic invertebrates rely on their innate immune system, with AMPs playing a crucial role in defending against pathogens. In aquatic environments, invertebrates are the primary source of AMPs. This study aims to investigate AMPs derived from aquatic invertebrates and their effectiveness against both aquaculture and human pathogens, including bacteria, fungi, and viruses. Additionally, it explores the potential use of these AMPs in the aquaculture industry.

To assess the diversity of AMPs obtained from aquatic invertebrates, an extensive review of public literature and databases was conducted. All invertebrates classified as having at least one phase of their life cycle in an aquatic environment, according to the World Register of Marine Species, were considered. Literature data retrieval involved using the taxonomy nomenclature in conjunction with specific search terms such as “antimicrobial,” “peptide,” and “activity”. Database information was sourced from various platforms, including NCBI’s GenBank, UniProt, Alphafold, and the Protein Data Bank. ProtParam provided physical and chemical parameters, Colabfold predicted protein/peptide structures for AMPs lacking this data, while ChimeraX offered protein/peptide molecular visualization.

AMPs from ten aquatic invertebrate phyla were gathered, originating from approximately 100 species across about 50 AMP families. These peptides vary in size, molecular weight, isoelectric points, and Grand Average of Hydropathicity values. Some AMPs exhibited antimicrobial activity against a wide range of important human and aquaculture pathogens. However, many of these AMPs lacked structural and functional information, which was further elucidated in the present study.

Despite increased interest in AMP research, there is a lack of data from *in-silico* analyses to *in-vivo* experiments. Information availability varies among different phyla and even within the same phylum, with important groups such as Porifera notably underrepresented. While bioinformatic tools like Alphafold2 advance AMP prediction, AMP isolation remains essential for understanding their structure and function. The application of AMPs in the aquaculture sector faces challenges, including stability at different pH levels, potential hemolytic side effects, and high production costs. However, their antimicrobial and immunostimulant properties offer a promising antibiotic alternative with sector-changing potential.

PROTEOMICS INSIGHTS INTO MOLECULAR RESPONSES IN THE SKIN OF THE COMMON OCTOPUS *Octopus vulgaris* UPON BACTERIAL CHALLENGE

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Aquaculture's importance within the global food industry offers immense potential driven by the escalating demand for enhanced production. In that sense, the common octopus (*Octopus vulgaris*) has emerged as a species of significant interest thanks to its short life cycle and remarkable reproductive capacity. However, the aquaculture of this cephalopod faces a noteworthy challenge in the form of bacterial infections due to the developed resistance to conventional antibiotics, resulting in substantial economic losses and endangering the welfare of these animals.

In invertebrates like cephalopods, stress triggers an innate immune response, encompassing cellular and humoral reactions, owing to the absence of an adaptive immune system. To improve animal welfare in aquaculture and delve into the immune defense mechanisms of cephalopods, investigating defense mechanisms in the skin, which features a protective mucus layer in the epidermis against pathogens, becomes imperative.

Thus, assessing defense responses upon bacterial challenge, particularly against multidrug-resistant pathogens such as *Vibrio parahaemolyticus*, holds immense potential, namely, to uncover forthcoming antimicrobial peptides (AMPs). Therefore, in the research developed in the Marie Skłodowska-Curie BIOPTAL project, we hypothesize that the immune system of the common octopus activates in response to pathogenic bacteria, potentially involving intricate molecular mechanisms, including the differential expression of several protein-coding genes and protein synthesis (Figure 1).

To address this hypothesis, ten octopi were divided into control and bacterial-challenged groups. Skin samples from both experimental groups were analyzed at both transcriptomics (RNA-seq) and proteomics (LC-MS/MS) levels. This integrative high-throughput approach promises invaluable insights into specific peptides with potential antimicrobial activity. Such insights not only contribute to our understanding of immune regulatory mechanisms in this species but also offer knowledge into the complex interplay between host and pathogen, paving the way for the discovery of novel strategies to strengthen disease resistance in aquaculture production.

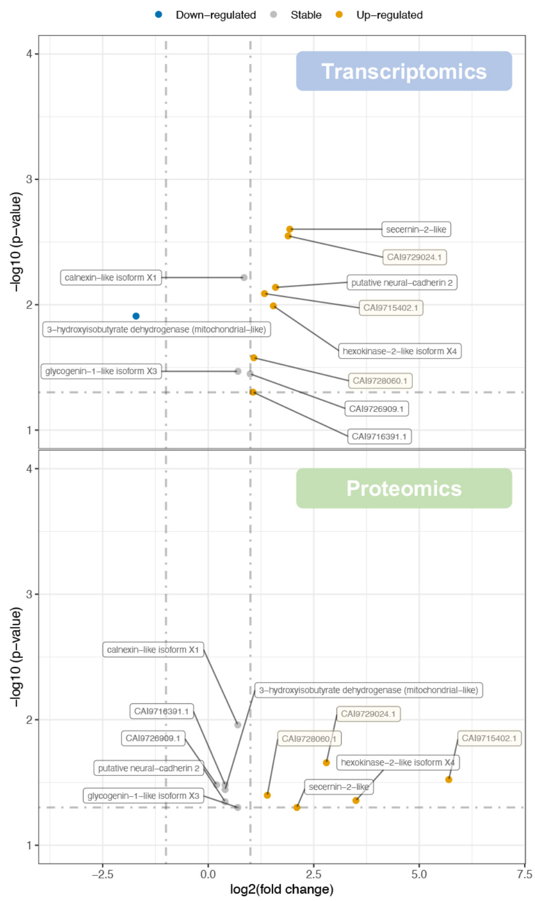

FIGURE 1. AMPs screening of *O. vulgaris* skin after a bacterial challenge.

EFFECT OF MINERAL-BOUND PREBIOTIC-PHYTOGENIC BLEND AGAINST ENDOTOXIN ON SURVIVABILITY AND IMMUNE RESPONSES OF *Liptopenaneus vannamei*

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Endotoxin, a lipopolysaccharide component of gram-negative bacteria's outer cell wall membrane, is a common denominator in many animal diseases. Its significant impact includes the suppression of immunity in various animals, including those in aquaculture. Despite reports of aquatic species' resilience to endotoxin, continual exposure to Gram-negative bacteria, prevalent in aquatic ecosystems, can compromise immune function and growth stimulation.

To address the challenges posed by endotoxin, a 45-day feeding trial was conducted in 100 L tanks focusing on *Liptopenaneus vannamei*. The study aimed to explore the potential benefits of a mineral-bound prebiotic-phytogenic blend (MBPB). Feeds containing three different levels of MBPB (0, 100, and 1000 g/ton feed) were tested alongside three doses of orally administered endotoxin (0, 10 ppm, and 100 ppm).

Twenty-seven shrimp tanks were randomly assigned to each diet, stocked at a density of 20 shrimp per tank (with an average body weight of 3-5 g per shrimp), and maintained in 25-30 ppt salinity. Feeding rates were adjusted based on body weight throughout the trial period. Survival rates were recorded at the trial's conclusion, and hemolymph samples were collected to assess immune parameters among dietary treatments (n=5 per treatment). Data analysis using the GLIMMIX SAS procedure revealed significant differences between shrimp-fed MBPB-containing feeds and those on the control diet. The effects were most pronounced with the highest MBPB levels (1000 g/ton feed), compared to the lower MBPB diet (100 g/ton feed), across varying endotoxin doses (Fig 1).

These results suggest that MBPB additives in shrimp feed enhance immune response and survivability.

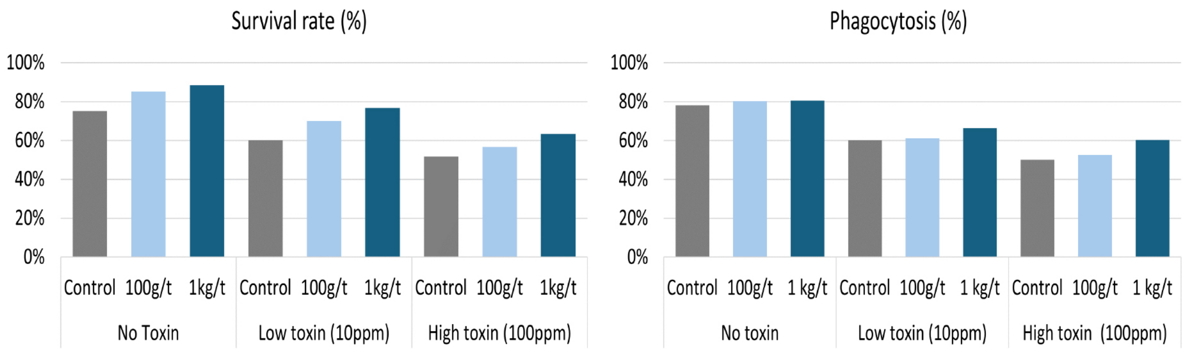

Figure 1. Survival rate and phagocytosis activity of shrimp fed MBPB diet at different levels challenged with three different doses of endotoxin.

AQUAUCLTURE VERSUS AGRICULTURE ROLES AND CONTRIBUTIONS IN FOOD SECURITY

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Foods produced from the ocean are essential for maintaining environmental friendliness, reducing starvation, and building nutritious, sustainable, and resilient food systems. Still, it is important to keep these in mind while discussing food. More money was made for developing countries by exporting blue foods than by exporting any other agricultural products. The central aim of this study is to analyze the impact of aquaculture production factors on food security within two distinct groups: European Union developing countries (EU13) and European Union developed countries (EU14), spanning the period from 1990 to 2023. To address the endogeneity issue, robust least squares (RLS), two-stage least squares (2SLS), and ordinary least squares (OLS) estimators were employed, yielding crucial insights. The analysis reveals that agriculture and aquaculture production exert a more pronounced influence on food security in EU13 developing countries compared to their EU14 counterparts. Moreover, the precise estimations from the three different methods highlight the significant roles played by fossil fuels, governance, and carbon gross domestic product (GDP) in fostering sustainable food security within EU13 countries in contrast to EU14 developed countries. Based on the study's findings, policymakers in the developed EU-14 countries are advised to provide policies targeted at advancing aquaculture production and agriculture production top priority. Additionally, this study suggests that authorities in the industrialized EU-14 countries improve the governance, economics, and efficiency of aquaculture's use of fossil fuels.

Table 9: Model 2. Panel Estimation for the EU-14 Region from 1990-2023

Variable	OLS		2SLS		RLS	
	Coefficient	Std. Error	Coefficient	Std. Error	Coefficient	Std. Error
AQ	0.017	0.075**	0.015	0.007***	0.047	0.053***
AG	1.065	0.028**	0.072	0.030**	0.120	0.046***
GDP	0.032	0.046***	0.024	0.040***	0.023	0.068***
FF	0.063	0.012***	0.053	0.012***	0.059	0.020***
GVR	0.049	0.016***	0.061	0.017***	0.085	0.023***
Constant	1.724	0.039***	1.744	0.038***	1.682	0.053***
R^2	0.164		0.162		0.076	
Adjusted-R^2	0.155		0.152		0.110	

- Note: ***, ** and * indicate significance at the 1%, 5%, and 10% levels respectively.

Table 10: Model 3. Panel Estimation for the EU-13 Region from 1990-2023

Variables	OLS		2SLS		RLS	
	Coefficient	Std. Error	Coefficient	Std. Error	Coefficient	Std. Error
AQ	0.050	0.032***	0.034	0.032***	0.018	0.083***
AG	0.143	0.016***	0.128	0.016***	0.089	0.037***
GDP	0.036	0.040***	0.033	0.039***	0.010	0.010***
FF	0.076	0.076***	0.077	0.023***	0.064	0.069***
GVR	0.079	0.013***	0.094	0.015**	0.034	0.045***
Constant	2.022	0.033***	1.942	0.037***	1.938	0.010***
R^2	0.468		0.427		0.022	
Adjusted-R^2	0.461		0.419		0.037	

- Note: ***, ** and * indicate significance at the 1%, 5%, and 10% levels respectively.

BUILDING TRUST: CONSUMER AWARENESS, ACCEPTANCE AND ATTITUDES RELATED TO EUROPEAN AQUACULTURE AND THE POTENTIAL EFFECTS OF GREENWASHING

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Greenwashing is a major challenge for the promotion of sustainable markets. This study examines consumer segmentation and preferences about farmed fish, with a focus on understanding consumer behaviour regarding greenwashing and its impact on perceptions.

The study utilized a cross-sectional online survey conducted in 2019, gathering responses from 2500 participants across the UK, France, Spain, Germany, and Italy. Its focus was to investigate European consumers' awareness, attitudes, and acceptance regarding aquaculture production methods. The results of cluster analysis identify three different consumer profiles that offer valuable insights for experts and policy makers. Moreover, findings show that consumers are concerned about the prevalence of greenwashing in sustainability communication in European aquaculture production. Despite a generally positive attitude and acceptance, the respondents' overall awareness of the various production systems is low.

As far as we know, this is the first study to analyse consumer behaviour towards aquaculture taking into consideration the potential effects of greenwashing, helping to enrich the economic literature.

FROM CATCH WELFARE INDICATORS TO CERTIFICATION AND COMMUNICATION; WHAT IS RELEVANT FOR CONSUMERS?

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The Catch Welfare Platform (CWP) is dedicated to improving catch welfare in the wild capture fisheries. Our objectives include empowering stakeholders, prompting evidence based discussions, encouraging knowledge exchange to help devising practical and profitable soloutions for better catch welfare.

The CWP operates as a collaborative network with spesialised working groups targeting specific aims. Such as formulating consumer centric criteria for certification and communication regarding catch welfare improvements. Existing knowledge on welfare in aquaculture will be integrated into CWP's initiatives.

To achieve our objective, we will gather input from CWP partners to establish indicators for evaluating catch welfare. This information will inform the design of a consumer study in the UK aimed at understanding consumer preferences and attitudes towards the welfare of animals for seafood production.

We will do an experiment within the survey to assess participants' willingness to receive additional information. During the survey, participants will be offered the option to access supplementary details by clicking on a provided link. This will help measure their inclination to seek further information. Participants who choose to access the additional information will then have the opportunity to read it. Following this, we will ask questions to confirm their understanding of the provided information.

The survey will also include questions aimed at helping us interpret the results, including participants' psychological, social, and demographic characteristics, as well as their consumption behavior.

Through this research, we aim to get an understanding of the challenges and opportunities associated with synchronized evaluation, certification, and communication of catch welfare, contributing to the sustainable development of the fisheries industry.

INVESTIGATION OF THE EFFECTS OF *Dictyota ciliolata* EXTRACT ON GROWTH PERFORMANCE, FEED UTILISATION, HAEMATO-BIOCHEMICAL INDICES, HEPATIC ANTIOXIDANT ACTIVITIES AND IMMUNE RESPONSES OF AFRICAN CATFISH *Clarias gariepinus*

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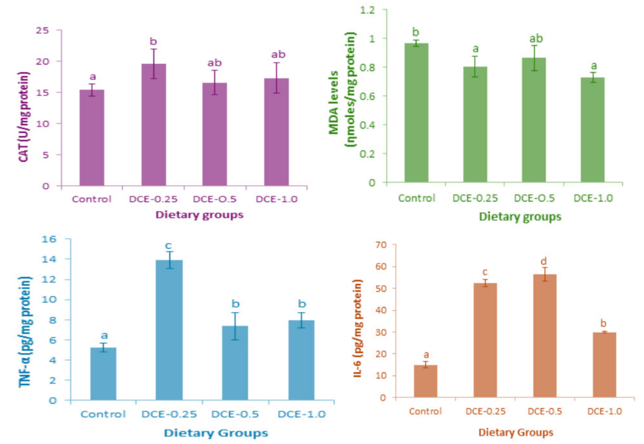
Seaweeds are amongst the most valuable resources in coastal ecosystems due to their nutritional composition and wide range of bioactive compounds. The inclusion of several seaweeds and their derivatives in aquafeed have proven to be beneficial to cultured fish species. However, the effects of the tropical brown seaweed, *Dictyota ciliolata* on the growth performance and health status of *Clarias gariepinus* remains unknown. Hence, this study investigated the effects of *D. ciliolata* extract (DCE) on growth, feed utilisation, antioxidant status and immune responses of *C. gariepinus*. A total of one hundred and eighty fish with an initial weight of 14.47 ± 0.06 g were allotted into four triplicate groups (10 fish/group) and fed diets containing DCE at inclusion levels of 0 g/kg (control), 0.25 g/kg (DCE-0.25), 0.5 g/kg (DCE-0.5) and 1.0 g/kg (DCE-1.0) for 28 days.

At the end of the feeding trial, significantly reduced feed conversion ratio and increased protein efficiency ratio were recorded in fish fed DCE-based diets when compared to the control ($p < 0.05$). Erythrocytes and leukocytes counts were significantly higher ($p < 0.05$) in *C. gariepinus* fed DCE-0.25 and DCE-0.5 diets compared to the control. The liver function enzyme, alkaline phosphatase (ALP), was significantly higher ($p < 0.05$) in fish fed DCE-0.5 and DCE-1.0 diets than fish fed control diet. Dietary DCE improved enzymatic and non-enzymatic antioxidant activities and decreased malondialdehyde (MDA) levels in *C. gariepinus*. Cytokines such as Tumour Necrosis Factor – alpha (TNF-□), Interleukin-1 Beta (IL-1□), and Interleukin-6 (IL-6) were elevated in fish fed DCE-based diets when compared to the control.

These findings suggest that dietary administration of DCE improved nutrient utilisation, impeded oxidative stress, and induced the production of immunoregulatory cytokines in *C. gariepinus*. Hence, DCE could be developed as a feed additive to enhance the health status of cultured fish species.

Table 1: Growth performance and feed utilisation of *C. gariepinus* fed with *D. ciliolata* extract at various inclusion levels.

Parameters	Control	DCE-0.25	DCE-0.5	DCE-1.0
IBW (g/fish)	14.47 ± 0.06^a	14.43 ± 0.06^a	14.47 ± 0.05^a	14.47 ± 0.03^a
FBW (g/fish)	44.10 ± 0.64^a	41.52 ± 6.03^a	42.19 ± 2.25^a	41.01 ± 3.61^a
WG (g/fish)	29.67 ± 0.58^a	27.05 ± 5.94^a	27.73 ± 2.32^a	26.57 ± 3.62^a
RWG (g/day)	0.85 ± 0.02^a	0.77 ± 0.17^a	0.79 ± 0.07^a	0.76 ± 0.11^a
FCR	1.36 ± 0.01^b	1.16 ± 0.06^a	1.26 ± 0.08^{ab}	1.26 ± 0.15^{ab}
PER	1.84 ± 0.01^a	2.13 ± 0.35^b	1.99 ± 0.12^{ab}	1.96 ± 0.04^a
FI (g)	40.79 ± 1.16^a	31.82 ± 6.61^a	34.87 ± 1.75^a	33.86 ± 4.55^a
PI (g)	16.31 ± 0.47^b	12.73 ± 2.64^a	13.95 ± 0.70^{ab}	13.54 ± 1.82^{ab}



REPRODUCTIVE PERFORMANCE OF WILD-CAUGHT SPINELESS CUTTLEFISH *Sepiella inermis* IN CAPTIVITY – PRELIMINARY RESULTS

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The aquaculture sector has recognized the potential of cuttlefish culture in the industry as it can reach market size quicker when compared to finfish. Several technological bottlenecks in cuttlefish culture were acknowledged, such as semelparity, low fertility and fecundity, and the inability to sustain consecutive generations. This work aims to assess the reproduction performance of *S. inermis* from a wild-caught breeding stock in a recirculation aquaculture system (RAS).

Thirty Sri Lanka wild-caught *S. inermis* were homogeneously distributed (body mass = 19.3 ± 3.9 g; mantle length = 5.0 ± 0.5; mean ± s.d.) into two 2700L tanks in a RAS. Water parameters were controlled daily (salinity, temperature, dissolved O_2 concentration, pH, and total alkalinity) and maintained on the following ranges: salinity – 34.75 ± 0.75 ppt, temperature – 27.25 ± 0.75°C, pH – 7.9 ± 0.1). During the acclimation period (15 days), environmental enrichment (sandboxes and fake plants) was added to the tanks to reduce the exhibition of stress behaviors. The animals were fed daily a quantified *ad libitum* of thawed *Euphausia superba*. After 9-12 days, all animals were observed eating during feeding sessions. At the end of the acclimation period, courtship behaviors were observed, and on the 21st day mating. In tank B, the reproductive stage started 7 days after mating with 11 cuttlefish, 4 of which were females. In tank A, 9 cuttlefish were remaining, with only 1 female that did not lay any eggs in captivity.

A total of 2008 eggs in 4 batches were obtained during this experiment. There were significant differences observed between the egg batches regarding the number of eggs and egg viability percentage (see Table 1). Additionally, significant differences were observed in the percentage of hatching rate between egg batches. In the first batch was observed that the mean hatchling wet weight was significantly higher (14.2 ± 3.9 mg) than the hatchlings from the other batches. RAS did not seem to impact reproductive performance. However, control over reproduction in captivity is fundamental for the sustainability of aquaculture production.

Table 1 - Data of eggs batches of wild-caught *Sepiella inermis* breeders maintained in a recirculation aquaculture system.

Egg batch	#1	#2	#3	#4
n eggs	762	1030	123	93
mean egg mass (g)	0.0778	0.1067	0.1048	0.0793
Maximum Individual Egg Mass (g)	0.1655	0.3890	0.1869	0.7410
Minimum Individual Egg Mass (g)	0.0252	0.0209	0.0463	0.0470
Total Egg Biomass (g)	59.31	109.92	12.90	7.37
% egg viability	47.1	45.1	82.1	78.5
n viable eggs	359	465	101	73
n non-viable eggs	403	565	22	20

BOOSTING GROWTH AND STRESS RESILIENCE OF GILTHEAD SEABREAM THROUGH INNOVATIVE PROTEIN HYDROLYSATE SUPPLEMENTATION IN AQUAFEEDS

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As aquaculture expands, intensified conditions raise animal stress and disease outbreaks, urging innovative prophylactic measures to promote fish robustness and welfare. Protein hydrolysates, rich in bioactive peptides and amino acids, emerge as stress-reducing functional ingredients in aquafeeds. Gilthead seabream (*Sparus aurata*) is highly susceptible to cold-induced "winter disease", characterized by a decrease in metabolic rate and hindering production. This study evaluates the potential of novel hydrolysates from local agri-food by-products to improve seabream growth and stress resilience in low water temperatures.

Four isoproteic and isoenergetic practical diets were formulated: a commercial-like diet containing 20% fish meal (CTRL) and three experimental diets (INSECT, FISH, SWINE) that replaced 3% of high-quality pre-digested fishmeal (CPSP90) with the corresponding hydrolysate. Each experimental diet was randomly assigned to triplicate homogenous groups of 65 fish (IBW 11 g), fed thrice daily for 88 days at 20ºC. Following the feeding period, fish were subjected to thermal stress by reducing water temperature from 20ºC to 15ºC and kept at 15ºC for 5 days. Plasma and liver samples were collected from stressed and unstressed fish.

At the end of the feeding trial, the SWINE group exhibited significantly higher body weight compared to the INSECT group, which in turn exhibited higher body weight than the CTRL. Feed intake and nutrient utilization remained unaffected by the dietary treatments, but a trend towards increased N and energy gain was observed in fish fed the hydrolysates compared with the CTRL. Intestinal integrity was maintained across all experimental groups. The FISH group exhibited significantly lower values for both VSI and HSI, whilst the SWINE-fed fish showed a decreased HSI compared to the CTRL group. Under thermal stress, plasma metabolites shifted: NEFA levels increased, cholesterol and cortisol dropped across groups. Lactate was significantly reduced in all experimental groups except SWINE group, which maintained lactate levels similar under stress and non-stress conditions. Triglycerides' levels were significantly affected by diet, with the INSECT group showing the highest values regardless of stress condition. Glucose remained unaffected. Oxidative stress results will be further discussed.

In conclusion, our findings suggest that diets supplemented with SWINE and INSECT hydrolysates hold potential as growth enhancers for seabream. Additionally, the SWINE diet influenced fish metabolism, resulting in a decrease in fish HSI by the end of the trial. Notably, following thermal stress, lactate levels in the SWINE group remained similar to those in the non-stress group, indicating the preservation of anaerobic metabolism and highlighting its potential to alleviate the metabolic disruptions triggered by the low temperatures.

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FISH BEHAVIOR IDENTIFICATION BASED ON COMPUTER VISION

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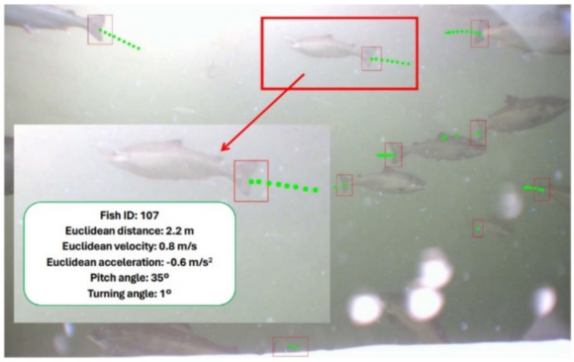
The aquaculture industry needs to address several challenges to secure the demand for increased seafood production and sustainability. When targeting increased autonomy and more exposed locations within the fish farming industry [1], and when introducing intrusive objects, sensors and robots into these environments care should be taken to ensure good fish welfare. This paper addresses this by developing, implementing, and comparing methods to identify fish behavior when affected by intrusive objects. A novel approach for detecting, tracking, and estimating the 3D position of individual fish has been created to reach this goal. The proposed method and its versions utilize a combination of stereo vision by SuperGlue, triangulation and RAFT-Stereo, image pre-processing including morphological area opening and discrete wavelet transform (MO-WT), object detection by YOLOv8, and multi-object tracking by ByteTrack to estimate the relative distance between a stereo camera system and detected fishtails. Additionally, these methods can by Savitzky-Golay smoothing of raw estimates derive indirect behavioral estimates of fish, as shown in Figure 1.

The methods have been tested on data collected from an industrial fish farm with Atlantic Salmon on the SINTEF ACE site [2] in 2023. A stereo camera system and two sonars were put on a structure coated with different color- and shape combinations (yellow, white, cube, big cylinder and small cylinder) placed at 8 m depth. Six replicate experiments of 12 min were conducted for each color-structure combination.

Results are shown in Table 1, concluding that fish stay further away from yellow and large objects than from white and small objects. These conclusions are aligned with those found when processing sonar data from the same cage [3]. Additionally, the system can estimate derived parameters close to real-time as well as over longer time periods. The proposed method is general and can thus be used to study both the collective and individual behavior of fish. This advantage makes the method especially useful, both for instant feedback in control systems and for observing altered fish behavior over time, and it can be an essential contribution in the future of fish farming.

Figure 1 Estimated and derived parameters of tracked fish.



Case		Mean distance [m]	
		Initial augmented	MO-WT
Big Cylinder	White	0.84 ± 0.13	0.85 ± 0.14
	Yellow	1.30 ± 0.29	1.29 ± 0.29
Small Cylinder	White	0.72 ± 0.15	0.73 ± 0.16
	Yellow	0.88 ± 0.24	0.89 ± 0.24
Cube	White	0.97 ± 0.17	0.97 ± 0.18
	Yellow	0.92 ± 0.19	0.98 ± 0.23
Average	White	0.84	0.85
	Yellow	1.03	1.05
	Big Cylinder	1.07	1.07
Average	Small Cylinder	0.80	0.81
	Cube	0.95	0.98

Table 2 Comparison Results

References
[1] E. Kelasidi and E. Svendsen, Robotics for Sea-Based Fish Farming. Springer Int. Publishing, 2023, pp. 1–20
[2] "Ace," https://www.sintef.no/en/all-laboratories/ace/, accessed: 2023-12-19
[3] Q. Zhang, et. al,, "Farmed atlantic salmon (salmo salar l.) avoid intrusive objects in cages: The influence of object shape, size and colour, and fish length," Aquaculture, p. 740429, 2023

UNRAVELING THE BLUE TRANSFORMATION: THE CONTESTED DEPOLITICIZATION OF THE FISHMEAL AND FISH OIL INDUSTRY

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A significant component of aquaculture technology requires fishmeal and fish oil to produce feeds for farmed species and forms the basis for current optimism about blue food or foods from marine ecosystems. Industry spokespeople state that blue food is the future of a sustainable global agri-food system. Yet, recent debates around blue transformation do not adequately engage the ethical and sustainable sourcing of fishmeal and fish oil. At the same time, the voracious demand for pelagic fish for the production of feed has raised concerns over a possible dependence on wild fisheries (or a so-called fishmeal trap), friction of consumption, and the implications for the proliferation of transnational governance initiatives, such as the Global Roundtable on Marine Ingredients, which seek to govern the industry through market-based solutions. However, we lack an understanding of how the capitalist logic of growth that underpins the industry challenges or reproduces unequal power relations. This paper addresses this gap by investigating the rapidly changing role of the fishmeal and fish oil industry in producing blue food. Drawing on the 'global ecological political economy' heuristic framework, which centers on the dialectical relationship between ecology and social change, and data from event ethnography during the United Nations Oceans Decade in Barcelona, and interviews with blue food actors, this paper advances a justice-focus approach to blue transformation centering on power relations inherent in blue food policies, including questions of who governs blue transformation and in what contexts, who sets the terms, who are excluded in decision-making, whose interests are served, and what implications blue food production and consumption have for global environmental change. It concludes with pragmatic recommendations for actors and decision-makers in food governance spaces (particularly in increasing equity in policy for vulnerable populations) while offering normative analysis of transformative change in the agri-food system.

DIETARY ALGINATE MICROCAPSULE ASSESSMENT IN MODULATING THE GROWTH AND HEALTH PROFILE OF *Labeo rohita* (HAMILTON, 1822)

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Fish growth and immune status can be improved by using encapsulation. Various microcapsules have been used in aquaculture to effectively utilize probiotics to achieve desired fish and shellfish production. Thus, to explore the efficacy of encapsulation, a feeding trial was planned to check the *in vivo* efficiency of *G. candidum* encapsulated with alginate (Alg) alone and in combination with starch (Alg-S) and xanthan (Alg-X), followed by simple chitosan coating (Alg-C, Alg-S-C, Alg-X-C) and nano-chitosan coating (Alg-CN, Alg-S-CN, Alg-X-CN). *Labeo rohita* (15.63±0.35g) fingerlings were evenly distributed in ten different groups. Control group was fed with basal feed while experimental group was given un-encapsulated probiotic, *G. candidum* @1×10^9 CFU per g, while remaining groups were given probiotic microcapsules based diet containing Alg, Alg-S, Alg-X, Alg-C, Alg-S-C, Alg-X-C, Alg-CN, Alg-S-CN and Alg-X-CN.

Results showed significant impact of probiotic microcapsules on growth, intestinal and serum enzyme activities, hematology, lipid profile: total cholesterol, high- to low-density lipoprotein and triglyceride value of fish as compared to fish fed control diet. Additionally, chitosan coated *G. candidum* microcapsules indicated improved performance as compared to fish fed uncoated-microcapsules based diet. Furthermore, among chitosan coated microcapsules, Alg-CN microcapsules showed highest efficiency for all growth and immunity parameters followed by Alg-C microcapsules. It was also revealed that alginate-nano chitosan microcapsule formulation is best to improve bioavailability and target release of locally isolated *G. candidum* in GI tract of *L. rohita*. Therefore, this investigation suggests a sustainable alternative for improving the growth and health profile of indigenous carp *L. rohita* by feeding the diet supplemented with alginate and nano-chitosan coated microcapsules of *G. candidum*.

WATER QUALITY ISSUES CAUSED BY ZINC AND GAS SUPERSATURATION DURING SIMULATED WELL BOAT OPERATIONS OF ATLANTIC SALMON

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Handling operations are a significant cause of mortality in salmon farming. Mortality incidents related to gas supersaturation (gas bubble disease) and heavy metal accumulation, have been suspected. During some well boat operations, fish are exposed to a lower than ambient pressure when they are transported by suction from the sea surface to the highest point in the loading line. As pressure decreases, total gas saturation increases proportionally, potentially leading to significant gas supersaturation. Gas bubble disease is caused by total gas supersaturation and subsequent bubble formation in the fish's blood and/or tissue fluids.

Previous studies of mortality incidents associated with freshwater treatments and closed transport of salmon in well boats have shown accumulation of Zinc (Zn^{2+}) and Copper (Cu^{2+}) in the water, with concentrations reaching high levels. These heavy metals could come from corrosion-protective equipment such as anodes or surface treatment in well boats in addition to other sources.

In controlled experiments using pressure chambers to simulate well boat operations, we demonstrated that gas bubble disease could be induced in salmon under conditions relevant to fish loading (0.4 ata and > 60 minutes duration). Behavioral changes were the first indication of the development of gas bubble disease. Ultrasound examinations and visual observations after exposure revealed bubbles in the bloodstream and fins of some fish. The proportion of fish displaying abnormal behavior and bubbles in the bloodstream (gills and heart) and fins increased with exposure time. Extensive bubble formation in systemic circulation was consistently associated with mortality.

Controlled laboratory experiments were also conducted to investigate the effects of elevated concentrations of Zn^{2+} (low, moderate and high doses) and Cu^{2+} in freshwater and seawater on fish physiology over eight hours of simulated transport and freshwater treatment. Before and after exposure, the post-smolts (~500 g) were kept in seawater to simulate a closed transport using seawater and two freshwater treatments in a well boat.

No fish died during the simulated seawater transport, but mortalities occurred during the freshwater treatments, with higher mortality rates in the treated raw water compared to untreated water. Mortalities in the untreated freshwater were observed after returning to seawater, illustrating that the combination of zinc exposure and osmotic stress can be fatal. For freshwater treatments, lower mortality was seen in untreated freshwater compared to treated freshwater with silicate lye. This is likely because the untreated water contained more humus, which binds Zn^{2+} and reduces its toxicity.

We have demonstrated that salmon can develop gas bubble disease under pressure conditions (loading) relevant to the salmon farming industry. Elevated levels of Zn^{2+} can lead to increased mortality, highlighting the importance of choosing an appropriate freshwater source during such operations.

Project NYBRØK (901788) – "Ny Brønnbåt Kunnskap", New well boat knowledge – biological risk factors when using well boats for transport and treatment of salmon. Funded by the Norwegian Seafood Research Fund (FHF).

IMPROVED NUTRIENT UTILIZATION IN RAS BY CO-FARMING SEA LETTUCE (*Ulva fenestrata*) WITH RAINBOW TROUT (*Oncorhynchus mykiss*) AND WOLFFISH (*Anarhichas lupus*)

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Co-farming species from different trophic levels is commonly referred to as integrated multitrophic aquaculture (IMTA), where nutrients from fed species are consumed by extractive species. Nitrogen budgets can be used to evaluate the nitrogen remediation efficiencies of different farming systems, for example in recirculating aquaculture systems (RAS). Nitrogen is of large importance since it affects both fish growth, health and welfare, water quality, and the surrounding environment. The nitrogen input to a system is typically through feed, which is the most climate impacting and costly factor in fish farming. Thus, nitrogen budgets can be used to evaluate both environmental and economic sustainability. In Sweden the production of farmed fish is dominated to almost 90% by rainbow trout while both Atlantic wolffish and macroalgae such as sea lettuce have been proposed as a promising cold-water species in aquaculture diversification, thus making them interesting for co-farming studies. The aim of this study was to evaluate the effects of co-farming rainbow trout and Atlantic wolffish with sea lettuce *Ulva fenestrata* on the performance of a closed seawater (SW) RAS through analysis of nitrogen budgets.

Rainbow trout and Atlantic wolffish were housed in a 18 m^3 SW RAS for four consecutive periods, each lasting four weeks. During the first and third period, only fish were held in the system and during the second and fourth periods, fish were held together with *Ulva* in the RAS loop. A nitrogen balance and budget were calculated for the RAS for each period. Nitrogen input to the system was through added feed. Nitrogen outputs included uptake by fish and *Ulva*, changes in dissolved inorganic nitrogen (DIN), DIN in wastewater and skimmer elute. The nitrogen uptake by *Ulva* was calculated from nitrogen in tissue content and biomass growth.

The nitrogen budgets varied between the periods and accounted for 47 – 68% of the added nitrogen. The exact total nitrogen held as fish biomass, in rainbow trout and Atlantic wolffish, varied over time between 87 - 91%. System DIN concentrations remained stable around 10 – 12% of the total nitrogen. The *Ulva* held 3 - 4 percent of total system nitrogen at the end of the co-farming periods and assimilated between 7 and 11% of the total nitrogen input. The nitrogen content of *Ulva* increased with 29-35% dry weight (dw) throughout both periods, thus indicating that the RAS water was beneficial for the macroalgae. Our results demonstrate that *Ulva* is an efficient nitrogen assimilator and that co-farming with *Ulva* in RAS improves nitrogen utilization.

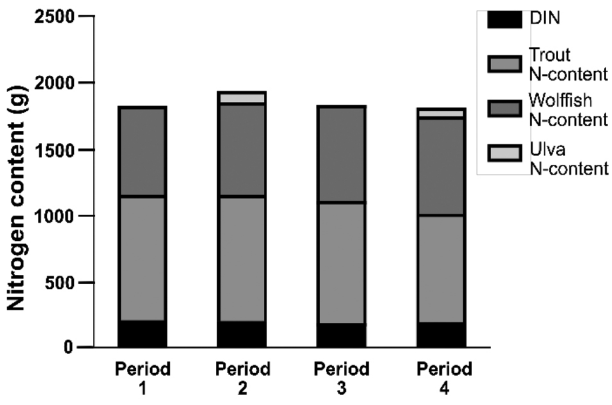

Figure 1. Composition of total nitrogen in the RAS at the end of each period.

NUTRIENT FLOWS OF CARBON, NITROGEN, AND PHOSPHORUS IN *Hediste diversicolor* (OF MÜLLER, 1776) (ANNELIDA: NEREIDIDAE) FED AQUACULTURE SLUDGE

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Introduction

With the continuous growth of Atlantic salmon (*Salmo salar* L.) aquaculture, appropriate management of nutrient-rich waste streams from both land- and sea-based production has become increasingly important (Turchini et al., 2019, Wang and Olsen, 2023). Following a circular approach, nutrients contained in aquaculture sludge can be utilized for biomass production of the polychaete *Hediste diversicolor* (Wang et al., 2019b, Anglade et al., 2023a). While the potential for upcycling nutrients has been established previously (Wang et al., 2019a, Anglade et al., 2023b), this study aimed to assess nutrient flows in *H. diversicolor* fed aquaculture sludge as understanding these will be crucial for optimizing future large-scale production of this species.

Material and methods

We investigated the effect of low and high feed supply of smolt sludge on the nutrient flows of carbon (C), nitrogen (N), and phosphorus (P) in *Hediste diversicolor* on an individual level. Hereby we assessed ingestion, defecation, excretion, respiration, and uptake for growth.

Results and discussion

Nutrient flows differed between the nutrients C, N, and P in the high feed supply group where C uptake for growth was significantly higher than N uptake, and N uptake was significantly higher than P uptake. In contrast, no significant differences were observed in the low feed supply group. In the high feed supply group most of the ingested C, N, and P by *H. diversicolor* was allocated to uptake for growth with smaller proportions allocated to fecal production and respiration/ excretion. The results from the low feed supply group suggest insufficient nutrient supply as most ingested C was allocated to respiration, while N was spent on uptake for growth, and ingested P was either taken up for growth or used for fecal production. Overall, nutrient uptake reflected the smolt sludge composition rather than nutrient requirements for *H. diversicolor*, which may be a short-term effect and needs to be investigated further. The flow of nutrients was strongly affected by feed supply, as ingestion, defecation, excretion, and uptake for growth were significantly higher in the high feed supply group than in the low feed supply group.

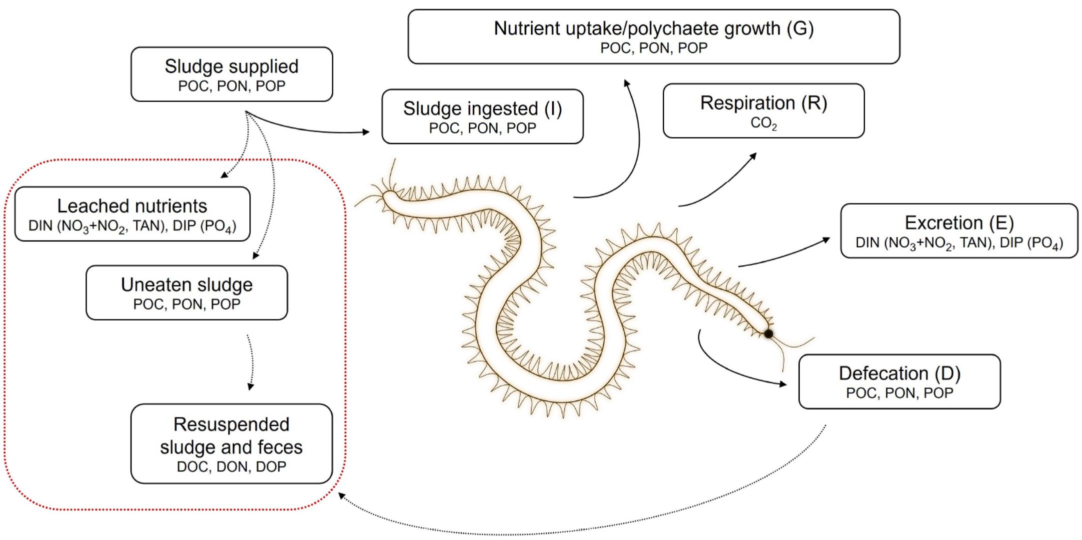

Figure 1 Nutrient budget of *H. diversicolor* adapted from Olsen et al. (2008). Polychaete illustration by Anglade (2022).

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References

Anglade, I. (2022). Illustration of the common ragworm *Hediste diversicolor* (OF Müller, 1776) (Annelida: Nereididae). *Unpublished work*.

Anglade, I., Dahl, T. H., Kristensen, B. S. B., Hagemann, A., Malzahn, A. M. & Reitan, K. I. (2023a). Biochemical composition of *Hediste diversicolor* (OF Müller, 1776)(Annelida: Nereidae) reared on different types of aquaculture sludge. *Front. Mar. Sci*., 10**,** 899. doi: 10.3389/fmars.2023.1197052

Anglade, I., Kristensen, B. S. B., Dahl, T. H., Hagemann, A., Malzahn, A. M. & Reitan, K. I. (2023b). Upcycling of carbon, nitrogen, and phosphorus from aquaculture sludge using the polychaete *Hediste diversicolor* (OF Müller, 1776) (Annelida: Nereididae). *Front. Sustain. Food Syst*., 7. doi: 10.3389/fsufs.2023.1278586

Olsen, L. M., Holmer, M. & Olsen, Y. (2008). Perspectives of nutrient emission from fish aquaculture in coastal waters. Literature review with evaluated state of knowledge. FHF project. doi:10.13140/RG.2.1.1273.8006.

Turchini, G. M., Trushenski, J. T. & Glencross, B. D. (2019). Thoughts for the Future of Aquaculture Nutrition: Realigning Perspectives to Reflect Contemporary Issues Related to Judicious Use of Marine Resources in Aquafeeds. *North American Journal of Aquaculture*, 81**,** 13-39. doi: 10.1002/naaq.10067

Wang, C. & Olsen, Y. (2023). Quantifying regional feed utilization, production and nutrient waste emission of Norwegian salmon cage aquaculture. *Aquac. Environ. Interact*., 15**,** 231-249. doi: 10.3354/aei00463

Wang, H., Hagemann, A., Reitan, K., Ejlertsson, J., Wollan, H., Handå, A. & Malzahn, A. M. (2019a). Potential of the polychaete *Hediste diversicolor* fed on aquaculture and biogas side streams as an aquaculture food source. *Aquac. Environ. Interact*., 11**,** 551-562. doi: 10.3354/aei00331

Wang, H., Seekamp, I., Malzahn, A. M., Hagemann, A., Carvajal, A. K., Slizyte, R., Standal, I. B., Handå, A. & Reitan, K. I. (2019b). Growth and nutritional composition of the polychaete *Hediste diversicolor* (OF Müller, 1776) cultivated on waste from land-based salmon smolt aquaculture. *Aquaculture*, 502**,** 232-241. doi: 10.1016/j.aquaculture.2018.12.047

QUALITY OF SPERMATOZOA EXTRACTED FROM SPERMATOPHORE REMOVED BY DISSECTION OR EXTRUDED BY ELECTROEJACULATION FROM WILD-CAUGHT REDCLAW CRAYFISH *Cherax quadricarinatus*

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In this study, we determined the efficiency of electroejaculation and assessed sperm quality using conventional and advanced reproductive biomarkers following the extraction of spermatophores by either dissection or electroejaculation from wild-caught redclaw crayfish. In Experiment 1, sexually mature male crayfish (> 40 g) were collected from Ross River Dam, northern Queensland and were subjected to electroejaculation to evaluate the efficiency of electrical stimulation to extrude spermatophores. In Experiment 2, 33 male crayfish weighing 99.4 ± 7.6 g (mean ± SEM) were subjected to sperm quality assessment following isolation from spermatophores collected by dissection and electroejaculation. Body weights ($p = 0.029$) affected the odds of extracting spermatophore and were greatest within crayfish weighing between 60 and 130 g. The success rate in extracting spermatophores from either gonopore using electroejaculation was 22.2 % ($n = 153$). The mean weight of spermatophores from left (0.016 ± 0.002 g) or right (0.011 ± 0.002 g) gonopores were comparable and were not significantly influenced by body weight ($p = 0.430$). A greater mean concentration of spermatozoa, sperm viability, total potential fertile sperm concentration (TPFSC) and lesser sperm DNA fragmentation were measured following the extraction of spermatophores by dissection than those by electroejaculation ($p < 0.001$). Together, these data suggest that the method used for spermatophore collection impacts sperm quality in redclaw crayfish. Spermatophores collected following dissection provided better sperm quality and are recommended for developing advanced breeding techniques, including sperm freezing and artificial reproduction techniques. However, electroejaculation is a simple, non-lethal alternative for collecting spermatophores for routine breeding and commercial production of redclaw.

EVALUATION OF IMMUNOMODULATORY PROPERTIES OF ANISAXIN A-2S, AN ANTIMICROBIAL PEPTIDE FOR USE IN AQUACULTURE

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Antimicrobial Peptides (AMP) are vital elements of the host's innate immunity due to their microbicidal and immunomodulatory properties. AMPs can be a promising solution for sustainable aquaculture development in preventing or treating infectious diseases. Specifically, anisaxins, cecropin-like helminthic AMPs from the zoonotic marine nematodes, *Anisakis simplex* and *Anisakis pegreffii*, express high potency against clinical and referent human bacterial Gram-negative isolates.

The immunomodulatory properties of annisaxin-2S (A-2S) firstly tested *in vitro* in the common carp (*Cyprinus carpio*) red blood cells (RBC) and white blood cells (WBC) exposed to fish pathogen, the myxozoan parasite *Spaher* were *ospora molnari*, over three time-points (0, 1 and 24h). Secondly, the carp was challenged *in vivo* with the parasite and/ or immunosuppressed by cortisol. Blood was collected before the challenge and 2, 3 and 4 weeks after challenge, and isolated RBC and WBC were exposed to A-2S. Expression of target cytokines (*il1ß*, *il6*, *il10*, *tnfα* and *infγ*) in RBC and WBC from both assays was measured by qPCR, and flow cytometry was used to determine the kinetics of reactive oxygen species production over time in the *in vivo* experiment.

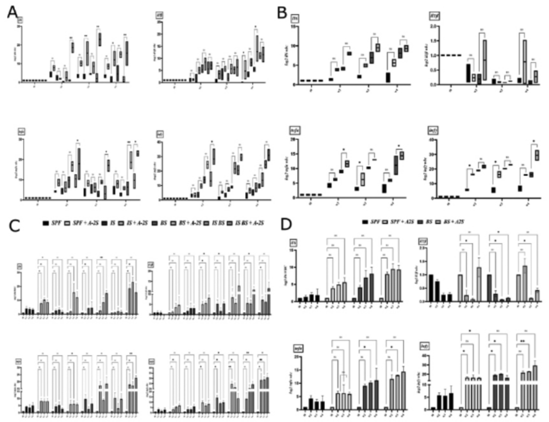

Fig. 1: Graphical representation of differences in cytokines' expression in RBC (A, C) and WBC (B, D) during the interaction between A-2S-stimulated and unstimulated cellular responses over time priorly *in vivo* challenged by *Sphaerospora molnari* and followed by A-2S stimulation, and among time points (0, 2, 3 and 4 week). SPF: untreated cells (negative control); SPF + A-2S: cells stimulated by A-2S; IS: immunosuppressed cells; IS + A-2S: immunosuppressed cells stimulated by A-2S; BS: cells infected by *S. molnari* blood stages; BS + A-2S: cells infected by *S. molnari* blood stages and A-2S-stimulated; IS BS: immunosuppressed cells infected by *S. molnari* blood stages; IS BS + A-2S: immunosuppressed cells infected by *S. molnari* blood stages and A-2S-stimulated.

The results revealed that all pro-inflammatory cytokines, especially *il1ß*, *tnfα*, and *infγ*, have shown statistically significant expressions in A-2S-stimulated RBC and WBC. ROS production and cell proliferation also increased significantly in both A-2S-stimulated cell lineages, progressing from weeks 2 to 4 (parasitemia). Anisaxin-2S demonstrates notable immunomodulatory properties in the blood cells of healthy fish. Initially, it boosts a pro-inflammatory reaction, which later is balanced by an anti-inflammatory response. In contrast, in the presence of a co-antigen, *i.e.*, fish infected with the parasite *S. molnari*, the environment becomes anti-inflammatory at an early stage. Overall, the expression of targets of the innate immunity increases towards 24h or over four weeks, in the case of the *in vivo* (Fig.1) and *in vitro* challenge assay with *S. molnari.* The pathway activating the cytokines production, however, still needs to be elucidated. Although immunomodulatory properties have been so far mainly attributed to the flatworms' AMPs, the nematode A-2S has indicated a considerable activation of innate cells. This could be valuable for designing aquaculture nutraceuticals and using anisaxins as an alternative against the treatment of infectious diseases.

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TRANSITIONING TO THE IMPLEMENTATION OF BIO-BASED ROPES (BIOGEARS) IN LOW TROPHIC AQUACULTURE: EMBRACING NATURE- BASED SOLUTIONS FOR MARINE CONSERVATION THROUGH SUSTAINABLE AQUACULTURE PRACTICES

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Introduction

Low trophic aquaculture, such as bivalve species (i.e., mussels), are candidate species for the minimization of environmental impact of worldwide growing aquaculture, while providing with ecosystem goods and services. 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 validated bio-based and compostable ropes (hereinafter referred to as "biogears") as more sustainable solutions than existing commercial counterparts for suspended mussel production in offshore longline systems. Integration of biogears into low trophic aquaculture infrastructures holds promise for enhancing the efficacy of these practices as nature-based solutions. However, defining and advancing the pathway for implementing such aquaculture gears remains a challenge. The aim of this study is to assess the route for the implementation of bio-based ropes in low trophic aquaculture alongside further appraising the effectiveness of such aquaculture practices as nature-based solutions.

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 functional properties similar to commercial mussel rope counterparts. The sustainability assessment of biogears was conducted by comparison with fossil-based counterparts regarding to technical characteristics (a one-year mussel longline production), environmental performance (compostability and Life Cycle Analysis) and economic outcomes (cost and benefit analysis along the value chain and ecoefficiency. The compostability tests were conducted in laboratory (58±2 ºC) simulating industrial composting conditions (UNE EN ISO 14855 /UNE-EN 13432). The co-efficiency indicator was calculated according to ISO 14045:2012 and LCA according to ISO 14040/14044, respectively. In the light of promising results of biogears, the next step is to implement them in the sector and mussel productions as nature-based solutions (NbS). In Biogears project the potential implementation of biogears in the European aquaculture sector was assessed considering, 1) the current context (considering technical, environmental, policy, etc., aspects of biogears), 2) the potential circular value chain, 3) a consultation to stakeholders (by online surveys and personal interviews), 4) a BlueLab concept (considering the principal stakeholders involved in the value chain), and 5) end-of-life (EoL) biogears best practices. Aligning this implementation context with NbS is a challenge that will be overcome in a subsequent project to BIOGEARS, TRANSEATION (Proposal ID 101135343): Advancing Ecosystem-Based Management through Hybrid Blue-Grey Infrastructures in Marine and Coastal Areas). Within this project, efforts are underway to enhance the mechanical properties of biogears prototypes to improve their durability in mussel aquaculture settings. Furthermore, the assessment of the effectiveness of low trophic aquaculture productions (mussel longline and raft cultures) as NbS including the implementation of biogears will be conducted.

Results and discussion

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 quality was not compromised by using biobased ropes (similar Condition Index, meat yield, proximal composition and fatty acid profiles as in mussel grown-out in fossil-based ropes). Mechanical properties (load at break and elongation) of biogears did not compromise rope functionality in one-year mussel production while holding a higher mussel weight. Compostability tests concluded that industrial composting of biogears is technically feasible and validated composting as EoL option for biobased ropes. Life Cicle Analysis showed that, considering rope aquaculture production (impact/kg mussels produced per rope linear meter), B2 was the best environmental performing rope, as reducing by 20% the environmental footprint (34% reduction of the carbon footprint and 63% reduction of the use of fossil resources) along the whole value chain compared to conventional fossil-based ropes. The economic evaluation revealed that the enhanced productivity of mussels in B2

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ropes could counterbalance the present cost disparity in biogears production, attributed to the relatively higher market price of biobased materials. Consequently, the adoption of biogears would render mussel production more economically viable compared to employing commercial fossil-based ropes. Additionally, the eco-efficiency analysis emphasized the importance of promoting the production and utilization of B2 prototype ropes due to their superior eco-efficiency indicators, considering both the environmental impact and economic value per kilogram of harvested mussel. From these results, 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.

The analysis of the current context of implementation of biogears in the European aquaculture sector concluded that, overall, current market, technology, social, and policy drivers are promising context for their implementation in the aquaculture sector. (Arantzamendi et al., 2023). Furthermore, the consulted stakeholders would support their implementation regarding their environmental benefits when providing with similar mechanical characteristics.

In TRANSEATION project new biogears with improved mechanical characteristics (to BIOGEARS project) are being developed. To evaluate the efficacy of the low trophic aquaculture infrastructures (longline and rafts) through the implementation of biogears as nature-based solutions (NbS), the following steps are being undertaken:

- The development of an Ecosystem-based Management (EMB) framework.
- Two demonstrators for low trophic aquaculture, utilizing biogears for mussel longline and raft cultures.
- A framework was established to evaluate the benefits and potential trade-offs of these NbS in both short and long terms, particularly concerning marine biodiversity and ecosystem services protection and restoration.
- The design of digital solutions was defined for monitoring, analysis, and social engagement to assess the performance of the NbS.

References

Arantzamendi L, Andrés M, Basurko OC, Suárez MJ. Circular and lower impact mussel and seaweed aquaculture by a shift towards bio-based ropes. Rev Aquac. 2023; 15(3): 1010-1019. doi:10.1111/raq.12816

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HEALTH MANAGEMENT OF FISH &SHRIMP RAS (RECIRCULATED AQUACULTURE SYSTEMS) PRODUCTION SYSTEMS

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Successful aquaculture requires practical and sustainable production methods.

The challenge is to develop profitable and sustainable culture facilities which will implement advanced technologies while minimizing adverse environmental effects.

This increasing concern regarding environmental effects of the industry and the increasing intensity of aquatic pathogens represents two of many motivations that have driven the interest of the aquaculture industry to examine alternatives to traditional methods of intensive aquaculture.

Recirculating aquaculture systems can be defined as an aquaculture system that incorporates the treatment and reuse of water with less than 10% of total water volume replaced per day.

Recirculating aquaculture system, or RAS, provides a constant and controlled environment for the fish or shrimp, allowing for optimal and fully manageable production of numerous aquatic species.

However, while Recirculating Aquaculture Systems offer fish producers a variety of important advantages over open pond and cage culture, they also present numerous challenges.

Many of these challenges are related to health management of the fish or shrimp which are cultured in very high densities while continuously exposed to a long list of stressors and sub-optimal environmental conditions. In these conditions, many disease-causing agents which are frequently regarded as chronic will exhibit aggressive behavior and a much more acute form of disease, resulting in low survival rates and severe financial losses.

In this presentation, Dr Ra'anan Ariav, a well – known Fish health specialist will describe in detail the specific veterinary management tools and measures for successful disease prevention and optimization of growth performance in Fish and Shrimp recirculated culture systems.

This presentation will be based on over 30 years of experience in veterinary management of recirculated culture systems in Israel and abroad.

HOLOGENOMIC ANALYSIS REVEALS COMBINED INFLUENCE OF HOST GENETICS AND GUT MICROBIOME ON FEED EFFICIENCY IN ATLANTIC SALMON *Salmo salar*

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The genetic improvement of feed efficiency (FE) is imperative to augment profits and promote sustainable production in aquaculture. The essential role that the gut microbiome plays in digestion and nutrient metabolism implies its significant involvement in the efficient utilization of feed. However, the influence of gut microbiota, and its potential interaction with host genetics, on FE has not yet been investigated in any aquaculture species. In this study, a hologenomic approach using a series of linear mixed animal models was applied to determine the effects of host genetics and gut microbiota on growth and individual indicator traits for feed conversion ratio (IFCR) and feed efficiency ratio (IFER) based on muscle ^{13}C (AMC) and ^{15}N (AMN) isotope profiles in Atlantic salmon.

Using a total of 690 samples, the host genetics was found to affect relative weight gain (RG) and FE indicator traits explaining 6-26% of the variations for the said traits (Figure 1). The gut microbiota also explained 14% and 32% of variations in RG and AMN-based FE indicator traits, respectively. On the other hand, the microbes in the gut showed no effect on AMC-based indicator traits for FE.

Comparison of heritability and microbiability using full (including both microbial and animal genetic effects) and reduced (including either microbial or animal genetic effects) models revealed almost no change in the estimates indicating independent effects of host genetics and gut microbiome on growth and FE. Results of microbiome-wide association analyses revealed that *Jeotgalibaca* were linked with growth and FE while *Lactobacillus* and *Hyphomicrobium* were associated with AMN-based FE traits exclusively. Among the identified microbes, *Jeotgalibaca* was also discovered to be heritable.

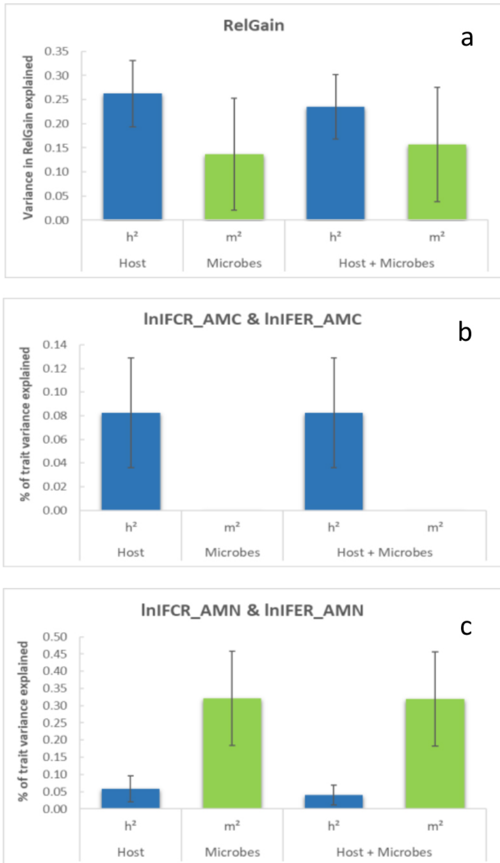

Figure 1. Heritability and microbiablity estimates for (a) relative weight gain, (b) AMC-based and (c) AMN-based feed efficiency indicator traits.

The present study confirmed the combined effects of gut microbiome and host genetics on growth and FE in Atlantic salmon. However, their influence on the analysed traits were independent of each other thus a separate management should be implemented.

ASSESSMENT OF DISEASE CONTROL MEASURES IN AQUACULTURE: APPLICATION OF A FLEXIBLE MODELLING FRAMEWORK

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Infectious diseases are a major threat to aquaculture sustainability. Once established aquatic diseases can spread rapidly due to the high connectivity between aquaculture sites. Control measures such as movement restriction, contact tracing, culling, disinfection and fallowing can be implemented to mitigate this spread. These measures are often associated with substantial costs. Therefore, evidence is necessary to provide epidemiological and economic justification for implementing such control measures. To this aim, mathematical modelling frameworks can be used to simulate disease epidemics within the aquaculture industry and evaluate the efficiency of control measures, providing valuable insights to inform biosecurity decision-making.

Here, we present the Aquaculture Network Model (referred to as Aqua-Net Mod), a mathematical modelling framework underpinned by a network representing aquaculture site connectivity via several mechanisms such as along river networks and live fish movements. Using real-world data representative of the salmonid aquaculture connectivity network in England and Wales, the model simulates disease spread, facilitating evaluation of the epidemiological impacts of disease outbreaks, and the cost-benefits of control measures.

Aqua-Net Mod outputs provide a better understanding of disease epidemics in highly connected aquaculture networks and offer essential insights into the most economically and epidemiologically effective measures to mitigate the impacts of important salmonid diseases. The model is thus a comprehensive and adaptable tool that supports preparedness and cost-efficient response to disease outbreaks in aquaculture.

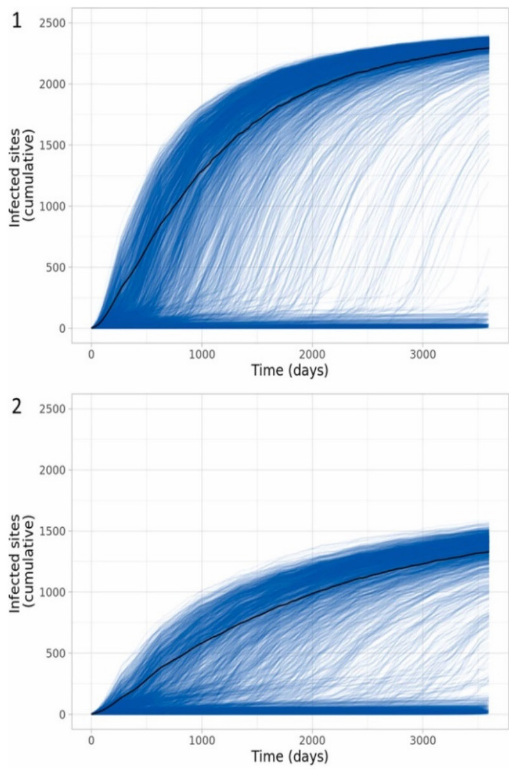

Fig 1. Cumulative number of infected site transmissions under viral haemorrhagic septicaemia (VHS) simulations under 1. No disease controls (top) and 2. Movement restriction for most connected sites (bottom). Black line corresponds to the mean.

HARNESSING EFFICIENT GENETIC IMPROVEMENT TO MAXIMIZE ECONOMIC RETURN

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Genetic improvement programs play a pivotal role in maximizing productivity and profitability in various industries, including aquaculture. This talk presents a comprehensive analysis of the Investment Returns (IR) for genetic improvement investments in shrimp, specifically focusing on different breeding strategies: mass selection, family selection, and genomic selection.

The findings demonstrate that all three breeding strategies contribute to long-term economic benefits for companies investing in genetic improvement. Mass selection, a traditional method based on phenotypic evaluation, allows for genetic progress over generations, leading to improved performance and profitability but with a lower IR than the other strategies. Family-based selection, which involves selecting and breeding individuals from superior families, provides enhanced genetic gains and economic returns. Genomic selection, a cutting-edge approach utilizing genomic information, enables rapid genetic progress by accurately predicting the genetic merit of individuals and by allowing for within family selection, producing the highest economic and genetic gains.

The comparative analysis reveals that genomic selection outperforms mass and family selection in terms of IR. By harnessing genomic information, companies can make more precise breeding decisions, resulting in accelerated genetic gains and higher profitability. However, mass selection and family selection remain viable options for companies with limited genomic resources or specific breeding objectives. Mass selection, despite having lower accuracy, still offers considerable genetic progress and cost-effective improvement opportunities. Family selection, on the other hand, provides greater control over specific traits and allows for customized breeding goals, making it suitable for specialized markets or niche product lines.

The analysis also reflects the challenges and considerations associated with each breeding strategy. Genomic selection requires substantial investment in genomic technology, data management, and skilled personnel. Mass selection may necessitate larger population sizes to maintain genetic diversity, while family selection demands careful family management and increased record-keeping efforts.

In conclusion, genetic improvement investments utilizing different breeding strategies offer substantial IR in the long term. While genomic selection stands out as the most efficient and accurate approach, mass selection and family selection remain viable alternatives depending on the specific needs and resources of the company. Understanding the economic impact and trade-offs of each breeding strategy enables companies to make informed decisions and optimize their genetic improvement investments.

ASSESSMENT OF EU REGULATORY LANDSCAPE FOR SEAWEED CULTIVATION AND MARKET

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In November 2022, the EU proposed 23 actions to facilitate the growth of a sustainable seaweed industry. These actions aim to enhance governance, improve the business environment, bridge research gaps, and raise social awareness. Despite its potential, the European seaweed industry faces systemic challenges.

A regulatory assessment made in January 2024 by the project SeaMark, identified some key obstacles for the expansion of a European seaweed market. These were mainly uncertainties in Novel Food status, strict health-claim requirements, unaligned regulations across borders in both authorization and cultivation, and a lack of separation between organic and inorganic arsenic limits. The obstacles differed between the three focus applications; green alginate, beta-glucan and fermented pig feed, which also highlights a difficult regulatory environment. Also, the study found that compared to the seaweed market in other continents, there is generally a lack of incentives in the EU for accelerating seaweed cultivation, such as subsidies and market development programmes. Compared to agricultural legislation (e.g. CAP), this makes the thresholds for success higher for aquaculture in general, and seaweed in particular due to the novelty aspect.

Recommendations to ease the regulatory challenges and foster the sustainable and circular economy seaweed offers in the EU are:

- Standardize and expedite licensing for seaweed farms.
- Speed up authorization processes while ensuring transparency and harmonization.
- Develop precise regulatory guidance for macroalgae.
- Provide targeted support, subsidies, and infrastructure funding, especially for SMEs.
- Encourage international collaboration and alignment with global standards.

Since the completion of the assessment, new species and derivatives from seaweed were given approvals on their novel food status, a stakeholder consultation on maximum levels for inorganic arsenic is under consultation, a green claims directive has been voted on in the parliament, and other reforms affecting the seaweed market are expected. This will be followed in SeaMark and AlgaeProBANOS looking at product commercialization, go-to-market strategies, sustainability assessment and general development of seaweed products. Also, the EU Algae Industry Study, supporting a sustainable EU algae industry, will examine the current regulatory ecosystem and the potential for strengthening the market deeper.

UNDERSTANDING FACTORS INFLUENCING THE ENVIRONMENTAL, ECONOMIC AND SOICAL SUSTAINABILITY OF AQUACULTURE SYSTEMS

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Globally, aquaculture production has been the food production technology with the highest growth rate in recent decades. While this is a strong indication of economic sustainability, there are significant differences between species and locations. Moreover, the rapid growth has led to the environmental sustainability of the industry being questioned, although also here the picture is getting nuanced as some sectors have better performance than others. The rapid production growth also leads to societal change, and this is creating a further sustainability challenge for the industry.

A challenge when one is interested in comparing different production systems is that comparable data is generally not available beyond production numbers. In this paper we will use data collected using the Aquaculture Performance Indicators for 58 aquaculture production systems to investigate which factors are most important for sustainability for the tree sustainability pillars. The analysis is conducted using regression trees and random forest estimation.

The preliminary results indicate that for all three pillars, general societal factors related to governance and economic conditions are more important than aquaculture specific measures. For both economic and societal sustainability, the ability for collective actions and viable industry organizations are also important.

THIAMPHENICOL AND FLORFENICOL COMBINATION IN NILE TILAPIA: SIMULTANEOUS DETECTION AND QUANTIFICATION IN PLASMA AND MUSCLE PLUS SKIN SAMPLES, AND PHARMACOKINETICS FOLLOWING SINGLE ORAL ADMINISTRATIONCOMBINATION OF ANTIMICROBIALS AS AN APPROACH TO REDUCE THEIR USE IN AQUACULTURE: EXAMPLE OF USING THIAMPHENICOL WITH FLORFENICOL AGAINST *Aeromonas hydrophila*

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The use of highly effective combination of antimicrobials (CA) is a promising strategy for increasing treatment efficacy, controlling the bacterial drug resistance and reducing the use of antimicrobials in aquaculture. In the present work, we investigated the *in vitro* activity and *in vivo* therapeutic effectiveness of thiamphenicol (TAP) combined with florfenicol (FFC) against *Aeromonas hydrophila* isolated from diseased Nile tilapia *Oreochromis niloticus*. Individual minimum inhibitory and bactericidal concentrations (MIC and MBC) of each antimicrobial were determined for 26 strains of *Aeromonas* isolated from pacu *Piaractus mesopotamicus* and Nile tilapia during disease outbreaks in Brazil. MICs were used to perform the checkerboard microdilution assay to evaluate the *in vitro* effect of the combination (fractional inhibitory concentration index – FICI). Recommended doses of each antimicrobial (G1: TF – 10 mg kg^{-1} bw, and G2: FFC – 10 mg kg^{-1} bw) and two doses of TAP combined with FFC (G3: TAP + FFC – 5 + 2.5 mg kg^{-1} bw, and G4: TAP + FFC – 2.5 + 1.25 mg kg^{-1} bw) correspondent to FICI deduction, were administered by intraperitoneal injection to Nile tilapia (70 ± 12 g) challenged by gavage with *A. hydrophila*, to evaluate the therapeutic effectiveness of CA.

Aeromonas was sensible to the antimicrobials individually (Table 1) and combined (FICI ≤ 0.75), although some strains of *A. caviae* and *A. hydrophila* were somewhat less sensitive to TAP (>8 µg mL^{-1}), indicating that resistant strains are gradually emerging even though TAP is not approved for use in aquaculture in Brazil.

In fish aeromoniosis model, combinations of TAP and FFC (G3 and G4) were effective as the standards doses of the single antimicrobial (G1 and G2), and all medicated groups were statistically different ($p < 0.05$) from the unmedicated group (G5) (Figure 1).

These findings suggests the potentiality of CA involving TAP and FFC as an effective approach to control aeromoniosis with lower doses of antimicrobials.

Table 1. Antibacterial activity of TAP and FFC against *Aeromonas*.

Strains used (n)	Fish specie*	Period of isolation	Antibacterial activity (µg mL^{-1})			
			TAP		FFC	
			MIC	MBC	MIC	MBC
Aeromonas caviae (1)	1	2014	8	8	-	-
Aeromonas hydrophila (17)	1 and 2	2011 - 2015	0.5 - 32	1 - 32	0.5 - 2	0.5 - 2
Aeromonas jandaei (4)	2	2011 - 2017	1 - 2	1 - 2	0.5 - 2	0.5 - 4
Aeromonas veronii (4)	1 and 2	2013 - 2015	0.5 - 1	1 - 2	0.25 - 1	0.25 - 1
ATCC 25922® *Escherichia coli***		-	128	512	8	32

*Fish species: 1-pacu, *Piaractus mesopotamicus* ; 2- Nile tilapia, *Oreochromis niloticus*.
** Quality control.

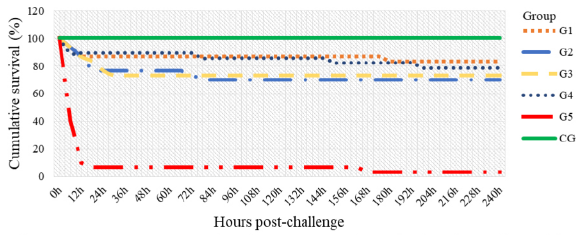

Figure 1. Cumulative survival (%) of Nile tilapia challenged with *A. hydrophila* and medicated with thiamphenicol (TAP) or florfenicol (FFC) alone and combined. G1: TAP (10 mg kg^{-1}); G2: FFC (10 mg kg^{-1}); G3: [TAP + FFC] (5 + 2.5 mg kg^{-1}); G4: [TAP + FFC] (2.5 + 1.25 mg kg^{-1}); G5: unmedicated; and CG: Control group (Phosphate Buffered Saline).

ENHANCED PROTECTION OF SHRIMP *Litopenaeus vannamei* AGAINST YELLOW HEAD VIRUS THROUGH DELIVERY OF VIRUS-SPECIFIC DSRNA VIA A COMPOSITE NANOMATERIAL

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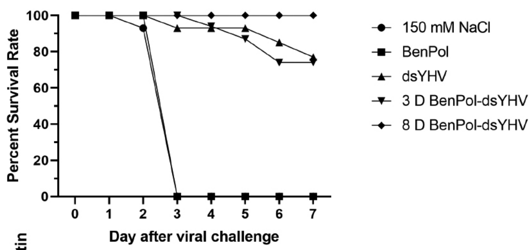
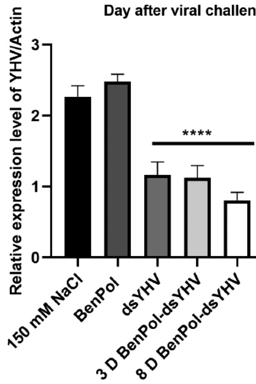
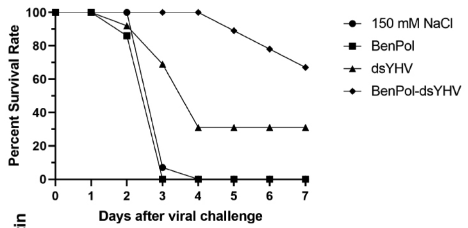
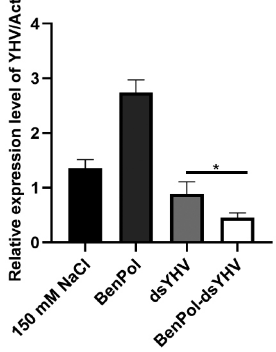
Crustacean aquaculture is vulnerable to viral diseases. RNA interference (RNAi) technology could increase resistance against these viruses, depending on effective delivery systems for double-stranded RNA (dsRNA). However, dsRNA's instability poses challenges, particularly in aquatic settings.

This study evaluated composite polymer-clay nanoparticles made from a bentonite biopolymer (BenPol) to protect dsRNA from degradation and enhance its delivery to *Litopenaeus vannamei* shrimp. Specifically, dsRNA targeting the protease gene of yellow head virus (dsYHV) was incorporated into BenPol nanoparticles, forming BenPol-dsYHV complexes. These complexes effectively protected dsYHV from nuclease digestion and maintained stability under simulated environmental conditions over time. To assess release efficiency, complexes were stored at 4°C for 3 or 8 days before being injected into shrimp, which were then observed for 7 days before a yellow head virus challenge. Shrimp treated with complexes prepared 8 days before injection showed a 100% survival rate, significantly higher than other groups. Additional tests on the 8-day prepared complexes determined the duration of protection offered against YHV infection. Shrimp injected with these complexes 7 and 21 days before a YHV challenge showed 33% and 69% mortality rates, respectively, indicating prolonged protective effects. These findings highlight BenPol's potential as a promising nanoparticle-based delivery platform for dsRNA, effectively protecting against viral infections in aquaculture.









COMPERATIVE EFFECT OF DIFFERENT PLASTICIZERS ON PHYSICOCHEMICAL PROPERTIES OF HYDROXYPROPYL METHYLCELLULOSE (HPMC)-BASED FILMS AND APPLICATION IN GILT-HEAD SEABREAM

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Edible packaging is an innovative method to address the problem emerged by petroleum based materials due to their non-toxicity and safety to improve quality by controlling microbial growth (Majumder and Balange 2023). Hydroxypropyl methylcellulose (HPMC) is a cellulose derivative which is used as packaging material due to biocompatibility, odorless and tasteless compound without affecting the fish organoleptic characteristics (Athanasopoulou et al. 2024). The aim of the study was to synthesize HPMC films with the best properties for preservation of gilthead seabream fillets.

2% HPMC-based films were produced according to the solvent casting method and glycerol was tested as a hydrophilic plasticizer while oleic and linoleic acid were used for their hydrophobic nature. Water barriers, hydrophobicity, mechanical, optical, and morphological properties, were evaluated HPMC-based films were applied on gilthead seabream fillets (*Sparus aurata*) stored isothermally at 4°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.

Moisture content showed that by adding hydrophobic materials used as plasticizers showed lower moisture content (8.49±0.26% and 13.42±1.91% when used linoleic and oleic acid, respectively), while moisture content was 77.26±2.75% for films with glycerol. All films showed high values of lightness and opacity. The replacement of conventional PVC films with the developed HPMC-based films did not affect the microbial growth of gilthead seabream fillets during refrigerated storage, resulting at shelf-life of 10 days at 4°C (same for all the tested conventional and alternative packaging films).

The results of the study show the potential of HPMC-based films for the effective preservation of chilled gilthead seabream, without affecting quality and shelf-life. The appropriate design of HPMC films and the selection of appropriate plasticizer for the desirable physicochemical properties of the developed packaging systems will contribute to the sustainability of the aquaculture sector towards a zero-waste future.

References

Athanasopoulou E, Bigi F, Maurizzi E, et al (2024) Synthesis and characterization of polysaccharide- and protein-based edible films and application as packaging materials for fresh fish fillets. Sci Rep 14:517. https://doi.org/10.1038/s41598-024-51163-y

Majumder RK, Balange AK (eds) (2023) Advances in fish processing technologies: preservation, waste utilization, and safety assurance, First edition. Apple Academic Press ; CRC Press, Palm Bay, FL : Boca Raton, FL

SCREENING, SELECTION, AND IDENTIFICATION OF AUTOCHTHONOUS POTENTIAL PROBIOTIC BACTERIA AGAINST THE RAINBOW TROUT PATHOGEN *Flavobacterium psychrophilum*

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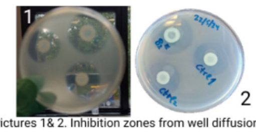
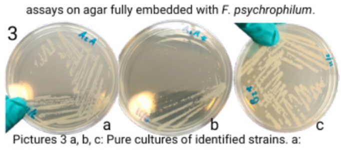
Introduction: *Flavobacterium psychrophilum* is a globally distributed pathogen affecting salmonid aquaculture and is responsible for the lethal Bacterial Cold Water Disease (BCWD) and Rainbow Trout Fry Syndrome (RBFS) in adult and fry/juvenile rainbow trout individuals, respectively. The prophylactic and therapeutic use of antimicrobials against this pathogen have resulted in the development of antimicrobial resistance (AMR), while there is no effective and commercially available vaccine for adults, to date. Therefore, the development of alternative sustainable biocontrol measures against this detrimental pathogen is required. The purpose of this study was to develop an assay for the primary identification of candidate probiotic strains against *F. psychrophilum*. These strains should be sourced from within the rainbow trout aquaculture environment, utilizing an element that already exists within the microbiome system, rather than introducing a new element, focusing on recirculating aquaculture units.

Methods: *F. psychrophilum* strain 950106-1/1 previously isolated from rainbow trout farms in Denmark was fully embedded in Tryptone Yeast Extract Salts agar (TYES-A) supplemented with glucose. Rainbow trout aquaculture tank biofilm and water samples were tenfold diluted and plated on TSA media. These plated samples were then replica plated on *F. psychrophilum* embedded plates. For this assay, *Pseudomonas fluorescens* AH2 was used as a positive inhibition control. Following the examination of the embedded plates for inhibition zones, the topologically corresponding colonies within the master plates were selected and purity-streaked to further confirm inhibitory activity through the same well diffusion assay, in triplicates. The colonies which produced clear inhibition zones were later selected for identification using MALDI-TOF via MALDI Biotyper. Whole Genome Sequencing of the inhibitory strains is underway, which, coupled with analyses of secondary metabolite potential (anti-SMASH), would reveal genomic structures pinpointing the molecules involved in the antagonistic activity of the strains.

Results: The MALDI-TOF results revealed 3 probiotic candidates following the screening of approximately 600 replica-plated colonies on the *F. psychrophilum* embedded agar. One of the candidates was identified to belong to the *Pseudomonas* genus, without a clear species identification. The other two candidates were identified as *Pseudomonas gessardii* and *Janthinobacterium lividum*. The latter has previously been identified as a member of the gut microbiome of salmonids, while *Pseudomonas gessardii* has also been retrieved from rainbow trout aquaculture units.



Pictures 1& 2. Inhibition zones from well diffusion assays on agar fully embedded with *F. psychrophilum*.



Pictures 3 a, b, c: Pure cultures of identified strains. a: *Pseudomonas* sp. , b: *Janthinobacterium lividum*, c: *Pseudomonas gessardii*

Conclusion: This assay paves the way for the continuation of exploration of autochthonous probiotic candidates against *F. psychrophilum*. The screening would follow the expansion of the microbial sample pool, including samples from more aquaculture units and healthy rainbow trout individuals.

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Acknowledgements: We would like to thank DTU Aqua senior researcher Lone Madsen and laboratory technician Kari Mouritsen for their assistance with strain identification, and University of Copenhagen professor Mathias Middelboe for providing the samples.

ASSESSMENT OF THE GROWTH AND NUTRIENT UTILIZATION OF *Clarias gariepinus* FINGERLINGS FED DIFFERENT INCORPORATION LEVELS OF PREPARED *Moringa oleifera* SEED MEAL

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Introduction: Exploration of alternative plant protein sources for their prospect as potential replacements for fish meal has been intensified in recent times and *Moringa oleifera* (Lam) has been identified as one of such prospective candidates (Nsofor *et al*., 2012). However, the elimination of undesirable anti-nutritional substances from plant protein sources is imperative to improve their nutritional quality and maximize their full potential as components in fish feed.

Methodology: The trial took place at the Teaching and Research Fish Farm within the Department of Fisheries and Aquaculture at Usmanu Danfodiyo University in Sokoto located on latitude 13^{0} 07' 47.6''N, longitude 05^{0} 12'11.3''E and altitude 275m above sea level (Google MAP, 2015). Five experimental diets were formulated, and fishmeal was replaced with the processed (B90min/S72hrs) *Moringa oleifera* seed meal at 0, 20, 40, 60 and 80% inclusion levels and fed to the fingerlings for twelve weeks. Fish growth, feed conversion and nutrient utilization indices were determined.

Results: Highest weight gain (12.77±1.14g) was recorded in the fish with diet T1 which is the control diet but, the difference was not significant from dietary treatment T2 with 20% MSM replacement level. Lowest FCR was recorded with fish fed diet T2 and there was no statistically significant difference in the PERs of all the treatments.

Discussion and Conclusion: Diet with 20% substitution level that contributed 8.33% by gross composition enhanced good growth, and the fingerlings were still healthy at 80% substitution level. The findings of the feeding experiment revealed a similar ($p>0.05$) survival rate across the treatments. However, the growth response decreased with increased inclusion level of the test ingredient MSM in the diets.

Table 1: Growth parameters of *Clarias gariepinus* fingerlings fed diets incorporating different concentrations of *Moringa oleifera* seed meal.

	Treatments				
Parameter	T1 (0%)	T2 (20%)	T3 (40%)	T4 (60%)	T5 (80%)
No of fish stocked	20	20	20	20	20
Mean initial weight (g)	1.50±0.00	1.50±0.00	1.50±0.00	1.50±0.00	1.50±0.00
Mean initial length (cm)	6.17±0.06^{a}	6.17±0.06^{a}	6.13±0.06^{a}	6.17±0.06^{a}	6.17±0.06^{a}
Survival rate (%)	78.33±2.89^{a}	78.33±2.89^{a}	73.33±5.77^{a}	70.00±13.23^{a}	75.00±0.00^{a}
Mortality rate (%)	21.67±2.89^{a}	21.67±2.89^{a}	26.67±5.77^{a}	30.00±13.23^{a}	25.00±0.00^{a}
Mean final weight (g)	14.29±1.12^{a}	12.35±0.43^{ab}	11.08±0.13^{b}	8.48±2.27^{c}	8.21±0.36^{c}
Weight gain (g)	12.77±1.14^{a}	10.87±0.40^{ab}	9.57±0.12^{b}	6.97±2.25^{c}	6.70±0.35^{c}
Percentage weight gain (%)	849.13±72.06^{a}	720.39±27.85^{ab}	636.71±10.24^{b}	463.72±151.55^{c}	446.03±24.56^{c}
Specific growth rate (%/day)	2.26±0.08^{a}	2.12±0.03^{ab}	2.01±0.01^{b}	1.74±0.26^{c}	1.73±0.04^{c}
Mean final length (cm)	10.17±0.45^{a}	10.13±0.55^{a}	10.07±0.42^{a}	9.20±0.56^{b}	9.20±0.17^{b}
Condition factor (K)	1.36±0.11^{a}	1.20±0.15^{ab}	1.09±1.14^{ab}	1.14±0.49^{ab}	1.05±0.01^{b}

Mean values in row having different superscripts are significantly different ($p<0.05$)

OFFSHORE MOORED FISH CAGE FLUID-STRUCTURE INTERACTION MODELLING

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Introduction and Motivation: Offshore fish cages are of strong interest due to availability of open space and the potential in reducing adverse effects on the environment. However, offshore fish cages also introduce significant technological challenges to withstand the harsh environment of the sea, provide sufficient water exchange with the sea while minimising any adverse effect on the sea environment (from altering sea currents and sediment to waste disposal and disease transmission). To assist the design of such a fish cage system, an accurate high-fidelity model of the cage's hydrodynamics and structural response is highly desirable. In this paper we present a new advanced high-fidelity computational model of the fluid-structure interaction (FSI) of a full fish cage moored and floating on the water surface, and use it for hydrodynamics-structural analysis.

Methodology: The computational fluid dynamics OpenFOAM software has been modified to simulate the moored floating fish cage FSI, where unsteady RANS or Large Eddy Simulation along with a volume of fluid is used as the flow solver. The structural solver includes 1D line (beam, cable, mass, spring) elements modelling the net, collar and mooring lines. Following our previous submerged cage study [1], an immersed boundary method is used to model the load and displacement interaction between the fluid (water) and the fish cage structure. Second order accuracy in space and implicit first order accuracy in time is preserved.

Results and Analysis: The new solver has been rigorously validated against several benchmark problems of multiple planar and circular nets subjected to a range of incoming surface waves and currents. The moored system has also been simulated as illustrated in Fig 1, achieving excellent agreement with known experimental results in terms of surface evaluation and mooring line tension forces. The drag force acting on the fish cage subjected to incoming regular surface waves have been analysed to show sharp peaks in the force's time variation for the collar and broader peaks for the net, indicating load absorption by the system. The effect of water exchange on waterborne diseases transmission will be briefly discussed in the conference using analysis of particle dynamics simulation (based on Lagrangian approach). Finally, the ability of this computational model to simulate adds-on to the system as a small kinetic turbine (wind/water) to supply green energy to the fish cage assembly will also be highlighted.

[1] Mi et al (2022) *Ocean Eng* 264, 112843

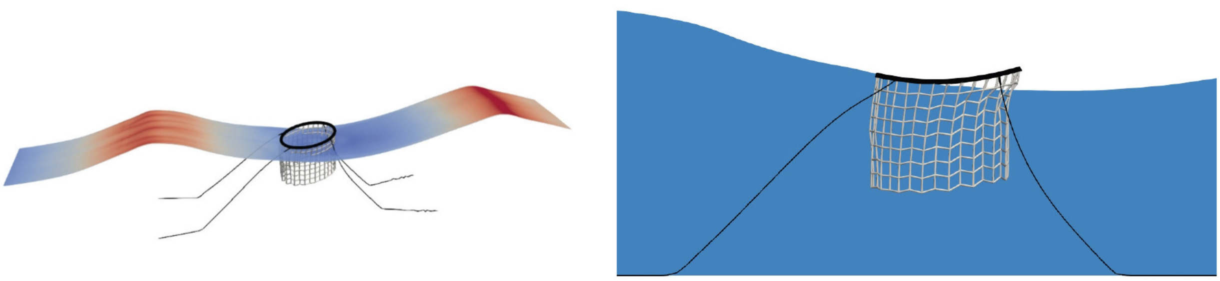

Fig 1*: Snapshots of the moored fish cage subjected to incoming regular free surface waves.*

CIRCULAR AQUACULTURE: INTRODUCTION

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Technion, Israel Inst of Technology

In order to satisfy expected population rise and need for proper and healthy nutrition demand, Aquaculture production has to double till 2050. However, suitable land, water and feed are limited. Undoubtedly, we need to efficiently use and recycle inputs that are rare and expensive, minimizing waste of resources and maximize production. In the session ahead of us we will learn of different means of achieving these goals and later' in the afternoon session, discuss potential means to work together along these lines, achieving intensive circular aquaculture.

Traditional aquaculture annual production is 20-40 ton fish/ha. Average annual evaporation is 35,000 m^3, i.e. about 1.3 m^3 per kg fish. In properly managed biofloc technology (BFT), annual production can reach 400 ton/ha, i.e. about 0.1 m^3 water /kg fish, enabling good production even under arid conditions. In traditional aquaculture farmers use to replace used water with newly fresh water, wasting water and polluting the environment. Using different modes of aquaponics you recycle the water between fish and plants compartments, saving water and using excessive nutrients by the growing plants.

In traditional aquaculture feed conversion ratio (FCR) is about 20%. With BFT, the excessive feed waste is harvested by micro-organisms, serve as a base to water biota and then eaten by fish. Protein utilization is doubled, feed is eaten by fish as feed pellets and then harvested as nutrient rich bioflocs. In addition, bioflocs improve disease resistance and immunity of fish and shrimp. Moreover, studies prove that the residual flocs can be drain, collected and used as a raw material for feed production. So we will be able to recycle the feed twice, a true circular process!

A way to maximize production, even for fish that require clear water for proper growth is by using RAS (Recirculation Aquaculture Systems), where the water in the fish compartment is continually drained, pass though filters and biofilters and returned as clear water to the fish compartment. In most such systems, the collected residues are disposed or expensively treated. In some new experimental RAS units, the fish tank water is are recycled through a biofloc tank, or possibly with an aquaponic system. IMTA (Integrated Multi Trophic Aquaculture), stocking different species in the pond, utilizing different feed sources improves FCR and raises total production. Aqua-mimicry systems used in SE Asia contain production and water treatment reservoirs, and external aerated reactors fed mostly with rice bran, releasing large quantities of copepods and other species into the production reservoir. Different technologies applied together offer new perspectives for aquaculture responding to development and environmental stakes.

The plan of the day ahead is to review the different technologies and develop among us a way to work together toward efficient circular aquaculture technologies,. based upon our common knowledge, to be further discussed in the afternoon session.

PHYTOCHEMICAL CONSTITUENTS OF *Azadirachta indica*, ITS IMPORTANCE AS ANTI-WATER FOULING AND ANTI-STRESSOR IN CULTURED *Clarias gariepinus* (BURCHELL, 1822)

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Plant phytochemicals have been known to boost the immunity of fish with the release of electrolytes in the cultured water body. This study evaluates the phytochemicals present in the neem plant that can acts as anti-water fouling and anti-stress for culturing fingerlings of African Catfish *Clarias gariepinus*. Fresh matured leaves of *Azadirachta. indica* (neem) were collected, air dried and aqueous-extract was used to screen for some phytochemical presence. Two hundred (200) fingerlings of *Clarias gariepinus* were purchased from LASU hatchery and stocked in a 500 litres tank for 3 weeks (21days) in order to induce stress on the fishes by overstocking, water was not changed and feeding was not regular and fed once per day. The leaf extracts were analyzed at the World Bank Step B Laboratory of the Federal Institute of Industrial Research, Oshodi, (FIIRO), Lagos State using the Atomic Absorption Spectrophotometer (SCHIMAZU-AA-7000). Five (5) grams of the air-dried and finely pulverized sample was weighed into a 100 ml of digestion flask. For proximate composition, moisture content was determined using the hot air oven, by drying the sample at 105 °C ± 2 °C until a constant weight was obtained. Total lipid was determined by Bligh and Dyer method using chloroform/methanol (1/1, v/v). Crude protein content was determined by Kjeldahl's method (Nx6.25). Ash content was determined after combustion. The result revealed the moisture (5.67±0.35%), Carbohydrate (82.15±0.92%), Protein (1.52±0.06%), Ash (2.96±0.03%), fibre (5.04±0.0.11%) and fat content (2.65±0.34%). The mean Temperature values observed was (27.92±1.24) mg/ L in the control tank (tank 1) and (26.95± 1.34) mg/L in the tank with neem leaf (tank 2). The dissolved oxygen recorded was (5.70± 1.23) mg/ L in tank 1 and (3.82±0.98) in tank 2. The pH value ranged from (6.65±1.08) mg/ L (minimum value), which was observed in tank 1 while the highest value of (7.66±1.17) mg/ L obtained in tank 2. All other water quality parameters measured such as Total suspended solids, CO_2, and Ammonia were within the permissible limit as stated by WHO/USEPA. The mineral content had Calcium, Magnesium, Potassium, Sodium, Iron, Zinc, Lead, copper, Manganese and Nickel. Magnesium (2019.125±11.349) recorded the highest value, while Nickel (0.033±0.297) was recorded with the lowest value, Ninety-eight (98) chemical compounds were obtained in *Azadirachta indica*. Some of the chemical compounds include 2,2-Dimethyl-1,3-dichloro-1-cyano-4 (trichloromethyl)cyclobutane, Tocopherol, Octadecanoic acid methyl ester, Propanoic acid 2-oxo- methyl ester, Cyclopentanol,5,7-dimethyl-6,8-dioxabicyclo[3.2.1]octane, 3,5,5-trimethyl-4-Amino-6-hydroxypyrimidine, Hexadecanoic acid methyl ester and n-Hexadecanoic acid In conclusion, neem leaf were able to reduce water fouling within thirty days as well boost fish immunity with low fish mortality when fish were stressed.

Table 1: Physical and chemical parameters of the water used during the experiment

	Dissolved Oxygen	Carbon iv oxide(mg/L)	Ammonia (mg/L)	Temperature (^{0}C)	pH
Control Tanks	5.70± 1.23^a	2.30± 0.86^a	0.50± 0.09^a	27.92± 1.24^a	6.65± 1.08^a
Tanks with neem leaves	3.82± 0.98^b	3.54± 1.48^b	1.45± 0.89^a	26.95±1.34^{ab}	7.66±1.17^{ab}

Mean with different superscript in the column are significantly different at ($p<0.05$)

Table 2: Mineral contents in *A. indica* (Neem) leaf

Minerals	(mg/100g)
Calcium (Ca)	1294.135±7.389^a
Magnesium(Mg)	2019.125±11.349^a
Potassium(K)	1308.180±13.081^a
Sodium(Na)	1283.350±13.944^a
Iron(Fe)	3.208±0.322^a
Zinc (Zn)	5.060±0.085^a
Lead(Pb)	0.262±0.071 ^{a}
Copper(Cu)	2.073±0.091^a
Manganese(Mn)	2.073±0.091^a
Nickel(Ni)	0.033±0.297^a

Mean with different superscript in the column are significantly different at ($p<0.05$)

SOCIO-ECONOMIC OPPORTUNITIES AND CHALLENGES OF SEAWEED POLYCULTURE (*Gracilaria* sp.) IN BREBES REGENCY, INDONESIA

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Indonesia accounts for nearly 30% of seaweed production worldwide and thereby is one of the primary global producers (Cai, 2021). The main seaweed genera produced in Indonesia representing about 20% of the weight produced and 18% of the value is *Gracilaria* sp. In 2021, national *Gracilaria* sp. production was approximately 1.9 million tonnes/year, worth around USD 370 million (KKP, 2023).

Part of Indonesian production is done in polyculture, defined as the joint cultivation of two or more species. This can improve pond productivity and increase environmental quality in the pond. Despite the potential benefits of polyculture, the socio-economic elements of the polyculture practices remain largely understudied. Investigated with means of literature review, interviews and a survey, this article aims to better understand the socio-economic aspects of polyculture seaweed farming. This study explores the case of seaweed culture (*Gracilaria* sp.) in Brebes Regency, Central Java Province, Indonesia.

The results presented illustrate that – from a financial perspective – there is no obvious incentive for farmers to switch to polyculture as there is no significant impact on operating profit (Figure 1). Two key considerations that influence adoption of polyculture practices are the relationship to other household productive activities and non-financial benefits of polyculture.

From societal perspective, polyculture is an alternative supporting farmers food resilience and offering the possibility to generate higher revenues per m^2 (Figure 2). It is needed to support farmers and farmer organisations in adopting polyculture practices. This can be done by increasing their bargaining power vis-à-vis the middlemen and/or financial support that enables farmers to do upfront investments in polyculture.

Figure 1. Average revenue, cost and profit per m^2

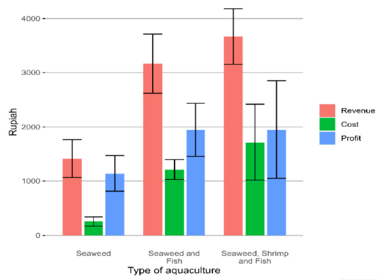
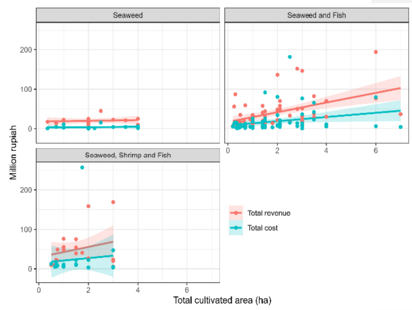


Figure 2. Total revenue vs total cost per ha across cultivation type



References
Cai, J. (2021). *Global status of seaweed production, trade and utilization.* www.fao.org/fishery/statistics/software/fishstatj/en.
KKP. (2023). Statistics KKP : fishery production. https://statistik.kkp.go.id/home.php?m=total&i=2#panel-footer. Retrieved on 5 May 2023.

NEUROPROTECTIVE AND ANTIOXIDATIVE PROPERTIES OF *Sargassum hornschuchii*, BROWN MACROALGA FROM THE ADRIATIC SEA

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The challenging environment of the Adriatic Sea, characterized by high salinity, temperature fluctuations, and intense UV radiation, has driven macroalgae to evolve bioactive compounds with unique properties and various potential applications in the cosmeceutical, pharmaceutical, and food industries. With such a hypothesis, *Sargassum hornschuchii* was sampled from the Adriatic Sea, extracted with methanol : dichloromethane and further fractionated by solid phase extraction to obtain less polar fraction. Chemical composition was determined by UHPLC-ESI-HRMS. Antioxidant activity of the obtained fraction was assessed using ABTS, DPPH, and FRAP assays, while neuroprotective activity was determined with acetylcholinesterase inhibition assay. In addition to its beneficial properties, the toxicity potential of *S. hornschuchii* fraction was determined *in vivo* using zebrafish *Danio rerio* embryos.

Tested fraction was found to effectively inhibit acetylcholinesterase, an enzyme associated with neurological disorders. Specifically, in concentrations of 1, 0.5 and 0.25 mg/mL, the fraction exhibited acetylcholinesterase inhibition rates of 61.11 ± 0.79, 41.48 ± 3.48 and 17.60 ± 1.18 %, respectively. Additionally, this research has demonstrated that bioactive molecules fractionated from *S. hornschuchii* exhibit significant antioxidant activity. The antioxidative and neuroprotective properties of bioactive compounds are often interlinked due to their role in mitigating oxidative stress, a significant factor in the development of neurological disorders.

This can be explained by the presence of bioactive molecules identified through UHPLC-ESI-HRMS analysis, such as fucoxanthin and chlorophyll derivatives pheophytin a and pheophorbide a, which have established antioxidant potential. It is important to note that the antioxidant activity cannot be attributed to a particular compound, as various bioactive molecules may act synergistically.

The dual antioxidative and neuroprotective activities of bioactive compounds detected in *S. hornschuchii* suggest significant potential in preventing or slowing the progression of neurodegenerative diseases and stress-related conditions. By reducing oxidative stress, these compounds protect neurons from damage. Additionally, by inhibiting acetylcholinesterase, they support neurotransmitter balance and neuronal health. Furthermore, toxicity assessment conduced on zebrafish *Danio rerio* embryos provided valuable insights into the safety of tested fraction, supporting its potential application across various fields.

A REGENERATIVE MARINE ECOSYTEM SERVICES THAT PAY FOR ITSELF: A PANACEA TO REVENUE GENERATION IN NIGERIA

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Nigeria is situated on the on the western Indian Ocean, where coral reefs are a vital component of marine ecosystems that support traditional fishing and livelihoods in coastal communities, as well as commercial seafood production. One of the fastest and most scalable approaches for marine ecosystem service and aquaculture is the cultivation of reefs using prefabricated structures.

In the global climate change context, carbon dioxide removal at gigaton scale is required to retain any hope of achieving the Parish climate accord objectives. Nigeria has an extensive coastline, with an Exclusive Economic Zone (EEZ) greater than 200,000 square kilometers. Using 1% for seaweed cultivation could remove about 7.6 million tons per year of carbon dioxide (CO2) from seawater, which is actual greenhouse gas removal or reduction.

This paper aim to focus on the rapid expand coral reef and seaweed ecosystems as part of regenerative marine aquaculture which provides climate-proof food security as part of the broader blue economy in Nigeria.

ENHANCING ω3 LC-PUFA BIOSYNTHESIS IN THE POLYCHAETE *Platynereis dumerilii* THROUGH SALINITY MODULATION

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Aquatic invertebrates, including polychaetes, are considered promising sources of ω3 long-chain polyunsaturated fatty acids (LC-PUFA) due to their endogenous synthesis capability facilitated by enzymes such as elongases, front-end desaturases (Fed), and methyl-end desaturases. The biosynthesis of ω3 LC-PUFA in these organisms can be modulated through the diet and environmental conditions such as temperature and salinity. Salinity variations can induce changes in osmotic pressure, leading to adjustments in membrane protein production and fatty acid composition to adapt to new conditions. This offers the opportunity to develop optimized cultivation protocols that enhance ω3 LC-PUFA biosynthesis. This study focuses on investigating the impact of salinity on fatty acid profiles in Platynereis dumerilii, a well-established model in Evolutionary and Developmental Biology. For this purpose, in vivo experiments were conducted by culturing P. dumerilii juveniles at different salinity levels and analyzing the impacts at molecular and compositional levels.

In vivo trials were carried out in triplicate using P. dumerilii juveniles maintained under standard culture conditions (18°C temperature, 16L:8D photoperiod, and frozen spinach used as feed), to evaluate the impact of three salinity levels, namely "low" (30 ‰), standard (35 ‰) and "high" (40 ‰). After one month, survival and growth were determined, and samples were collected to assess the modulation of the ω3 LC-PUFA biosynthesis pathways by analyzing lipid and fatty acid profiles, and gene expression of key desaturases and elongases by qPCR.

Despite the uniform diet, P. dumerilii grown under high salinity conditions (40 ‰) exhibited elevated levels of ω3 LC-PUFA, notably eicosatetraenoic acid (20:4n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosapentaenoic acid (22:5n-3), compared to those maintained at standard and low salinity conditions (35 and 30 ‰, respectively). Such variations in fatty acid profiles suggest a regulatory role of salinity on the P. dumerilii lipid metabolism. Consistently, gene expression analyses revealed a significant upregulation of a Fed desaturase, with Δ6/Δ8 activity, under high salinity (40 ‰). Indeed, fatty acid analyses indicated a decrease in specific substrates of this enzyme, particularly eicosadienoic acid (20:2n-6) and eicosatrienoic acid (20:3n-3), in worms grown at high salinity, in agreement with an enhanced Δ8 desaturase activity as shown in the gene expression results. Collectively, these findings suggest that cultivating P. dumerilii, and potentially other polychaetes, in high salinity environments can enhance their nutritional value by increasing the contents of the essential and health-beneficial ω3 LC-PUFA.

This work is part of the IMPROMEGA Project (RTI2018-095119-B-I00) funded by MCIN/ AEI /10.13039/501100011033/ and FEDER - A way of making Europe. Additionally, this study is part of the ThinkInAzul program funded by the European Union NextGenerationEU (PRTR-C17. I1) and the Generalitat Valenciana (THINKINAZUL/2021/026).

INVESTIGATING THE LC-PUFA BIOSYNTHESIS OF FRESHWATER POLYCHAETES: THE CASE OF *Namalycastis rhodochorde*

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Many aquatic invertebrates, such as polychaetes, serve as excellent sources of the essential long-chain polyunsaturated fatty acids (LC-PUFA), namely eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6). Recent research highlights the presence of complete sets of LC-PUFA biosynthetic enzymes, including elongases (Elovl), front-end desaturases (Fed), and methyl-end desaturases (ω des), in marine polychaetes, with freshwater species being overlooked. We hypothesize that, compared to marine counterparts, freshwater-adapted polychaetes possess an enhanced capacity for LC-PUFA biosynthesis to compensate for the limited availability of these essential nutrients in their environment. The present study aims to test this hypothesis in the nereid polychaete Namalycastis rhodochorde, a species commonly found in freshwater, and highly valued as bait.

Searches were conducted in the N. rhodochorde transcriptome assembly to identify complete sequences of elongases and desaturases homologous to those previously characterized in marine polychaetes. Conserved motifs were identified, and phylogenetic relationships were established. Functional assays were performed in yeast. Conversions of the Elovl, Fed and ω des toward the exogenously supplemented polyunsaturated fatty acids (PUFA) substrates were assessed by analyzing the fatty acid profiles of transgenic yeast.

N. rhodochorde has 3 Elovl, 2 Fed, and 2 ω des involved in LC-PUFA biosynthesis. The elongases, identified as Elovl2/5, Elovl4, and Elovl1/7 based on their homology with vertebrate Elovl enzymes, extend PUFA substrates from C18 to C22. Functional assays showed Δ5 desaturase activity for Fed1 and dual Δ6/Δ8 activity for Fed2. Specifically, Fed1 is a Δ5 desaturase that synthesizes ARA and EPA from their precursors 20:3n-6 and 20:4n-3, respectively. The Δ6 desaturase activity of Fed2 allows the conversion of linoleic acid (LA, 18:2n-6) and α-linolenic acid (18:3n-3) into 18:3n-6 and 18:4n-3, respectively. Furthermore, Fed2 showed Δ8 activity on 20:2n-6 and 20:3n-3, converting them into 20:3n-6 and 20:4n-3, respectively. Two genes encoding ω des have been demonstrated to exist in N. rhodochorde; a Δ12 desaturase enabling the production of LA (de novo synthesis of PUFA), and an ω3 desaturase, with the ability to convert various ω6 PUFA into the corresponding ω3 PUFA products. These findings imply that N. rhodochorde possesses the requisite enzymatic machinery for synthesizing nutritionally valuable LC-PUFA, such as ARA and EPA. This capacity offers the possibility of cultivating these polychaetes using nutritionally poor substrates to create a new alternative and sustainable source of ω3 LC-PUFA-rich ingredients.

This work is part of the POLYPUFA project (PID2022-136234OB-C21), funded by MICIU/AEI /10.13039/501100011033 and by FEDER, UE. Additionally, this study is part of the ThinkInAzul program funded by the European Union NextGenerationEU (PRTR-C17. I1) and the Generalitat Valenciana (THINKINAZUL/2021/026)

NNV RECRUITS PHOSPHO-EIF4E AND INITIATES CAP-DEPENDENT TRANSLATION IN REMODELED MICROTUBULE-ORGANIZING CENTER

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Nervous Necrosis Virus (NNV), the causative agent of massive outbreaks in fishes worldwide, is a bipartite positive-stranded mRNA virus that replicates in mitochondria. Despite decades long studies on NNV life cycle, the mechanisms involved in translation of its proteins remain unexplored. In this study, we observed that newly synthesized NNV RNA replicate in mitochondria via double-stranded RNA intermediate. In addition, both RNA1 and RNA2 move out of mitochondria towards microtubule-organizing center (MTOC) during late infection stage. Using puromycin labeling, we demonstrated that neo-synthesized proteins correlate with NNV RNA2 and coat protein in nascent viral factories (VFs) providing evidence for active translation. By analyzing crucial proteins which are involved in cap dependent translation, we showed that NNV RNA2 and coat protein colocalize with phospho-eIF4E and its regulatory protein p-eIF4E-BP indicating that NNV translation initiation occurs by recruiting and binding of p-eIF4E in these VFs. Moreover, NNV coat protein also correlated with p38 MAPK, ERK1/2 and MNK1 in their phosphorylated forms and activated MNK via p38 MAPK and ERK1/2 signaling cascades as inhibitor treatment for these respective proteins showed significant decrease in relative coat protein expression. Furthermore, we observed that ribosomal protein RPS6 also correlated with coat protein and not its phosphorylated form (p-RPS6) which reduced and gradually degraded after infection. Finally, we demonstrated remodeled MTOC act as VFs and its architecture is supported by host cytoskeletal proteins as confirmed by transmission electron microscopy. Our results may help in expanding the knowledge on NNV-host interactions and pave the way for developing preventive measures and future therapeutic strategies against NNV to enable improved disease control.

SAFETY EVALUATION OF COLD ATMOSPHERIC PLASMA (CAP) FOR DECONTAMINATION IN RECIRCULATING AQUACULTURE SYSTEMS

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The development of novel decontamination technologies in aquaculture, is important due to rising microbial resistances against the commonly used processes based on UV and ozone [1]. Cold atmospheric plasma (CAP) is a physical technology, leading to physico-chemical reactions responsible for the inactivation of microorganisms. Previous studies on the antimicrobial efficacy showed promising results against genera like *Vibrio* and *Aeromonas* which are known to be harmful to fish [2]. This suggests CAP may be suitable for decontamination in recirculating aquaculture systems (RAS). But besides a sufficient efficacy and the sustainability of a new technology, the fish welfare has to be ensured or even improved. Thus, the safety of CAP was examined by various assays determining the potential toxicity and mutagenicity.

Therefore, a possible risk of the plasma application was analysed by an acute fish cell toxicity test of the Organisation for Economic Co-operation and Development (OECD) [3] and a bioluminescence assay based on *Aliivibrio fischeri* [4]. Mutagenic potential was determined by the test, using the microorganism *Escherichia coli* WP2 uvrA [5]. To assess the plasma application, the possibility of a bromate formation was also investigated. Bromate may be produced by plasma-mediated oxidation processes and can be toxic to aquatic organisms. For the plasma treatment, a model aquaculture water based on analytical results of RAS water from a baltic sturgeon stock was designed. A volume of 500 ml was treated with a pin-to-liquid discharge for 30 minutes. This plasma-treated solution was used for all test assays.

The results of the toxicity tests showed a consistent high vitality of the fish cells (RTgill-W1 cell line) up to 50% CAP treated solution. For the bioluminescence assay whereas, lower amounts of treated solution led to a toxicity, quantifiable by the luminescence intensity correlating to the bacterial vitality. The bacterial mutagenicity testing revealed no indication for a mutagenic potential. No generation of the toxic agent bromate by plasma was detected.

In conclusion, the performed tests showed a low toxicity potential of CAP treated model aquaculture water under the chosen test conditions. Based on the results, a concentration between 20% and 50% of CAP treated solution should be used for decontamination in RAS, while not harming the fish. To finally ensure a safe use of CAP for aquaculture additional tests are necessary. The results of the preliminary experiments show, that CAP is a promising technology that can be suggested for use in RAS after further investigations.

1. Liltved, H., et al., *High resistance of fish pathogenic viruses to UV irradiation and ozonated seawater.* Aquacultural Engineering, 2006. **34**(2): p. 72-82.
2. Halpern, M. and I. Izhaki, *Fish as Hosts of Vibrio cholerae*. Frontiers in microbiology, 2017. **8**: p. 282-282.
3. OECD, *Test No. 249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay*. 2021.
4. DIN EN ISO 11348-3:2009-05 Water quality - Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) - Part 3: Method using freeze-dried bacteria (ISO 11348-3:2007); German version EN ISO 11348-3:2008
5. Gatehouse, D., *Bacterial Mutagenicity Assays: Test Methods*, in *Genetic Toxicology: Principles and Methods*, J.M. Parry and E.M. Parry, Editors. 2012, Springer New York: New York, NY. p. 21-34.

1

A BALANCED FATTY ACID COMPOSITION IN THE EARLY LIFE CYCLE STAGES OF *Sander lucioperca* (L., 1758)

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Introduction
Live feed for fish larvae has already previously been studied. *Artemia*, the most common early live feed, lacks certain fatty acids such as DHA (omega-3 fatty acid) and thus is not adequate for all cultivated fish species. Omega-3 fatty acids are not produced *de novo* by vertebrates, and therefore must be provided through the diet.

Material and methods
We analysed the impact of two species of rotifers, a copepod, micro *Artemia*, *Artemia* sp., and microdiets on the fatty acid profile of pikeperch during early larval development. The study aimed to unravel the intricate dynamics of fatty acids of early life cycle stages of pikeperch within Recirculating Aquaculture Systems (RAS).

Results
The rotifers *Brachionus* spp. and the copepod *A. panamensis* had favourable effects on the fatty acid composition of the pikeperch larvae when they were applied in the right quantity and period. They had a high quality as live feed for pikeperch.

Discussion
The results of our study showed balanced lipid phenotypes throughout the early life cycle stages. Especially rotifers as first live feed provide a favourable fatty acid profile. We propose a balanced minimum relative content of 39.0% PUFA, 12.6% DHA, and a content of 8.8-12.4% LA for optimal larval development and rearing in RAS, equalling a metabolic match with a n-3/n-6 ratio of approx. 2.5.

Table 1. Fatty acid composition of pikeperch larvae and juveniles on the different periods considered in this thesis.

Dph	Diet	SFA/MUFA/PUFA	PUFAs	n-3/n-6	LA	DHA
On dph 10	*B. calyciflorus*	1.7 / 1.0 / 2.5	47.4%	2.4	8.1%	15.2%
On dph 10	*B. plicatilis*	1.0 / 1.4-1.6 / 1.6-2.0	38.8-46.2%	2.5	8.8-12.4%	12.6-18.5%
On dph 16	*A. panamensis*	1.0 / 1.5-2.0 / 2.3-4.1	48-58%	1.6-3.2	7.3-10.9%	25.0%
On dph 25	*Artemia* + microdiets	1.2 / 1.0 / 1.6	42.0%	2.6	8.2%	16.7%
On dph 41	Microdiets	1.0/1.1/1.5	41.2%	1.8	13.9%	15.7%
Dph 56	Microdiets	1.0/ 1.5/ 1.3	36.9%	1.2	14.7%	10.4%

PRELIMINARY INVESTIGATION OF CHEMICAL COMPOSITION OF BALLAST WATER SEDIMENTS IN SELECTED SHIPS WITHIN THE LAGOS HARBOUR, NIGERIA

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Ballast water is important for safe and efficient operation of vessels, helping to maintain stability during voyage in seas and docking in harbours. However, ballast pose considerable environmental challenges because they are often laden with polluted sediments and invasive species. The Lagos Harbour, which houses in largest port in West Africa, receives about five thousand vessels annually which discharges ballast water and sediment into the water with the potential to cause environmental pollution. In the Nigerian context there is high potential for pollution due to weak regulations, poor enforcement and limited research of this kind in other to understand their composition, fate and impacts. This study aims at characterizing the chemical composition (Heavy metals- Arsenic, Cadmium, Chromium, Copper, Lead & Zinc, as well as carbon and nitrogen content) of the ballast sediment because of their positive correlation with the survival of invasive species in the ballast water and sediment tanks. A total of four ships (2 cargos and 2 tankers) were surveyed within Lagos Harbour and surface (1-5 cm) sediment samples were scooped using a specialized grab for sampling ballast sediments. The sediments were transferred in specialized cool boxes to the University of Portsmouth in United Kingdom for analysis. Total metal concentrations were analysed using X-ray fluorescence (XRF). They were subjected to two acid digestions: *Aqua regia* (AR) and 1 M hydrochloric acid (1 M HCl) following the procedures set out by the Canadian National Water Research Institute (NWRI) and the United States Environment Protection Agency (US-EPA Method 3050B). Total carbon and nitrogen contents were determined using an in-line Yanaco MT-5 CHN analyser. The results obtained were compared with established guidelines and subjected to correlation and Principal Component Analysis (PCA). The findings indicated high concentrations of Arsenic, Lead, Cadmium, Carbon and Nitrogen in the sediments. Tanker vessels contained relatively higher concentrations of the pollutants than cargo vessels. The findings from this study provides interesting baseline data which will guide a more intensive investigation of chemical composition of ballast water and sediments for effective comparison with what is obtainable elsewhere in the world. The maritime industry needs to pay close attention to ballast management particularly in developing countries like Nigeria where there is limited regulation for management of coastal areas with respect to shipping activities as a potential source of marine pollution. The IMO needs to direct further effort at investigating the non-biological components of ballast water and sediments in other to protect valuable biodiversity.

MOLECULAR INVESTIGATION OF MAJOR VIRAL PATHOGENS OF THE GIANT TIGER SHRIMP *Penaeus monodon* (Fabricius, 1798) ALONG THE COAST OF GHANA

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Shrimps are decapod crustaceans of high economic value. Most wild-caught shrimps and all farm-raised shrimps belong to the Penaeidae family. In Ghana's infantile shrimp farming industry, the only cultured shrimp species is *P. monodon*. The gravid females are obtained from the wild, spawn in the hatchery and the post-larvae used for their grow-out operations. Globally, *P. monodon* is documented as highly susceptible to viral pathogens such as White spot syndrome virus (WSSV), Monodon baculovirus (MBV), Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), Infectious myonecrosis virus (IMNV) among others and the need to screen the wild-caught brooders for these pathogens cannot be overemphasized.

The study sampled the giant tiger shrimps from three coastal regions along the 570 km Coast of Ghana and screened for the presence of four globally important penaeid shrimp viruses; White Spot Syndrome Virus (WSSV), Monodon Baculo Virus (MBV), Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV) and Infectious Myonecrosis Virus (IMNV) using molecular methods.

Fifty (50) samples were collected from each of the following regions in Ghana: Greater Accra (GR), Volta Region (VR) and Central Region (CR). DNA and RNA extractions were performed on the gills, muscles, midgut and hepatopancreas using Qiagen extraction kits. Conventional PCR and real-time qPCR were used to screen for the presence of these viruses.

The initial PCR screening of some few collected hetopancreas and muscles samples indicate non detection of the viruses. However, DNA and RNA extractions and PCR analysis of the rest of the samples continues in order to confirm presence and otherwise of the four WOAH-listed viruses in Ghanaian shrimps.

Table 1: Quality of extracted DNA and Viral detection by PCR

Sample	DNA Conc (ng/µL)	Detected Virus
Hetopancreas	117.7	Negative
Hetopancreas	62.0	Negative
Muscles	90.4	Negative
Muscle	32.3	Negative
Muscle	54.6	Negative
Muscles	67.9	Negative
Hetopancreas	34.6	Negative
Hetopancreas	49.8	Negative
Hetopancreas	69.0	Negative
Hetopancreas	93.9	Negative

SYNTHESIS, CHARACTERIZATION AND APPLICATION OF PH-SENSITIVE INDICATORS FOR FRESHNESS MONITORING OF GILTHEAD SEABREAM *Sparus aurata* FILLETS DURING REFRIGERATED STORAGE

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Significant amounts of fish and seafood are wasted worldwide due to spoilage and degradation during various stages of the cold supply chain (EUMOFA, 2022). An essential aspect of fish and seafood quality is the rapid and accurate identification of quality level and remaining shelf life (Tsironi et al., 2018). The development of novel smart packaging solutions to monitor fish spoilage could contribute to accurately inform consumers about the freshness of fish products and, consequently, reduce fish waste (Mohebi et al., 2015; Li et al., 2022).

The pH-sensitive indicators were prepared by the immobilization of methyl red (MR) and bromothymol blue (BB) dyes in polylactic acid (PLA) and polyhydroxybutyrate (PHB) separately according to the solvent casting method. After fabrication, the smart films were evaluated for optical and mechanical properties, UV–Vis barriers, film wettability and microstructure, infrared spectra, and sensitivity to volatile amines commonly produced during fish spoilage (ammonia, trimethylamine and dimethylamine). The indicators were then placed into the fish packages (attached inside the headspace of sterilized sealed pouches containing 50 g of fish). Samples were stored at constant refrigerated and abused temperature conditions for shelf-life evaluation based on chemical (pH, TVB-N) and microbiological (spoilage bacteria) quality indices of the fish. Color response (ΔE value) as well as gas composition in the package headspace were monitored during the storage experiment.

Scanning electron microscopy confirmed quite adequate microstructural integrity and compatibility between the dye and the polymer carrier. The successful incorporation of the dye was confirmed by Fourier transform infrared spectroscopy. The fabricated films had adequate optical and mechanical properties. The addition of the dyes in the polymer matrix enhanced the wettability of the films (drop of contact angle from 75° to 57°). The sensitivity test indicated that the colour response of the developed smart films successfully monitored volatile amines during fish storage (the MR/PHB film turned yellow from red, and the BB/PLA film turned blue from yellow).

Preliminary results of the application of the indicators inside the fish package indicated that the fabricated smart films could monitor fish spoilage by a visual color change providing shelf-life prediction of fish fillets ranging from 5 days at 5°C to 16 hours at 25°C.

References

1. European Market Observatory for fisheries and aquaculture. (2022). The EU Fish Market.
2. Tsironi T, Taoukis P. (2018). Current practice and innovations in fish packaging. *J. Aquat. Food Prod.*, 27, 1024-1047.
3. Mohebi E, Marquez L. (2015). Intelligent packaging in meat industry: An overview of existing solutions. *J. Food Sci. Technol.*, 52, 3947-3964.
4. Li, N., Zhou, S., Yang, X., & Lin, D. (2022). Applications of natural polysaccharide-based pH-sensitive films in food packaging: Current research and future trends. *Innov. Food Sci. Emerg. Technol.*, 82, 103200.

EFECTO DEL GLIFOSATO SOBRE EL DESEMPEÑO REPRODUCTIVO DEL BOCACHICO *Prochilodus magdalenae*

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El Bocachico (*Prochilodus magdalenae*) es una especie fundamental para la pesquería continental en Colombia, la cual ha visto una reducción notable en su captura en las últimas décadas. Esta disminución se atribuye a factores como la degradación ambiental producida por la descarga continua de aguas residuales y residuos agropecuarios entre otros aspectos. El objetivo del presente estudio fue evaluar el efecto de glifosato sobre el desempeño reproductivo y letalidad en larvas de bocachico *Prochilodus magdalenae*. Glifosato en cinco diferentes concentraciones de exposición (2.5; 5; 10; 20; 40 mg/L) y un tratamiento control con agua Milli-Q fue evaluado por duplicado en tres réplicas. El semen fue incubado en una solución con cada concentración y glucosa 6% como diluyente y medio inactivador. Durante 7 horas de incubación fueron tomadas muestras en los tiempos: 0, 1, 2, 5 y 7 horas. El semen fue analizado con ayuda del Sperm Class Analyzer (SCA).

Las variables movilidad total, tipos de movilidad, progresividad, velocidad lineal, velocidad curvilínea, y duración de la movilidad fueron analizadas. La capacidad fertilizante fue estimada con pruebas de fertilidad y eclosión, mediante la activación e hidratación de los productos sexuales con agua contaminada con las diferentes concentraciones de glifosato. De igual forma 6 horas después del proceso de fertilización fue evaluado el diámetro ovocitario con la medición de aproximadamente 50 ovocitos por tratamiento. Un total de 60 organismos en microplacas de seis pocillos, diez larvas de 1 día posteclosión fueron sometidas por un período máximo de 24 horas a cada concentración de glifosato para estimar la sobrevivencia larval.

Todas las concentraciones de glifosato luego de 7 horas de exposición generaron reducción entre el 20 y el 40% de la movilidad total en comparación con los controles. Fue observada una relación inversa entre la concentración de glifosato y el diámetro en ovocitos de bocachico, con valores que oscilan entre 3.1 ± 0.09 (control) y 1.7 ± 0.1 (40 ppm) ($p<0.05$). Luego de 24 horas de exposición a glifosato en concentraciones superiores a 10 ppm, generan letalidad entre el 35% y el 100% en larvas de bocachico ($p<0.05$). Los resultados permiten sugerir que concentraciones de glifosato iguales o superiores a 2.5 ppm afectan la cinemática espermática y la capacidad fertilizante de bocachico, de igual forma concentraciones glifosato de 20 ppm y 40 ppm generan letalidad del 100% en larvas de bocachico.

BLACK SOLDIER FLY LARVAE OIL AS LIPID SOURCE IN DIETS FOR *Dicentrarchus labrax* AND *Sparus aurata* JUVENILES: A COMPARATIVE APPROACH

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Black soldier fly larvae oil (BSFLO) is a co-product derived from processing *Hermetia illucens* into meal. It is characterized by presenting high levels of lauric acid (30-40%), a fatty acid suggested to enhance hepato-metabolic status and promote glycolytic muscle fibre formation, improving growth in mammals. The study herein presented aims to appraise the potential of this new ingredient to replace another terrestrial animal fat already in use in aquafeed formulations. A comparative approach with European seabass and gilthead seabream was established to evaluate the impact of increasing levels of BSFLO on fish growth performance, feed efficiency, and nutrient utilisation.

A commercial formulation including 6% fish oil and 5% poultry fat (PF) was used as a control diet (CTRL). Three additional experimental diets were formulated to replace 33%, 66% and 100% PF with BSFLO supplied by Entrogreen® - Ingredient Odissey S.A., resulting in the BSFLO33, BSFLO66 BSFLO100 diets, respectively. These experimental diets were tested in triplicate groups for both seabass and seabream juveniles (11-16g), at a temperature of 23^{o}C. Fish were fed three times a day to visual satiety until the CTRL groups quadrupled their initial body weight. Subsequently, the feed efficiency, growth performance, and nutrient balance of both fish species were assessed.

In European seabass, the dietary inclusion of BSFLO to totally replace PF (BSFLO100 diet) improved feed conversion ratio (FCR), without affecting voluntary feed intake (VFI), resulting in significantly higher final body weight. In gilthead seabream, both partial (i.e., 66%) and total PF replacement by BSFLO resulted in enhanced growth performance of fish without significant alterations in both VFI and FCR. Seabass fed BSFLO66 exhibited lower whole-body (WB) energy content compared to those fed CTRL and BSFLO100 which was associated with lower energy gain and retention. Although there were no significant alterations in the somatic indexes or in WB macronutrient composition among dietary treatments, a slight decrease in lipid utilization was observed in fish fed BSFLO66, indicating changes in lipid and energy metabolism in these fish. Conversely, in seabream, although the WB nutrient composition, gain and retention were not affected by dietary inclusion of BSFLO, the total PF replacement induced alterations in somatic indexes, also suggesting potential alterations in the animals' metabolism. The metabolic pathways affected by the dietary inclusion of BSFLO and the resulting impact on flesh fatty acid profile will be discussed in both species.

Acknowledgments
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DEVELOPMENT OF A NEW STERILE MICROALGAE DIET TO APPLY IN RELAYING AND DEPURATION OF OYSTER *Magallana gigas*

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The aquaculture of bivalves is a fast-growing worldwide food production sector. Prior to commercialization, bivalves reared or harvested in locations with high microbiological contaminations require depuration for safe human consumption. Bivalves' depuration is performed in clean seawater with total absence of food for 1-3 days or longer in relaying facilities. However, starvation usually leads to a reduction of survival, physiological condition, and nutritional value, particularly in essential fatty acids (e.g. DHA and EPA) of the bivalves. Therefore, the development of microbiological-safe and nutritionally balanced diets suitable for bivalve's depuration systems is crucial to improve bivalve quality for human consumption, animal welfare and stakeholders' revenues. To achieve this, a concentrated microalgal liquid diet (8.5% dry weight (DW)) was formulated containing *Tetraselmis* sp., *Schiziochytrium* sp. and cornstarch. Thermal treatments were tested for diet sterilization: 1) autoclaving (121°C, 40 min) and 2) low-temperature long-time (LTLT) (75±2ºC, 1h followed by a 10ºC cold shock). A control diet was performed without any thermal treatment. The diets microbiology (TSA and TCBS), decantation and photosynthetic pigments were evaluated. Autoclaving promoted the highest bacterial reduction, but increased product decantation and reduced chlorophyll *a* and carotenoids. The LTLT treatment significantly lowered the diet total bacteria (2.75±0.16 Log CFU/g) compared to control (5.48±0.26 Log CFU/g). Thus, LTLT diet was selected for *Magallana gigas* depuration trials in three closed 250L depuration modules, with ultrafiltered seawater recirculation (15±1ºC and salinity 35) and a protein skimmer. Oysters were fed with live microalgae (*Tisochrysis lutea* and *Tetraselmis* sp., 3:2 ratio) (1.5% of microalgae/g of oyster DW per day), LTLT treated diet (5% of diet DW/g of oyster DW per day) and with food omission (control). Organisms were fed twice a day for 14 days, and then stored at 4±1ºC for 7 days (shelf-life). Water microbiology (TSA, PCA and TCBS) and oyster (*Escherichia coli*) were monitored. Condition index (CI) was estimated weekly. Water microbiology revealed that after 3 days of depuration, the treatment without feed showed significantly higher total bacteria compared to the other treatments. In all treatments, *E. coli* was effectively reduced from 1300MPN/100g (0h) to <18MPN/100g (18h). CI did not differ significantly among treatments at the end of the experiment (14 days), nor even after the shelf-life (21 days). In the future, the optimization of new LTLT diet dosage will be performed and supported by biochemical analysis of diets and depurated oysters. Moreover, a sensory analysis of oysters will be performed to ensure an adequate palatability of oysters fed with LTLT diet. The development of diets to be applied during depuration and relaying facilities represents a highly relevant strategy to maintain the animal's condition, prevent undesirable mortality and onshore microbiological safety of bivalves for human consumption.

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COMPARISON OF MICROALGAE HARVESTING EFFICIENCY BETWEEN INDUSTRIAL CENTRIFUGATION AND A MEMBRANE FILTRATION SYSTEM

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One of the most important steps in the production of microalgae is the harvesting process, responsible for up to 30% of the total production cost due essentially to its high energy demand. Two commonly used methods to harvest microalgae biomass were compared in this study, namely, membrane microfiltration and centrifugation. Microfiltration usually provides a high cellular concentration efficiency, with the possibility of varying the pore size of the filter membrane to deal with different size microalgae. It also allows the handling of more delicate species, avoiding cell disruption. On the other hand, centrifugation also provides a high cell harvesting efficiency, but it is more energy intensive, possibly causing cellular damage that makes cells inadequate for numerous applications.

In this study, both harvesting techniques were compared at a relevant scale in an industrial context, namely, 1) microfiltration, using a membrane filtration system (SANI Vibrio-I™ module) and 2) centrifugation, using an industrial batch centrifuge (Westfalia). To assess harvesting efficiency, microalgae cultures of *Nannochloropsis* sp. cultivated in 19m^3 tubular photobioreactors (TPBR) were used. These cultures were harvested from the TPBR and processed separately by the membrane system (2.5m^2 with a pore size of 0.6µm) and centrifuge (n=4 for each method). The concentration process of each method was followed by measuring several parameters such as optical density (750 nm) and cell countings (Neubauer chamber). Microscopic observations were performed to assess the presence of cells in the permeate, cell integrity and eventual presence of contaminants or debris in the culture. Each method (membranes and centrifuge) was compared using different performance analysis such as permeate flow rate (Q), volumetric concentration factor (VCF), final cell concentration and dry weight.

An average Q of 61.7±4.1 L/h and an extremely low Q decay of 3.1±2.5%/h for the membrane system revealed a steady state performance while the centrifugation exhibited a 10-fold increase in the average Q (626.59±2.78 L/h). The final cell concentrations were $4.27 \times 10^9 \pm 2.52 \times 10^9$ and $5.16 \times 10^{10} \pm 3.51 \times 10^{10}$ cells/mL for the membrane system and centrifugation, respectively. An economic analysis will be shown to determine the best cost-efficient harvesting technique. This will include the possibility of a synergetic effect of both methods for best harvesting performance. This constitutes one of many essential steps to reduce operational costs and optimise microalgae production processes with the aim of making microalgal products more sustainable and available worldwide.

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AQUSENS: AI-POWERED PLATFORM FOR AUTOMATED QUANTIFICATION OF PATHOGENS IN WATER

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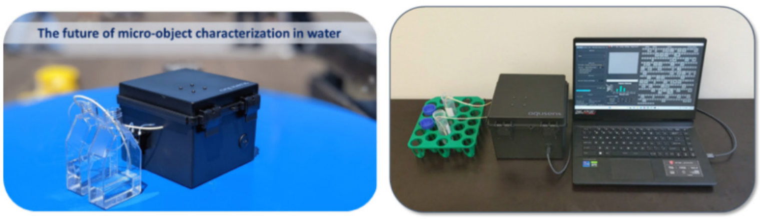
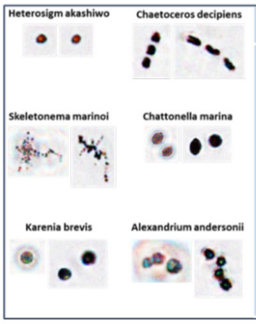
One of the most significant challenges in aquaculture is to mitigate or avoid production losses due to disease agents caused by parasites (such as sea lice), algae, bacteria, viruses, and fungi. This creates a critical need for the early detection of dangerous microorganisms and disease-causing agents in the water. Currently, the samples are collected manually and analyzed by trained technicians in a laboratory, often centralized, using conventional lens-based microscopes. This approach is expensive, laborious, time consuming and limiting scalability of operations. Furthermore, delays with disease agent identification often eliminate many mitigation strategies forcing approaches leading to production losses or premature harvesting.

To address this critical need, Lucendi has developed Aqusens – an AI-based holographic microscopy platform capable of rapid automated monitoring of water samples for the presence and accurate quantification of dangerous organisms, such as harmful algae, sea lice and others. Unlike conventional lens-based microscopy systems, Aqusens relies on capturing interference patterns as objects are passing through a pulsating light field. These patterns are then processed and characterized by deep learning. With this novel approach, Aqusens can identify objects anywhere from 2 micron to 4 mm range and above. The system screens the water with unprecedented 100 mL/hr throughput, which can be increased to liters per hour depending on the application needs. For every object Aqusens generates an intensity and phase images which are then analyzed by custom AI to determine the object type and to compute concentration. Collected data can then be immediately shared with key decision makers to provide advanced warning, optimize operations, and save costs.

Aqusens is currently deployed at several in-field facilities. It was validated in the laboratory studies demonstrating accurate identification of various harmful algae and sea lice. Aqusens can also be customized for specific operational conditions and applications, and is developed to support scalability and safety of the operations.





Actual \ Classified	Ocean	Alexandrium andersonii	Chattonella marina	Heterosigm a akashiwo	Skeletonem a marinoi	Chaetoceros decipiens
Ocean	189	0	5	3	2	1
Alexandrium andersonii	9	236	0	0	2	5
Chattonella marina	2	0	506	0	0	0
Heterosigma akashiwo	1	0	0	338	0	0
Skeletonema marinoi	0	0	0	0	181	0
Chaetoceros decipiens	1	1	0	0	0	118

Figure 1: Aqusens is an AI-powered cost-effective, high-throughput platform for automated identification and characterization of harmful micro-organisms in water. It can be deployed in-field with minimal maintenance requirements and provide advanced awareness to optimize

AQUSENS: AI-POWERED PLATFORM FOR AUTOMATED QUANTIFICATION OF LIVE FEED

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Many fish hatcheries rely on live feeds (i.e., microorganisms, such as copepods, rotifers, and artemia). One of the critical capabilities missing today is to enable automated live feed counting and characterization. Introducing automation, will result in elimination of manual processes, will improve accuracy, will promote standardization, and will reduce errors and culture crashes.

Existing solutions to live feed quantification and characterization are based predominantly on manual counts performed by trained experts on conventional microscopes. Furthermore, re-purposing existing lens-based imaging flow cytometers for automated live feed characterization is not ideal due to the high cost of the equipment and performance limitations of these solutions, including low-throughput processing, significant maintenance requirements, issues with adapting for large size range of organisms that is the case with live feed species.

Lucendi has developed the Aqusens platform, shown on Figure 1, which is capable of rapid and label free evaluation of the live feed samples with throughputs of anywhere from 100 mL/hr – 5 L/hr, depending on use case needs with sufficient resolution to characterize organisms in a diverse size range from single microns to millimeter scale. Furthermore, since the Aqusens is built primarily from the off the shelf components, the cost of the hardware solution is significantly lower than any of the existing alternatives.

Aqusens AI infrastructure is capable of automated live feed characterization by type (i.e. copepods, rotifers, artemia, etc.), and by phase of development (i.e. egg, nauplii, copepod). Aqusens can also identify cross-contamination to avoid production inefficiencies and losses.

Aqusens has been deployed in-field and was demonstrated to provide continuous live feed sample monitoring and quantification that is at least on par or better and more repeatable than that of the human operator.

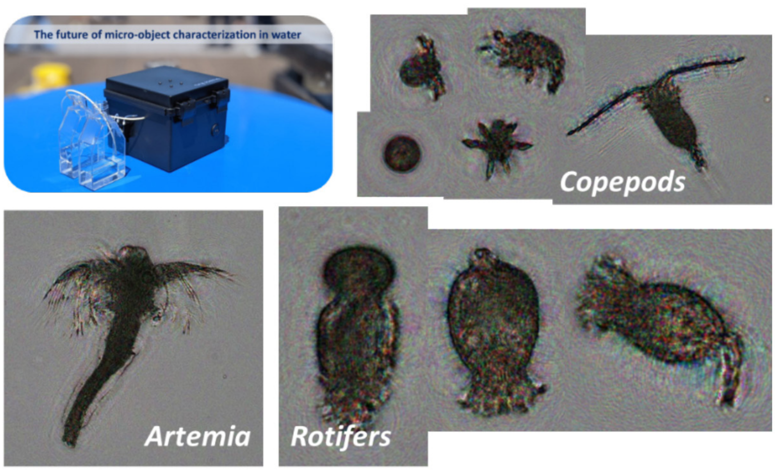

Figure 1: Aqusens platform leverages AI and unique holographic imaging flow cytometry to provide automated high-througput accurate identification of live feed organisms, including classification of the development phase, to optimize hatchery operations and empower

ASSESSING THE ROLE OF HEART MORPHOLOGY IN DETERMINING CARDIAC FUNCTIONALITY IN ATLANTIC SALMON *Salmo salar* L.

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Introduction: The aquaculture industry is experiencing alarming mortality rates. In Norway alone, mortality is approaching 20 % in the sea phase and still increasing. A large portion of this mortality is due to cardiac pathology. Atlantic salmon is known to display cardiac plasticity at different life stages, but abiotic and biotic factors such as temperature, light cycle, and nutrition can impact cardiac health. Though the extent is not recorded, the prevalence of deviating cardiac morphology is a common observation in farmed salmon and scientific studies mimicking industry-like conditions. Although such deviations were observed already in the early 2000s, few studies have investigated the functional consequences of altered heart morphology. Thus, we investigated the impact of deviating cardiac structures, such as ventricular size and shape, along with the alignment of the bulbus arteriosus on cardiac function.

Methods: Cardiac structure was assessed using established *ex vivo* qualitative and quantitative methods, while heart function was evaluated *in vivo* by cardiac ultrasound. Comparative statistical tests between wild-type (WT) and deviating-type (DT) hearts, along with partial regression analyses, were applied to examine the influence of distinct morphological traits on cardiac function.

Results: Significant functional differences were observed between WT and DT hearts. In general, DT hearts have lower heart rates, accompanied by increased stroke volume and cardiac contractility, resulting in maintained cardiac output. Additionally, heamodynamics were significantly affected by cardiac deviations in both systole and diastole, coinciding with larger atria and smaller outflow tract diameters. By analysing individual deviations separately, we observed that hearts with curved ventricles and skewed bulbi had increased ventricular contractility. On the other hand, short ventricles displayed both increased contractility and ventricular blood velocity and output. All types of deviations were associated with increased diastolic blood velocities. Regression analyses revealed that relative ventricular width affects systolic and diastolic function alike. However, ventricular length seems to have less effect. Lastly, bulbus size appears to impact cardiac function less than dimensions of the ventricular outflow tract and bulbo-ventricular valve.

Conclusion: A large fraction of mortality in Atlantic salmon aquaculture is due to cardiac pathology, including changed heart morphology. We show that altered cardiac structure alone is a key determinant of cardiac function and performance.

LOWER COST AND MORE SUSTAINABLE PRODUCTION OF HIGH VALUE SINGLE CELL PROTEIN THAT HAS BEEN OPTIMISED AS A FISHMEAL REPLACEMENT

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As the rapidly growing aquaculture industry expands to meet humanity's ever-increasing appetite for protein, the demand for fishmeal – a primary ingredient in aquafeeds - is on the rise. Unsustainable harvesting practices have depleted wild fish stocks and has led to a surge in fishmeal prices over recent years. Deploying protein alternatives that are more sustainable, higher value to the customer and cost-effective is needed. The protein alternative must be comparable to fishmeal in terms of protein content, amino acid composition, palatability and digestibility and this has posed significant challenges which MicroBioGen is addressing through its deep technology, elite genetics development program.

MicroBioGen leads the world in the development of novel and improved strains of *Saccharomyces cerevisiae.* This yeast is the world's leading microorganism workhorse with over US$2 trillion in products annually relying on it for production. After 20 years of technology development, MicroBioGen has successfully partnered with Novozymes (Novonesis) and now leads the world in supplying superior yeast strains to produce 1st and 2nd generation bioethanol. Billions of litres of extra biofuel are being produced as a result of utilising MicroBioGen developed yeast strains. MicroBioGen's elite genetics technology is now being optimised and deployed in the production of single cell protein+ from large scale biofuel and dairy waste/side streams. These waste/side streams include biodiesel, dairy and bioethanol which are rich in compounds such as glycerol and organic acids.

Some of the additional features that have been added to *Saccharomyces cerevisiae* yeast by the MicroBioGen team to improve sustainability, lower production costs and increase value to the customer, include the following:

- Ability to grow at industrial rates and productivities on glycerol, xylose and various organic acids as well as retain typical C6 sugar yields and productivities
- Protein content of 15% to 20% higher than typical *Saccharomyces cerevisiae* yeast.
- An excellent amino acid profile (to be disclosed in the presentation).
- Accumulation of enzymes to replace exogenously added enzymes, e.g. phytase.
- Palatability, and the option to add or accumulate antioxidants, e.g. glutathione.
- The organism has been 100% developed utilising non-GM technology, thus reducing compliance issues and increasing unit value.

The above features allow single cell protein to be grown on non-sugar waste/side streams at industrial rates, making it cheap to produce and higher value than other single cell protein options. This paper will highlight the value of various added features, amino acid profile and impact on production costs and unit value amongst others.

SOURCES AND PERSISTENCE OF FECAL BACTERIA AND ANTIMICROBIAL RESISTANCE IN FRESHWATER FISH FARMS FROM NORTH-WEST FRANCE

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Aquaculture is an essential food production sector worldwide and is currently experiencing a significant growth. However, the pollution of surface waters with fecal bacteria from humans and terrestrial animals may pose a food and environmental safety risk in aquaculture settings relying on surface waters. This study aims at identifying sources of antimicrobial resistance carried by bacteria classically considered fecal indicators (*Escherichia coli*, *Enterococcus faecium*, *Enterococcus faecalis*), and at estimating their persistence in freshwater fish farms from north-west France.

Four freshwater fish farms were recruited for this longitudinal study: two farms operating as flow-through systems and two farms operating as recirculating aquaculture systems (RAS). For each type of system, we selected a farm located downstream from a wastewater treatment plant. On two sampling dates per farm (autumn and winter) we collected fish feed, water, sediment, and biofilm samples from several points of the fish farm, following the sampling scheme designed by Novais et al (2018). Bacterial isolates were identified with either MALDI-TOF or PCR. Antimicrobial susceptibility testing was performed on a selection of 43 confirmed *Escherichia* strains with the broth microdilution method (determination of Minimum Inhibitory Concentrations) against a set of 15 antibiotics.

Out of 234 samples, 64 were positive for *Escherichia coli* (25.6%) and 20 for *Escherichia marmotae* (8.5%), including water, sediment and biofilm. Concerning water, 26 out of 81 samples were positive for *E. coli*, which suggests that water is an important vehicle of this bacterium into fish farms. The fact that 31 out 85 biofilm samples carried *E. coli* suggests that it may persist in this ecological compartment. Furthermore, with the use of epidemiological cut-off values we found resistance to sulphonamides (34/43), ampicillin (4/43), tetracycline (4/43), gentamicin (3/43) ciprofloxacin (2/43) and colistin (1/43). Nine *Escherichia* isolates showed an antimicrobial multiresistant profile. *Enterococcus* results are pending.

These preliminary results underscore the importance of monitoring and addressing fecal pollution in surface waters used by aquaculture and aquatic leisure activities. Further testing is needed on the strains showing resistance to antibiotics commonly used in aquaculture such as tetracycline and sulphonamides: if these resistance phenotypes were conferred by mobile genetic elements transferable to bacterial fish pathogens, the effectiveness of these drugs in controlling bacterial infections in fish populations would be compromised. Understanding the broader implications of fecal pollution in surface waters is highly important for ensuring aquaculture sustainability and safeguarding public health.

Reference: Novais et al (2018) Water supply and feed as sources of antimicrobial-resistant *Enterococcus* spp. in aquacultures of rainbow trout (*Oncorhyncus mykiss*), Portugal. Science of the Total Environment 625: 1102–1112

WOOD AS AN ALTERNATIVE TANK CONSTRUCTION MATERIAL IN SUSTAINABLE SHRIMP AQUACULTURE

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Shrimp (*Penaeus vannamei*) aquaculture faces growing demand coupled with increasing sustainability concerns, requiring innovative approaches. This study highlights possible low-tech approaches to tackle these challenges by investigating the potential of using wood in tank construction. In this study, we assessed the suitability of regionally grown wood, *Pseudotsuga menziesii* (Douglas fir) and *Quercus robur* (pedunculate oak) as a low carbon and ecologically friendly replacement for fiberglass-reinforced plastic (GRP) in shrimp rearing tanks, focusing on environmental impact and shrimp growth performance. Our results show that shrimp growth metrics in wooden tanks, particularly Douglas fir (figure 1), are comparable to GRP, indicating its potential for tank construction to promote sustainable aquaculture practices. Moreover, the presence of tannins in wood offers additional benefits such as antiviral and antibacterial properties, potentially fostering a healthier aquaculture environment. This research contributes to the exploration of alternative materials for aquaculture systems and addresses the pressing need for environmentally responsible practices in the industry. The study underscores the potential of wood as a sustainable alternative to plastic in shrimp aquaculture tank construction and paves the way for further research and implementation of environmentally friendly practices in aquaculture operations.

Figure 1: Douglas fir shrimp farming tanks.

CAN THE HYPOXIA TOLERANCE OF TRIPLOID SALMONIDS BE IMPROVED?

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Triploids are sterile and therefore cannot reproduce if they escape from farms. While this has obvious management benefits, triploids are not used in aquaculture to any extent because they have reduced high-temperature and hypoxia tolerance. This presentation summarizes some of our on-going research on developing practical husbandry approaches to improve the hypoxia tolerance of triploid salmonids. These experiments use an acute hypoxia challenge with loss of equilibrium as the endpoint, and with brook charr (*Salvelinus fontinalis*) as a model salmonid species. Triploids (3n) are always compared to sibling diploids (2n) within an experiment.

A: Acclimation to moderately elevated temperature (3°C above opt.) improves hypoxia tolerance in both ploidies; triploids are still inferior to diploids

Jensen, R.R. & T.J. Benfey. 2022. Acclimation to warmer temperature reversibly improves high-temperature hypoxia tolerance in both diploid and triploid brook charr, *Salvelinus fontinalis*. Comp. Biochem. Physiol. 264A: 111099.

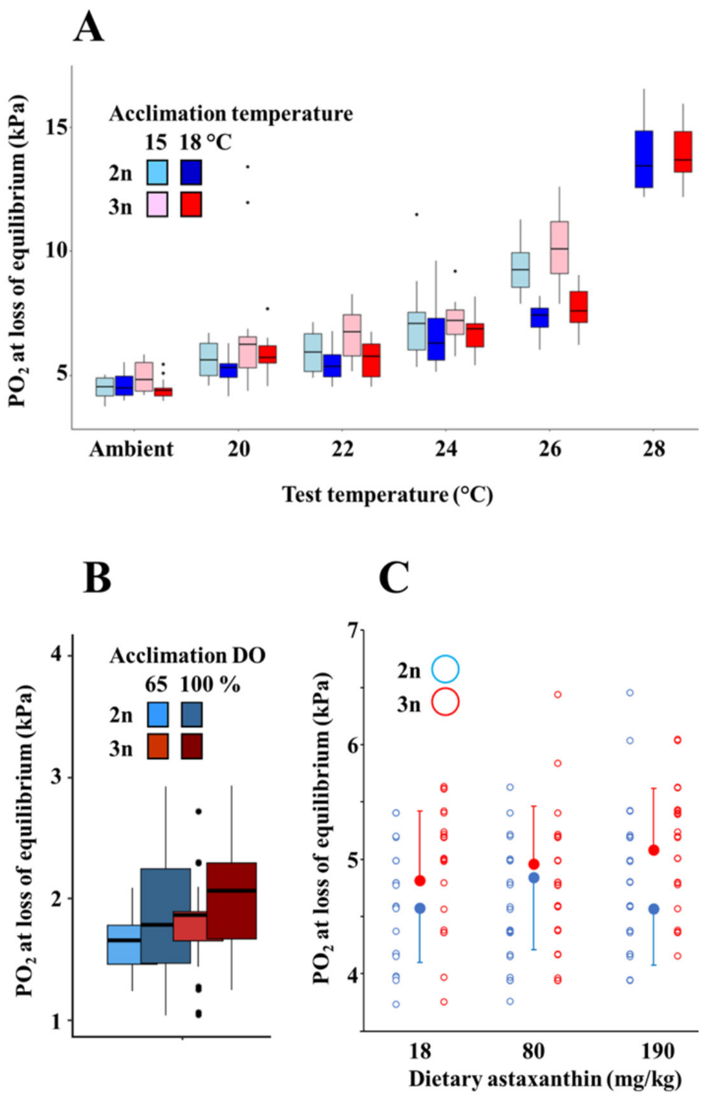
B: Acclimation to moderate hypoxia improves hypoxia tolerance in both ploidies; triploids are still inferior to diploids

McGeachy, S.A., 2022. Hypoxia tolerance of triploid brook charr, *Salvelinus fontinalis*. MSc thesis; manuscript in preparation.

C: Antioxidant supplementation does not improve hypoxia tolerance; triploids are still inferior to diploids

Baker, C.A., 2023. The effect of dietary supplementation of astaxanthin on acute hypoxia and thermal tolerance in triploid and diploid brook charr, *Salvelinus fontinalis*. MSc thesis; manuscript in preparation.

We are also investigating whether high-temperature and hypoxia tolerance of triploids can be improved through selection within family-based breeding programs that include sibling diploids and triploids within each family.



DOES PERSONALITY MATTER FOR THE WELFARE OF FARMED FISH ?

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There is growing interest in animal welfare from both the public and the scientific community. This is a complex concept with no formal consensus regarding definitions and methods to approach it, which becomes even more complicated in fish due to their vast diversity; what holds true for one species may not apply to another. Furthermore, fish farming is a much more recent activity compared with that of terrestrial animals. Studies on welfare aim to determine what is harmful or undesirable for the animal and try to mitigate negative impacts on their well-being. Extensive research has been conducted on behavioral, physiological, growth, and health indicators of welfare impairment. However, welfare also concerns what is beneficial or desired by the animal, which relates to positive welfare. Research on positive welfare in fish is lagging. Animal personality refers to behavioral tendencies that affect behavior in different contexts, vary across individuals in a given population, and are consistent within individuals across time.The purpose of this talk is to examine how the field of personality can be implemented in the welfare framework of farmed fish.

BIOACTIVE PEPTIDES FROM SALMON AQUACULTURE PROCESSING BY-PRODUCT AFFECT GROWTH PERFORMANCE, PLASMA BIOCHEMISTRY AND GUT HEALTH OF GILTHEAD SEABREAM (*Sparus aurata*)

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The objective of this study is to evaluate the effects of incorporating bioactive peptides (BPs) from salmon aquaculture by-product into the diets of gilthead seabream (*Sparus aurata*), focusing on growth performance, blood biochemistry and gut health and their interlink. Furthermore, the investigation explores potential advantages within the climate change context, providing a comprehensive understanding of the diverse implications associated with this dietary approach. For this purpose, three diets with varying BP levels (0%BP0, 5%BP5, 10%BP10) as substitutions for fish meal (FM) were administered to triplicate fish groups over a 58-day trial period. Successively, fish were exposed to suboptimal environmental conditions (high water temperature, low oxygen) for 7 days.

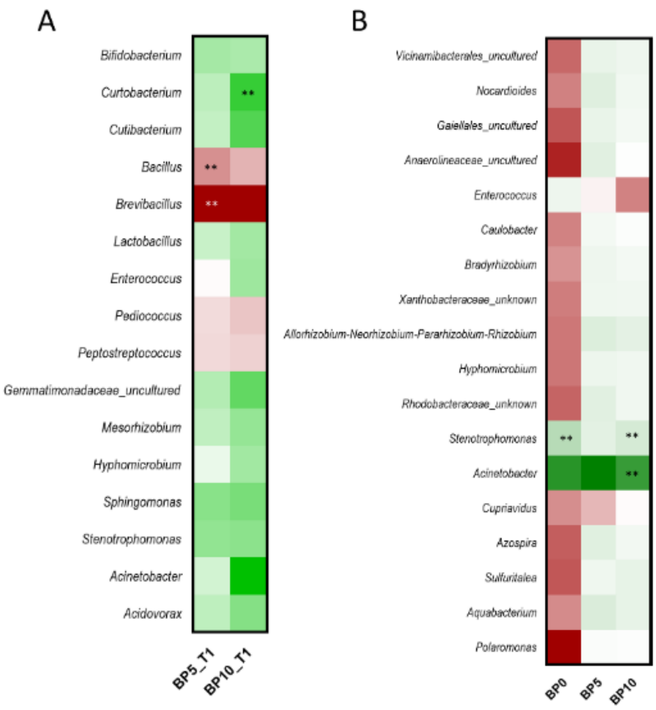

Figure 1 Heatmap showing log2 fold change of genera relative abundance affected by diet (A) and the combination of dietary and stress exposure(B).

No significant difference between fish fed the three experimental diet was observed for growth performances parameters. The highest dosage of BP (BP10) induced a significant increase of bacterial α-diversity, suggesting a potential benefits. Additionally, samples belonging to the experimental diet groups (BP5 and BP10) seemed less responsive to stressing conditions, in terms of microbiome variations. However, considering the microbiome profiles at T1 only (Fig. 1A), fish fed BP5 and BP10 showed a significant decrease in abundance of *Brevibacillus* and *Bacillus*, potentially indicating a reduced fish health. Moreover, challenging environmental conditions combined with high dietary inclusion of BP (10%) triggered an elevated plasma glucose, creatinine and uric acid levels, potentially indicating impairment of kidney function and reduced fish fitness. Furthermore, it was observed that in fish fed BP10, opportunistic fish pathogen as *Stenotrophomonas* and *Acinetobacter* increased after environmental stress and this data were positively correlated to higher lactate hematic concentration, suggesting a physiological distress exacerbated by adverse environmental conditions. Overall, while BP inclusion offers a sustainable alternative protein source, incorporating more than 5% in the diet might compromise fish health under challenging environmental conditions.

GLASSHOUSE AQUAPONICS OF MARIGOLD (*Calendula officinalis* L.) AS MEDICINAL PLANT WITH AFRICAN CATFISH (*Clarias gariepinus* BURCHELL, 1822) IN A HYDROPONIC EBB AND FLOOD SUBSYSTEM

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Marigold (*Calendula officinalis*) is a medicinal plant that is known and used for centuries. The substances of interest contained in marigold include flavonoids, triterpene and triterpene saponins. Extracts of the drug are used externally for the treatment of mild inflammation of the skin and oral mucous and to promote wound healing. It is regularly cultured on fields with potassium fertilization. The aquaponic production of medicinal plants in combination with African catfish offers the possibility of cultivating medicinal plants under controlled conditions while saving resources. However, its performance under glasshouse cultivation must be demonstrated.

In order to investigate the suitability of aquaponically produced marigolds as medicinal plants, marigolds were cultivated in three experimental groups and triplicates for 57 days in pot culture with perlite/vermiculite. The experimental groups were kept in ebb and flood systems with a regular commercial fertiliser solution (control), process water from intensive catfish aquaculture and process water from intensive catfish aquaculture with additional potassium fertilisation. The flowers were harvested at three points in time, each with a one-week interval between them. The quality of the harvested petals was analysed in accordance with European Pharmacopoeia 10.1.

Significanlty best growth was achieved in the group with intensive process water, followed by intensive process water with potassium and the control group. The first plants reached flowering after 26 days, with similar flowers in all three groups. Analyses of the valuable ingredients demonstrated that content of flavonoids were similar high compared with other commercial plants, reaching levels of about 0.4 %. This study demonstrates that the aquaponics production of marigold in combination with African catfish under glasshouse conditions is possible and that the quality determined compounds reach similar levels required for its use as a medicinal plant.

Figure 1 Aquaponically produced marigold experimental setup (upper picture) variety of the produced flowers (lower pictures)

IMPROVING FISH PRODUCTION IN CENTRAL EUROPEAN AQUACULTURE: EVALUATING PERIPHYTON COMMUNITIES ON DIFFERENT SUBSTRATES

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Periphyton is a fundamental component of freshwater ecosystems, significantly contributing to water quality enhancement, nutrient cycling, and overall productivity. Additionally, periphyton serves as a valuable food source in aquaculture. Periphyton communities were compared on two artificial substrates - heather mats and geotextile, each in four fishponds, examining their potential advantages in supporting predatory fish production in aquaculture under Central European conditions. The abundance and biomass of periphyton macroinvertebrates on heather mats were higher than on geotextile, and this difference increased with time. These differences resulted from significantly different dipteran larvae abundances (biomasses) on both substrates (Fig. 1). The analysis suggested a higher autotrophic component on geotextile, possibly indicating an increase in cyanobacteria during rearing. The presence and consumption by benthic organisms explained the lack of autotrophic growth on heather mats, supported by a higher abundance and biomass of the heterotrophic component. Installing heather mats can enhance the availability of otherwise poorly available benthic food to the water column during the ontogenetic switch from a planktonophagous to an ichthyophagous mode of food intake in predatory farmed fish species. The experiment was financially supported by NAZV project (QK22020144).

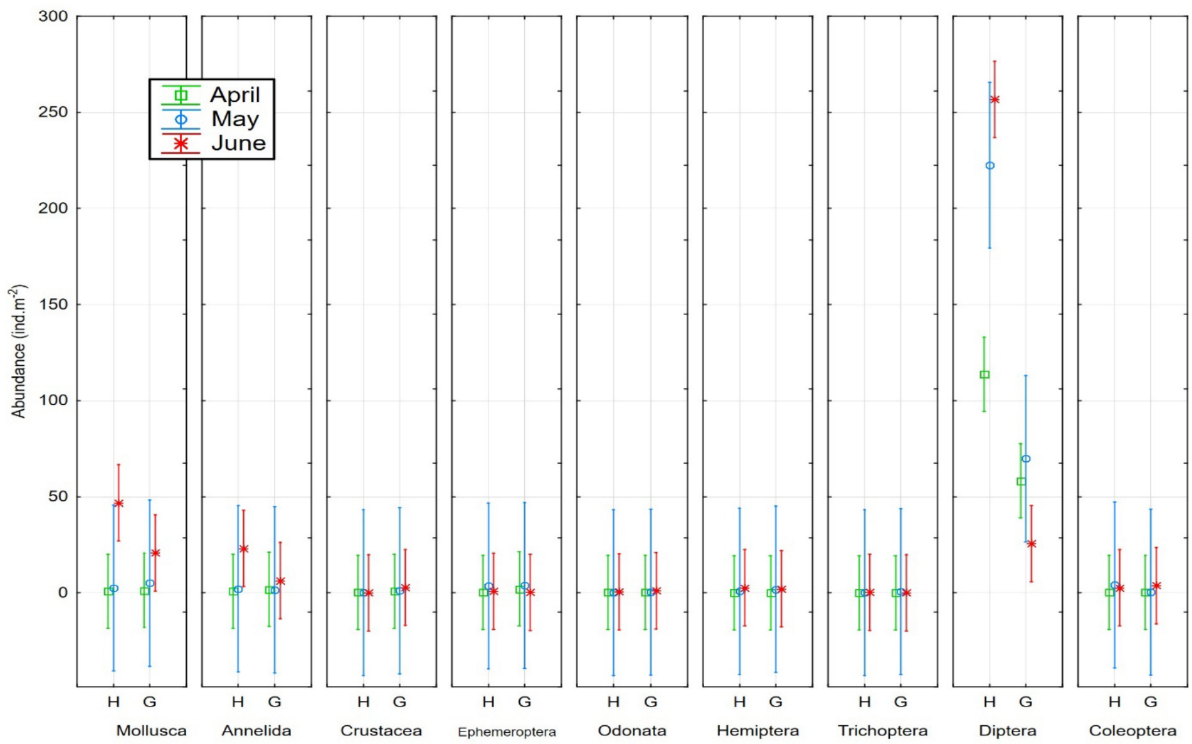

Fig. 1. Abundance of different systematic groups of periphyton macroinvertebrates on heather (H) and geotextile (G) substrates during the experiment.

TOWARDS SUSTAINABLE COEXISTENCE OF OFFSHORE WIND AND AQUACULTURE WITH FISHERIES AND OTHER MARINE ACTIVITIES

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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 and offshore wind are expected to increase competition for ocean space with existing activities, such as fisheries, shipping, oil and gas. Together with growing ambitions for marine protection, these developments emphasize the need for improved Marine Spatial Planning (MSP). To account for the lack of knowledge about key effects on ecosystems from e.g. offshore wind and/or offshore aquaculture development, careful consideration of uncertainties will be crucial to reduce the considerable trust gap among ocean stakeholders.

In the MARCO (MARine CO-existence scenario building) project, we utilize GIS (Geographic Information System) based data from different sources on Vulnerable Areas , spawning areas, and important areas for fisheries. These data are coupled with a system dynamics simulation model for the blue economy (i.e. wind, aquaculture, fisheries, and shipping activity). The project uses the North Sea part of the Norwegian Economic Zone as a case study.

The 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 utilize GIS technologies for mapping out plausible trajecto

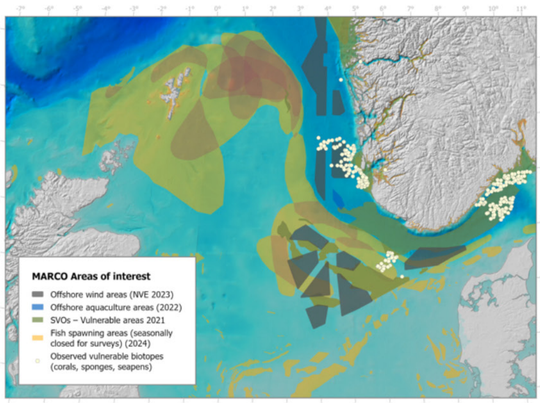

FIGURE 1. Suggested areas for development of offshore wind farming and offshore aquaculture in the Norwegian part of the North Sea. Vulnerable areas, observed vulnerable biotopes and spawning areas are also plotted.

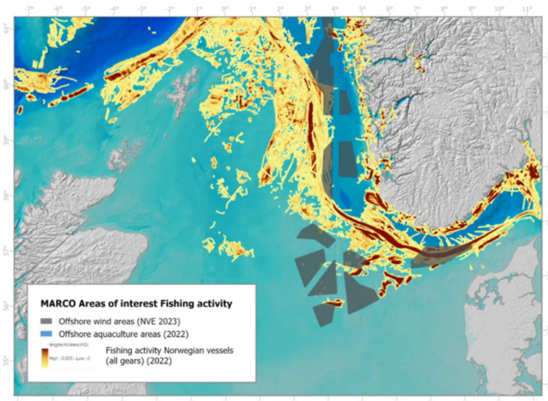

FIGURE 2. Fishing activity (in this case limited to Norwegian vessels) in the Norwegian part of the North Sea, with suggested areas for development of offshore wind farming and aquaculture added.

MULTI-USE REALISATION OF LOW-TROPHIC AQUACULTURE WITH OFFSHORE WIND FARMING OR FISH FARMING IN THE NORTH SEA AND BALTIC SEA

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The need for more efficient use of marine area has highlighted the potential for multi-use, i.e. the use of area for more than one purpose. The recent expansion of offshore wind power has emphasized conflicts among different stakeholders. At the same time, aquaculture of low-trophic organisms, such as bivalves or seaweeds appears an attractive way to increase biomass production. Bivalves can feed on microalgae and particles, and seaweeds utilize nutrients, such as nitrates, phosphates and CO_2. In the OLAMUR project, we are developing initiatives for multi-use involving low-trophic aquaculture (LTA) in the Baltic and North Seas.

The seaweed and mussels produced at the tree sites will undergo extensive analyses for nutrient content as well as contaminants.

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

Thereby, LTA can improve water quality and ecosystem services. Furthermore, LTA adds to a circular bioeconomy, utilizing excess nutrients from land and from fish farming.

LIGNOCELLULOSIC FILTRATION TECHNOLOGY FOR VALORIZATION OF RAS FISH MANURE

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Aquaculture waste can exacerbate eutrophication in both marine and freshwater ecosystems. Mitigating nutrient emissions from the aquaculture industry is crucial for enhancing the sustainability of the industry. Fish manure produced from recirculating aquaculture system (RAS) consists of uneaten feed and feces, and contains a significant portion of phosphorus and micronutrients, making it a valuable resource as fertilizer. However, due to its high-water content and metals, it poses challenges for transportation and utilization. Therefore, the manure water and metal content need to be reduced to obtain a higher valuable material.

In this study, we investigated the efficacy of lignocellulosic and cellulose filtration materials for reducing the water content of fish manure. We assessed the performance of the technology in freshwater and saltwater conditions. Our findings demonstrate that the technology can produce a fish manure cake with a dry matter content ranging from 25% to 35%, resulting in an 80% reduction in final weight. Additionally, concentrations of organic matter, phosphorus, and nitrogen in the treated manure were removed by 80%, 85%, and 78%, respectively.

Moreover, heavy metal concentrations, particularly cadmium and nickel, were reduced by up to 10-fold compared to typical levels found in fish manure. This enables its utilization in crop production while meeting regulatory standards for heavy metal concentration disposal.

The technology also has the potential to be tailored and additional elements such as struvite can be added to increase the fertilizer properties. Finally, to showcase its versatility, we utilized fish manure cakes as a substrate for growing mushrooms, demonstrating their potential as compost or fertilizer material and as a possible treatment method for fresh and saline fish manure.

THE EFFECT OF ALGAE SUPPLEMENTED FEEDING REGIME ON THE SPERM QUALITY IN COMMON CARP (*Cyprinus carpio*) MALE BROODSTOCK

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The aquaculture sector is facing a lack of natural resources and new challenges (decline of fishmeal, lack of water in extensive production, replacement of non-renewable energy sources, reducing of ecological footprint, monitoring of fish meat quality, reduction of pathogens, etc.). Common carp is one of the most cultured freshwater species in Europe and worldwide as well. Nowadays, it is difficult to predict the success of common carp propagation. In some cases, a variable or low individual fertilization capacity in breeders is observed. Alternative feed additives (such as algae, vitamins etc.) can increase gamete quality and stabilize the propagation success.

The aim of our study was to investigate sperm quality in common carp male broodstock following a 1% algae supplemented feeding regime (*Chlorella* spp, *Scenedesmus* spp, *Coelastrella* spp, *Acutodesmus* spp). The individuals were fed with experimental and control (carp broodstock feed, Haltáp Ltd., Szarvas, Hungary) feed in two different earthen ponds for one month prior to the spawning season. Six-six male was sampled at three different period (water temperature: 16, 20.5 and 22 °C) from both experimental and control group during the spawning season. Common sperm quality parameters (total volume, volume per body weight, motility parameters, osmolality, pH, concentration, total sperm production and total sperm production per body weight), glucose and ion content (glucose, fructose, sodium, potassium, calcium, magnesium), and fatty acid content of sperm (total, saturated, unsaturated, poly unsaturated, omega-3 and -6 fatty acids) were comprehensively analysed in both groups at each sampling period.

Significantly lower result was recorded only in pH and curvilinear velocity (kinetic sperm parameter) by the experimental group in comparison with the control. No significant difference was observed between the two groups in the glucose, ion and fatty acid content. The water temperature (sampling period) had significant effect on various common sperm quality parameters, glucose and ion content. In contrast, fatty acid content of sperm was not affected by the water temperature. This comprehensive sperm quality study highlighted the future opportunity of algae supplementation in the feeding regime of common carp male broodstock prior to the spawning season. However, the water temperature can affect notably several quality parameters.

The study was supported by the project 2020-1.1.2-PIACI-KFI-2020-00161 and the project "Support for talent management programmes in colleges" (NTP-SZKOLL-23-0043), commissioned by the Ministry of Culture and Innovation and funded by the National Cultural Fund of Hungary. Our work was also co-financed by the project 2022-1.2.6-TÉT-IPARI-TR-2022-00002 within the framework of the National Research, Development and Innovation Fund announced by the Ministry of Culture and Innovation. The experiments were also funded by the ÚNKP-22-3 New National Excellence Program of the Ministry of Culture and Innovation from the source of the National Research, Development and Innovation Fund.

ANALYZING QUALITY OF MONITORING DATA ON DISSOLVED OXYGEN AND SEA TEMPERATURE FROM MULTIPLE NORDIC OPEN SEA CAGE FARMERS

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Introduction

The interest to introduce digital technologies, such as AI and digital twins, into aquaculture practices is growing, for the purpose of automating and streamlining operations, including stabilizing dissolved oxygen (DO) levels and optimizing feeding [1,2]. These technologies rely on quality data to provide value. Ambient sea temperature (T) and DO are central to animal welfare and growth [3,4] and farmers collect vast amount of data on these parameters. However, due to the high variability of these water quality parameters within and between sites, standardizing methods to collect data is challenging. While reporting on the parameters are regulated, a lack of trust in the quality of the data prevents it from being used operationally. This phenomenon occurs in several industries [5]. For open sea cages, we have found no existing framework on how to collect and verify that the data collected in operations are of sufficient quality. In this study, we have gained access to production data from 18 open sea cage farmers to investigate the quality of data and potential operational values.

Methods and materials

In total, the 18 companies have 105 sites listed with 236 sensors. 137 sensors are active and 99 are inactive, and the active and inactive periods range from days to multiple years. The sites are spread across a large geographical area along the Norwegian coast and the Faroe Islands. The data is analyzed in an exploratory manner, using systematic data quality frameworks developed for other industries [5], here applied to aquaculture practices.

Results and Discussion

In our preliminary results, we find a range of quality issues in the data from the majority of the sensors. Physical granularity is the first main issue we identify, as 85% of the sites have four or less sensors across the space of the entire site. DO, especially, can vary greatly across short spaces [6], requiring a high level of granularity. The second pervasive issue we identify is accuracy. The data is noisy, and often only one sensor is placed in a cage, making validation of abnormal variations difficult, as it could be due to local variations that, albeit unusual, can occur naturally. Two examples of this are visualized by graphs 1 and 2 in Figure 1, with the first example showing unusually large variations in T and the second showing unusually similar variations in DO and T across all depths of the cage, which is often an indication of sensor issues. In this case, however, variations could be real and caused by a homogenous environment. Other sensor measurement errors are easier to detect, with examples in graphs 3 and 4 in Figure 1. Graph 3 in this example shows too stable DO values over time, within the optimal range. While we can visually observe an issue in this case, detecting it in real-time can be more challenging, as the values are not identical over time and within the positive range. Sensor 4 shows clear sensor errors in both T and DO. Abnormal values that are not caused by sensor errors can be due to production routines when the sensors are e.g., moved, such as net cleaning, sensor cleaning, disease treatment or counting. For digitalization using this data to provide value to the organization, more information is required. These early results indicate that current methods to monitor the sea cage environment is insufficient and provide low operational value. Methodical improvements for systematic monitoring are necessary before implementing digital technologies to extract value from this type of data.

References

[1] C. Wang, Z. Li, T. Wang, X. Xu, X. Zhang, and D. Li, "Intelligent fish farm—the future of aquaculture," *Aquac Int*, vol. 29, no. 6, pp. 2681–2711, 2021, doi: 10.1007/s10499-021-00773-8.

[2] M. Føre et al., "Precision fish farming: A new framework to improve production in aquaculture," Biosystems Engineering, vol. 173, pp. 176–193, Sep. 2018, doi: 10.1016/j.biosystemseng.2017.10.014.

[3] M. Remen, F. Oppedal, A. K. Imsland, R. E. Olsen, and T. Torgersen, "Hypoxia tolerance thresholds for post-smolt Atlantic salmon: Dependency of temperature and hypoxia acclimation," Aquaculture, vol. 416–417, pp. 41–47, Dec. 2013, doi: 10.1016/j.aquaculture.2013.08.024.

[4] M. Remen, M. Sievers, T. Torgersen, and F. Oppedal, "The oxygen threshold for maximal feed intake of Atlantic salmon post-smolts is highly temperature-dependent," Aquaculture, vol. 464, pp. 582–592, Nov. 2016, doi: 10.1016/j.aquaculture.2016.07.037.

[5] J. Morewood. "Building energy performance monitoring through the lens of data quality: a review" Energy & Buildings, vol. 279, pp. 582–592, Jan. 2023, doi: 10.1016/j.enbuild.2022.112701.

[6] D. Solstorm et al., "Dissolved oxygen variability in a commercial sea-cage exposes farmed Atlantic salmon to growth limiting conditions," Aquaculture, vol. 486, pp. 122–129, Feb. 2018, doi: 10.1016/j.aquaculture.2017.12.008.

(Continued on next page)

Figure 1: Examples of data from four sensors. In graphs 1, 2, and 4, the dotted lines represent T and the solid DO, while the different colors represent different depths. In graph 3, the blue line represents DO and the black T, at one depth.

BEHAVIOURAL METRICS OF INDIVIDUAL BROODSTOCK *Penaeus Monodon:* BASELINE DEVELOPMENT AND RESPONSE TO HUSBANDRY INTERVENTIONS

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Crustacean behaviour research has advanced in the last decades, with research focused on lobsters and crabs paving the way. However, the behaviour of the bulk of farmed crustaceans, i.e., Penaeid species, is poorly understood. Additionally, for shrimp species such as *Penaeus monodon*, there is still heavy reliance on wild-caught broodstock for seedstock production in hatcheries, and for all shrimp species, eyestalk ablation is still commonly employed by industry to induce satisfactory reproductive outcomes yet is attracting growing welfare concern. Understanding the behavioural profiles of *P. monodon* facilitates the assessment of the welfare impacts of husbandry interventions and strategies that may mitigate these impacts. The experimental system consisted of large aquaria with sand substrates, each equipped with individual custom computer modules to capture HD infrared recordings. Individual broodstock shrimp (N=16 for baseline behaviours) were stocked in sand bottom tanks for 72 hours. An ethogram was developed based on previously defined behaviours for other Penaeid species and refined with the identified behaviours in *P. monodon*, including walking, swimming, digging, feeding, grooming, eye beats, antennal scale flexion, forward antennal position, spawning and inactivity. Video analysis was quantified using Behavioural Observation Research Interactive Software (BORIS v8.22.6). The ethogram was then used to assess the response to husbandry interventions including ablation (N=8), ablation with topical anaesthetic (Lidocaine hydrochloride 20mg/g, N=8), handling (N=8), undisturbed (N=8), and a pleopod biopsy (N=8). These findings will contribute to our understanding of the impact of routine husbandry practices on the behaviour of broodstock *P. monodon* and provide a methodology for assessing welfare to be applied in future research on strategies to optimise broodstock management.

TARGETED ANALYSES OF A CANDIDATE GENOMIC REGION DETERMINING THE LACK OF PIGMENTATION IN GILTHEAD SEABREAM

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A major issue in gilthead seabream aquaculture is the high rate of phenotypic abnormalities during early development. Fry that do not meet specific standards are discarded, leading to significant economic losses. Increased defects in inbred populations suggest genetic factors may be involved. While skeletal deformities have been extensively studied, pigmentation discolorations also pose economic concerns by affecting consumer acceptance. Fish pigmentation results from the activity of up to six types of pigment cells or chromatophores, including melanophores (black or brown pigments), xanthophores (red, orange, and yellow pigments), erythrophores (red, orange, and yellow pigments), and iridophores (iridescent, blue, silver, or gold structural colours). These chromatophores are common in many fish species, including S. aurata, and are influenced by various genetic controls. However, the exact genetic mechanisms behind fish pigmentation are not fully understood.

We previously identified a strong signal on chromosome 6 (17-23 Mbp) linked to a lack of pigmentation, but the annotation of the gilthead seabream genome was limited at that time. Here, we leveraged recent progress in genome annotation and additional samples to characterize this genomic region. We compared genomic pools of 21 non-pigmented fry with three pools of normally pigmented fish (two related to the non-pigmented group and one unrelated) to confirm the peak. Variant calling was performed with CRISP, and Single Nucleotide Variants (SNVs) and Insertions/deletions (INDELs) were screened with VEP (Variant Effect Predictor). We compared the list of genes within the region with a comprehensive list of pigmentation-related genes in humans, mice, and zebrafish (https://www.ifpcs.org/colorgenes/), which includes nearly 700 genes. The analyses identified 4,810 SNVs and 541 INDELs, with six genes involved in pigmentation (dstyk, erbb3, fancd2, parp3, rab7, and slc2a11). Notably, the slc2a11 gene has been linked to the absence of xanthophores in zebrafish and medaka. Within these genes, 27 SNVs showed high divergence in allele frequency between the non-pigmented and pigmented pools, indicating that the non-pigmented pool contains carriers for the linked mutations. Most of the SNP consequences are linked to intronic and upstream/downstream gene variants, with no SNVs or INDELs identified as having significant impacts (Figure 1).

In this work, we identified several candidate genes for lack of pigmentation and concluded that the causative mutations may be linked to regulatory regions rather than functional changes in protein structure. Further analyses of these genes are necessary.

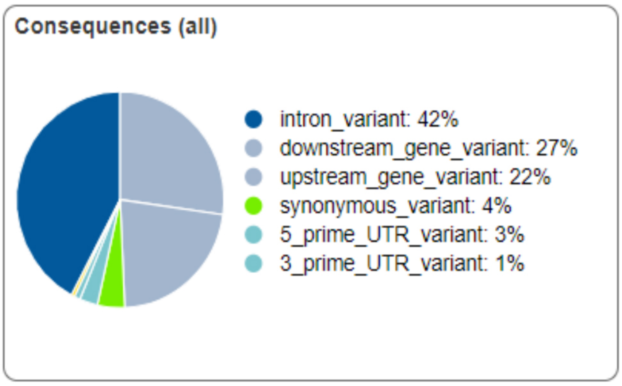

Figure 1: Percentage of predicted consequences from VEP of the SNVs with the highest allele frequency divergences within the six selected

IMPACT OF GLOBAL CLIMATE CHANGE ON *Paracentrotus lividus* LARVAE: DEVELOPMENTAL AND MOLECULAR MARKERS OF TOXICITY

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This study investigates the combined impact of microplastics (MP) and Chlorpyriphos (CPF) on the growth and development of sea urchin larvae (*Paracentrotus lividus*) under the backdrop of ocean warming and acidification. While the individual toxic effects of these pollutants have been previously reported, their combined effects remain poorly understood. An experiment was conducted using the Effective Concentration 50 (EC50) based on previous studies from our group. MP were adsorbed in CPF to simulate realistic environmental conditions. Additionally, water acidification and warming protocols were implemented to mimic future ocean conditions. Sea urchin embryo toxicity tests were conducted to assess larval development under various treatment combinations of CPF, MP, ocean acidification (OA), and temperature (OW). Morphometric measurements, molecular, and biochemical analyses were performed to evaluate the effects comprehensively.

Results indicate that combined stressors lead to significant morphological alterations, such as increased larval width and reduced stomach volume. Furthermore, biochemical biomarkers acetylcholinesterase (AChE), glutathione S-transferase (GST), and glutathione reductase (GRx) activities were affected, indicating oxidative stress and impaired detoxification capacity. The gene expression of these enzymes was upregulated showing the necessity of produce more protein in response to pollutants. The different isoforms of each gene (*ache*, *gst*, *grx*) present differences in their expression. Interestingly, while temperature was expected to enhance larval growth, it instead induced thermal stress, resulting in lower growth rates. This underscores the importance of considering multiple stressors in ecological assessments. Biochemical and molecular biomarkers provided early indications of stress, complementing traditional growth measurements, although molecular markers should be further studied to determine which isoforms better describe the behaviour of the enzymatic activity.

This study underscores the importance of holistic approaches in evaluating impact of environmental stressors on sea urchin aquaculture. Comprehending the interplay between pollutants and environmental stressors is essential for devising effective conservation measures. Further investigation should focus on understanding the effects at lower biological levels and examining adaptive responses in sea urchins confronting various stressors. Through this approach, we can enhance our ability to predict and mitigate the negative consequences of anthropogenic pollutants on sea urchin aquaculture and on the health of marine ecosystems.

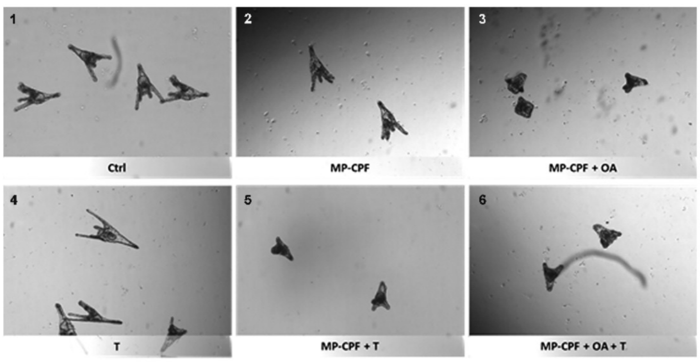

Figure 1. Images represent larvae morphology under each treatment from the experiment.

BENEFITS AND DRAWBACKS OF GENETICALLY SELECTING MORE EFFICIENT SEA BASS : IMPLICATIONS FOR LIPID TRAITS

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We previously showed that individual feed efficiency in the aquariums had a significant genetic basis in sea bass (genomic $h^2 = 0.47$). However, further exploration of the physiological factors influencing individual variations in feed efficiency, especially regarding lipid distribution and composition, is crucial. It is commonly assumed that leaner animals are expected to exhibit greater efficiency. Surprisingly, our study revealed that sea bass with higher global fat content exhibit greater efficiency, although further exploration of this relationship was not pursued. Therefore, the objective of the present study was to delve deeper into the relationship between individual efficiency and lipid distribution and composition in European sea bass.

First, 458 sea bass from the commercial breeding program of EMG-Ichtus were phenotyped in aquariums to estimate their feed efficiency as the residual body weight gain (rBWG). All fish were genotyped to estimate genetic parameters. Based on estimated breeding values, two categories were created: rBWG+ gathered the 120 fish with the highest EBVs while rBWG- gathered the 120 fish with the lowest EBVs. These 240 fish were reared in 6 groups of 40 fish (each group containing fish from the same EBV category) until 748g. Then, we measured several harvest traits: harvest weight, muscle fat content, fillet weight, head weight, viscera weight and headless carcass weight. Fatty acid composition was determined using gas chromatography on samples extracted from the right fillets. The left fillets underwent MRI analysis () to estimate the repartition of lipids in the filet (Figure 1).

rBWG was heritable (0.22 ± 0.08). There were no difference in fillet yield and headless carcass yield between rBWG+ and rBWG- fish suggesting no adverse effects of improved feed efficiency on these traits. However, rBWG+ fish exhibited significantly greater viscera-to-body ratio and lower head-to-body ratio compared rBWG- fish. MRI analysis indicated that rBWG+ fish were fatter (13.5% fat) than rBWG- fish (11.6% fat). These findings confirm a link between higher feed efficiency and increased fat content in sea bass, a unique observation in current literature. Elevated levels of palmitic and palmitoleic acid in more efficient fish suggest a higher tendency to convert protein into lipids. One hypothesis suggests that these efficient fish exhibit higher protein retention, but surplus protein may be converted into lipids in the absence of structural growth, as indicated by higher viscera-to-body ratio in more efficient individuals. However, all fillet areas were equally rich in lipids for both groups. Finally, there were promising results regarding EPA and DHA content, which were richer in more efficient fish. Notably, EPA and DHA also displayed heritability (0.17 and 0.07 respectively) suggesting the potential for selective breeding to enhance favorable fatty acid composition.

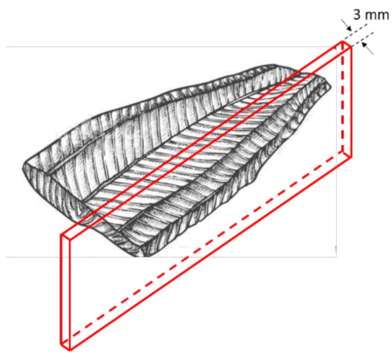

Figure 1. Orientation of MRI sections relative to the fillet. Several sections were taken to cover the full coronal plan (from the belly to the back) of the fillet.

SELECTION FOR GROWTH OR CARCASS YIELD BEFORE THE ONSET OF THE SPAWNING SEASON WILL NOT IMPACT EGG PRODUCTION IN RAINBOW TROUT *Oncorhynchus mykiss*

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The rainbow trout *(Oncorhynchus mykiss)* is a key species for the European freshwater aquaculture with nearly 185,000 tons produced in 2020 (EUMOFA 2022). There are currently at least 7 selective breeding programs operating in Europe on this species. These programs have mainly been improving growth and slaughter traits, and some have been dealing with sexual maturity issues. The first results on the genetic basis of spawning performance date back to the 1970s. There is now a need to understand the genetic links between egg-production traits, growth performance and carcass quality traits.

Our experiment focused on 1600 females from a selected commercial line of rainbow trout (Aqualande, France). They were produced through partial factorial matings of 100 sires and 84 dams. The females were individually tagged and recorded for body weight (BW) at nine months of age (355 g), sixteen months of age (1700 g), twenty months of age (2600 g) and at spawning (2600 g, twenty-one months of age). Six periods of thermal growth coefficients (TGCs) were evaluated until spawning. The fat content in the fillet (Fat) was recorded three times, spawning included. The non-lethal predictor of carcass yield (PCY) was measured at sixteen and twenty months of age using ultrasound tomography. At spawning, the following traits were recorded: weight of the spawn (on average 342 g), number of eggs (~7100), weight of an egg (~48 mg), relative fecundity (~3300 eggs per kilogram of live female) and gonado-somatic index (~13%). The heritabilities and genetic correlations between TGCs, BWs, Fats, PCYs and spawning traits were estimated using linear animal models.

The genetic correlations between BWs and spawning traits were all either not different from zero or positive (depending on the trait, from 0.05 ± 0.12 to 0.70 ± 0.06), as for the TGCs (-0.20 ± 0.13 to 0.63 ± 0.07) and PCY at sixteen months of age (-0.03 ± 0.14 to 0.24 ± 0.14). On the contrary, PCY at twenty-months was genetically negatively correlated with egg-production traits (-0.16 ± 0.14 to -0.47 ± 0.11). The genetic correlations between the Fats and the spawning traits ranged from -0.20 ± 0.11 to 0.32 ± 0.10.

In this population, selecting for growth up to twenty months of age should not impact the spawning performance. It may even contribute to slightly enhance it. Improving the carcass yield should not impair the egg-production traits as long as the target age does not exceed twenty months. On the contrary, a selection pressure applied on carcass yield during the late period of the ovaries development may be detrimental. We did not evidence a clear impact on the evolution of Fat during rearing up to spawning. This may be due to the fact that the feed fulfills the requirements are highly enough respected for good egg production.

We recommend that in selective breeding programs of rainbow trout that do not target egg-production traits, there is some individual spawning performance follow-up. Indeed, this would enable to observe changes in the genetic correlations between traits and then adapt the index of selection, the pressures to be applied on the different traits of interest and at what age.

FranceAgriMer (National Institute for agricultural and sea products) and the European Marine and Fisheries Fund co-founded this work through the Best'Ov project (n°2014-0823). Authors thank warmly Marc Vandeputte and Mathilde Dupont-Nivet for useful brainstorming sessions.

TRANSGENIC OMEGA-3 AND CAROTENOID-ENRICHED CAMELINA OIL ON FILLET QUALITY OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Terrestrial oils, which are used to replace fish oil in aquafeeds, are devoid in the health promoting omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) eicosapentanoic and docosahexaenoic acid (EPA and DHA, respectively). Consequently, n-3 LC-PUFA levels have also decreased in the fish fillet. Concomitantly, a reduction in the fillet deposition of astaxanthin (AST), the pigment that donates the pinkish colour, was observed. Recent studies on a novel source of n-3 LC-PUFA using the oilseed crop *Camelina sativa* as a vehicle to produce EPA and DHA gave promising results to boost n-3 PUFA levels in fish fillet. This trial tested a new iteration of this transgenic oil containing EPA, DHA and also ketocarotenoids, predominantly AST, with the potential to increase fillet pigmentation in parallel to EPA and DHA levels.

Rainbow trout (*Oncorhynchus mykiss;* initial weight: 294±32 g) were divided into groups of 40 fish and allocated into quadruplicate tanks per treatment. Two experimental diets were tested, a control diet (CTL) containing 8% fish oil and 12% plant oils, according to commercial common practices, and a camelina oil diet (CAM) with only the transgenic oil at 20% inclusion. In addition, CTL, but not CAM was supplemented with synthetic AST (50 mg/kg; Carophyll pink 10%, DSM). Analysis of the experimental diets revealed that n-3 PUFA accounted for 21% of total fatty acids in CTL compared to 30% in CAM. Although AST levels were considerable higher in CTL compared to CAM (39±1 mg/kg vs 16±2 mg/kg), total carotenoid levels were comparable between feeds (46±1 mg/kg in CTL vs 42±4 mg/kg in CAM). The fish were fed the experimental diets over a period of 10 weeks. At the end of the feeding period, 10 fish were sampled for chemical and molecular analysis. In addition, 6 fish per tank were challenged in a swim tunnel prior to sampling. Performance parameters collected during the trial were complemented with measures of flesh quality including fatty acid and carotenoid levels as well as fillet colour profile.

Final body weight was not significantly different between treatments, although fish fed CAM were in tendency (*p=0.06*, t-test) smaller compared to CTL (513±91 vs 537±106 g). Diet had no significant impact on exercise performance in the swim tunnel including tail beat counts or critical swimming speed. The fatty acid profile of the fillet reflected that of the diet with higher EPA and DHA levels in CAM compared to the CTL treatment. Fillet AST levels were significantly lower in CAM compared to CTL (Fig 1). In accordance, colorimeter measures gave a significantly lower red value in CAM compared to CTL.

In conclusion, the transgenic Camelina oil effectively increased fillet EPA and DHA levels compared to a fish/terrestrial oil mix but resulted in lower fillet colour compared to synthetic AST supplementation.

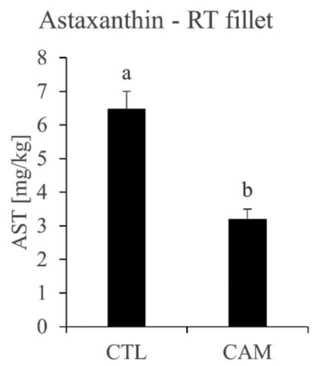

Figure 1 Astaxanthin (AST) levels in fillet of rainbow trout fed either a diet formulated on fish oil, terrestrial oils, and synthetic AST (CTL) or a transgenic EPA, DHA and AST-enriched Camelina oil (CAM)

PRELINE'S EXTENDED SMOLTFARM & PIPEFARM – (ENVIRONMENT)^4 SEMI CLOSED/ CLOSED TECHNOLOGY WITH ENVIRONMENTAL IMPACT IN 4 DIMENSIONS

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Our approach is holistic. Considering the laws of nature; physics (gravity, hydro dynamics, material properties), chemistry (water properties, nutrients & organic salts), biology (species characteristics, welfare, health, pathogens, parasites, predators) climate (sea state, temperature, moon phases, seasons) we design and build fishfarms.

The FOUR dimensions with improved Sustainability and Environmental impact are:

- PLANT/STRUCTURE: A circular approach to design and production minimises impact.
- FISH: The physical environment in the farm optimizes fish health and welfare.
- WASTE: Collection of faeces and excess feed is maximized.
- NATURE: The external environment is shielded by minimizing and diluting emissions.

The circular production approach is based on strong materials with a long life span Combined with a module based design, enabling efficient exchange of equipment when worn-out or out-dated. GRP (Glass reinforced plastic) is the material of choice, with an expected (escape free) life span of 40 to 60 years. We build for 20 years of certified use after which the structure will be docked, stripped, repaired and new equipment installed for productive use for another 20 years when the procedure is repeated till the plant is recycled. Equipment is kept in step with the technological development. Result: Effective life cycle management, Minimized cost pr kg fish produced and Minimized environmental impact.

To optimize fish health and welfare a flow of temperate water with high levels of Oxygen (O_2) and low levels of Carbon Dioxide (CO_2) is used. A speed of 1 fish length pr second is achieved with water collected below the lice belt and delivered back at about the same depth after flowing through the fish chamber. The flow is driven by low energy thrusters. The GRP walls protects farmed fish from surface pathogens, parasites, and predators. Speed keeps the fish focused on swimming and eating – reducing disputes. Mortality below 1,0% for a post smolt period of 100 to 150 days is normal. Low mortality and high quality will contribute to increased value creation at harvest. Exercise results in a robust fish with lower mortality, higher growth and a Netpen phase as short as 7 months.

Through a circular approach to operations, we achieve: Minimal use of additives such as (O_2) in production – due to high flow of good quality fresh seawater. Minimal feed waste, achieving a Feed Conversion rate (FCR)* of 0,8-0,9; reducing feed costs. We collect faeces and dead fish in traps along the bottom of the chamber. 100% of dead fish and 60 to 95% of solids – depending on technology version are collected. Our solutions and technology for collecting discharge is in constant development/improvement. We deliver solids to available circular value chains. Contributing to development of future income streams.

We protect the environment and life in the sea from our operations by minimizing farm discharge. Dissolved nutrients, organic salts and the 5-40% of solids not collected is diluted to very low concentrations and reintroduced at a depth of 20 to 40 meters. Financial gains will materialize when taxonomy is implemented in aquaculture.

Our Preline Extended smolt farm has been in production since May 2015, 3 million+ Postsmolt in 17 batches. 18th batch in production. Documentation of results are available.

* FCR = Kg feed / Kg fish produced

STRATEGIES TO REDUCE *VIBRIO* CONTENT IN THE GUT OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Shrimp farming, a significant contributor to global aquaculture, faces substantial challenges due to diseases caused by pathogens like *Vibrio* species. Antibiotic use, once common, is now restricted due to environmental and health concerns. This study aims to evaluate alternative strategies, particularly focusing on the influence of feeding regimens and additives on *Vibrio* spp. in the gut of Pacific white shrimp (*Litopenaeus vannamei*).

In the first part of this study, various feeding regimens (single feeding, four feedings per day, and continuous feeding) were tested to assess their effect on *Vibrio* counts on TCBS plates in both shrimp gut and feces collected. Sixty shrimp were randomly selected and allocated to three tanks (HI, H2, and H3), each containing 150 litres of culture water, and were fed over a five-day period. Examination of both gut and feces samples indicated that continuous feeding resulted in the lowest *Vibrio* spp. count, indicating a reduction in bacterial growth, whereas feeding once daily had the least influence on the bacteria population. The experimental results are depicted in Figure 1. This finding highlights the potential influence of feeding frequency on gut bacteria dynamics in shrimp aquaculture.

The subsequent experiment examined the inhibitory effects of a feed additive containing a blend of short-chain fatty acids, carpic carpylic, butyric and lauric acid on *Vibrio* growth. The shrimp were fed a reference diet or reference diet supplemented with 0.4% fatty acid blend for five days. Analysis of bacteria inside the gut and feces samples revealed that the addition of fatty acids in the feed resulted in significantly lower *Vibrio* counts on TCBS plates, measuring only 1.39×10^5 and 7.30×10^5 (CFU.ml-1) in the gut and feces respectively, compared to 2.23×10^5 and 1.13×10^6 isolated from gut and feces samples of shrimp fed the reference diet only (see Figure 2). Understanding the relationships between bacterial count influenced by the given diet is crucial for developing sustainable disease management practices in shrimp farming.

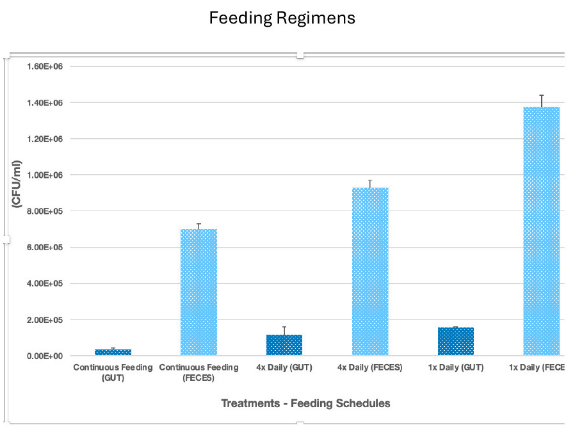

Figure 1. *Vibrio* counts (CFU/mL) isolated from gut (GUT) and feces (FECES) samples collected from *Litopenaeus vannamei* fed with different feeds (Continuous, 4x Daily, 1x Daily). Error bars represent standard deviation around the mean *Vibrio* counts, indicating data variability.

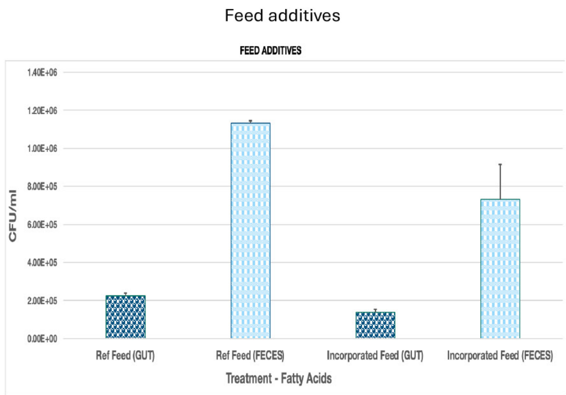

Figure 2. *Vibrio* counts (CFU/mL) isolated from gut (GUT) and feces (FECES) samples collected from *Litopenaeus vannamei* fed with reference diet (Ref Feed) and diets to which fatty acid blends were added (Incorporated Feed). Error bars represent standard deviation around the mean *Vibrio* counts, indicating data variability.

APPLICATION OF ADVANCED BIOGEOCHEMICAL MODELS FOR THE ASSESSMENT OF IMPACT ON MARINE PROTECTED AREAS

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In this study, generic models for the assessment of marine aquaculture in relation to marine protected Natura 2000 areas (N2000) was developed and applied, in accordance with the guidance on the European Habitat Directive and the biological quality elements used in the EU Water Framework Directive.

The habitat assessments involved five consecutive years of full-scale 3D hydrodynamic and biogeochemical modelling, use of satellite images/Earth observation data (EO) of benthic vegetation and extensive GIS mapping. The complex was validated and documented using five years monitoring data from the Danish NOVANA program (the national monitoring program for the aquatic environment and nature).

The hydrodynamic model includes an integrated dynamical description of waves, currents, shear stress, salinity, and temperature. The biogeochemical model includes an integrated dynamical description focusing on pelagic and benthic impacts. It also comprises an advection/dispersion module.

For the study of eutrophication impacts, the biogeochemical model includes state 80 variables describing the release and fate of inorganic and organic of nutrients (nitrogen and phosphorus) and organic carbon to the pelagic and seabed. The pelagic part of the model describes changes in concentrations and processes related to phytoplankton, zooplankton, nutrients, dead organic matter (detritus), and dissolved oxygen, arising from the fish production. The benthic part of the model describes spill, deposition and fate of organic nutrients and carbon from fish cages. At the seabed, waste is mineralized and/or resuspended by shear stress (sum of wave and current energy).

The present study illustrates that advanced biogeochemical models is a key toll for scienced based and scientifically sound environmental impact assessment of marine aquaculture. Further, the study illustrates how aquaculture can be environmentally sustainable and compatible with nature protection provisions, if developed and located with respect to the biological structure, function and integrity of the protected habitats and species.

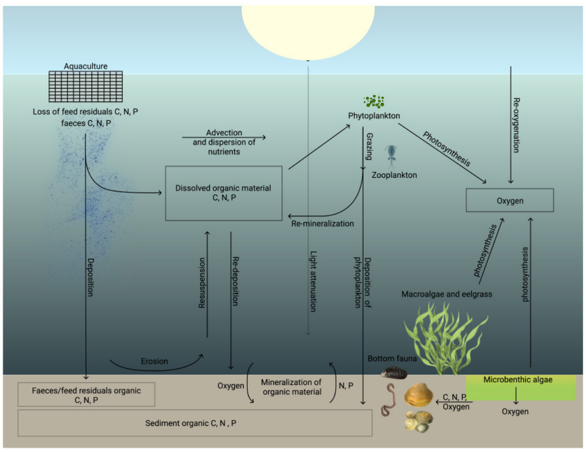

Figure 3 Schematic presentation of the model. The diagram is greatly simplified in relation to the actual complexity of the model.

GROWING SUSTAINABLY, ADVANCING MARINE AQUACULTURE IN THE UNITED STATES

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The National Oceanic and Atmospheric Administration (NOAA) is charting a bold course for American aquaculture to grow sustainably and reach its full potential. At an agency level, the "Office of Aquaculture's Strategic Plan (2023-2028)" provides a framework to manage efficiently and effectively, lead science for sustainability, educate the public, and support economic growth. This informs "NOAA's National Seafood Strategy", which outlines strategies for resilient seafood in our changing climate--encompassing both wild-capture fisheries and aquaculture. In addition, NOAA's science, including environmental interactions, climate change adaptations, and development of new candidate species, supports a resilient and sustainable industry.

Aquaculture is also becoming recognized as critical to American food security and climate resilience at the very highest levels of government. Executive Order 13921 "Promoting American Seafood Competitiveness and Economic Growth" called for, among other measures, the development of Aquaculture Opportunity Areas--geographic areas environmentally, socially, and economically appropriate for commercial aquaculture. The 2023 White House "Ocean Climate Action Plan" identifies aquaculture as a critical component of climate resilience, and the 2021 Homeland Security report "Threats to Food and Agricultural Resources" states the need for the U.S. to increase domestic aquaculture production. Additionally, aquaculture is recognized as pivotal to domestic food security in the White House "National Security Memorandum on Strengthening the Security of Resilience of United States Food and Agriculture." As aquaculture moves further into national discussions, NOAA will continue to provide science and services that ensure that the U.S. aquaculture industry grows sustainably.

EFFECTS OF RAPESEED AND PALM OIL ON GASTROINTESTINAL NUTRIENT SENSING AND APPETITE-REGULATORY MECHANISMS IN RAINBOW TROUT

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The diets used to feed aquaculture fish species have historically relied on high levels of fishmeal (protein source) and fish oil (lipid source). However, the continuous growth of aquaculture over the last decades has led to the need to reduce the dependence on these products. Plant-derived ingredients, and specifically rapeseed oil and palm oil, have been used as alternative lipid sources for a number of fish species. Nevertheless, while both have shown encouraging results for their use in aquafeeds, very little is known on the mechanisms involved in sensing these ingredients in the gastrointestinal tract (GIT) and on their putative physiological role in modulating feed intake. This study aimed to characterize the putative effects of rapeseed and palm oil on the mRNA abundance of several lipid sensors and appetite-regulatory hormones in the GIT of rainbow trout (*Oncorhynchus mykiss*). For this, fish were fed *ad libitum* with a diet containing high levels of either fish oil (control), rapeseed oil or palm oil, and samples of proximal and distal intestine, as well as plasma, were collected at 5 days, 3 weeks and 12 weeks for analysis.

As shown in **Fig. 1A**, *ad libitum* feeding with rapeseed oil- and palm oil-enriched diets required a significantly higher amount of feed to reach fish satiety, which indirectly suggests increased feed intake levels compared to fish fed a high fish oil diet, especially during the last weeks of the trial. Fish fed with both diets rich in a vegetable lipid source showed significantly higher plasma triglyceride and fatty acid levels compared to control (fish oil) fish (**Fig. 1B and C**).

Changes in the mRNA abundance of gut lipid sensors in response to rapeseed and palm oil were more notorious in the distal intestine, where increased levels of mRNAs encoding the fatty acid transporter Cd36, free fatty acid receptor 1 (Ffar1), members of the Ffar2 family, and the G-protein coupled receptor 119 (Gpr119) were observed compared to fish fed a high fish oil diet (**Table 1**). Interestingly, rapeseed oil- or palm oil-evoked changes in the mRNA abundance of gastrointestinal appetite-regulatory hormones (colecistokinin -*cck*-, peptide tyrosine tyrosine -*pyy*-, and glucagon- like peptide 1 -*glp1*-) showed an increase during the first weeks of trial (suggesting anorexigenic potential) but a decrease during the last weeks (suggesting orexigenic potential, as in Fig. 1A) (**Table 1**).

These results offer a view of physiological appetite-regulatory mechanisms activated upon feeding vegetable diets, which can serve for better and more efficient aquafeed formulations.

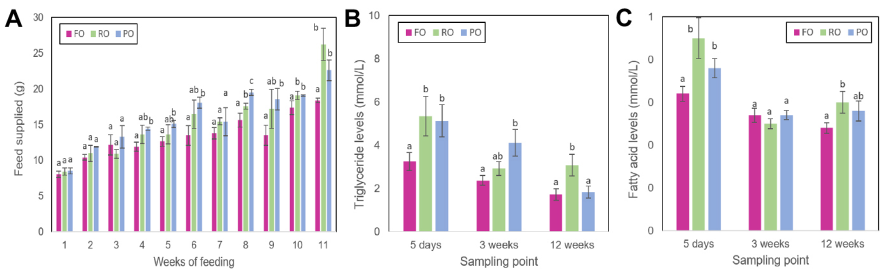

Figure 1. (A) Amount of feed supplied to trouts during the feeding trial. **(B,C)** Plasma triglyceride (B) and fatty acid (C) levels in trout fed a diet rich in fish oil (FO; control), rapeseed oil (RO) or palm oil (PO). Different letters denote statistical differences among diet groups within a given time point (p<0.05).

	Prox. Int.		Distal Int.	
	RO	PO	RO	PO
cd36	▲	≈	▲	▲
fatp4	≈	≈	▼	▼
ffar1	≈	≈	▲	▲
ffar2-family	≈	≈	▲	▲
gpr84	≈	≈	nd	nd
gpr119	≈	≈	▲	▲
cck	▲/▼	≈/▼	▲/▼	▲/▼
pyy	≈	≈	nd	nd
glp-1	≈	≈	▲/≈	▲/≈

Table 1. Differences (▲, increase; ▼, decrease; ≈, none) in mRNA levels of lipid sensors and appetite-regulatory hormones in proximal and distal intestine of fish fed rapeseed oil- (RO) or palm oil- (PO) enriched diet compared to control (fish oil) fish. nd: not detected.

ASSESSMENT OF MICROPLASTIC RELEASE FROM CLEANING OF AQUACULTURE NETS

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Aquaculture uses large amounts of plastic for infrastructure and equipment, which likely contributes to microplastic (MP; <5 mm) release to the environment. In addition to feed pipes and cage collars, pen nets are considered to be a potential source for MP release. *In-situ* net cleaning, typically using dedicated pressure washers, may exacerbate abrasion of nets and their coatings, leading to higher MP emissions during cleaning periods. As a result, fibres as well as synthetic particles from net coatings may be dispersed into the surrounding seawater.

A laboratory experiment simulating abrasion damage through net cleaning was performed to quantify and compare MP release from common and alternative net (Nylon, HDPE, Dyneema, ENTEX) and rope (polyolefins, HDPE, Dyneema) materials, both new and used. For the net material, samples with two coatings used to extend material lifetime were also included. The abrasion tests were conducted using a Buraschi abrasion simulator system (Figure 1). Particles released into the water were washed and filtered on 500 and 10 µm filters to isolate 'large' and 'small' MP fractions. The collected material was then quantified and physicochemically characterised using a combination of gravimetric measurements, microscope imaging and chemical fingerprinting by pyrolysis GC-MS.

Results indicate increased release from nylon nets compared to the HDPE, Dyneema and Entex nets. Coatings interacted differently with the various net materials, where there was no change in MP release from the coated Dyneema nets, but an increased particle release from the coated nylon nets. For ropes, we observed increased release from new Dyneema compared to new polyolefins and HDPE. For both nets and ropes, used materials released more MP than new ones.

The next step will be to conduct measurements in the field to validate the laboratory data. However, the outcomes of the laboratory study already indicate that the aquaculture industry may reduce current MP releases to the marine environment by selecting specific combi-nations of net and rope materials/polymers.

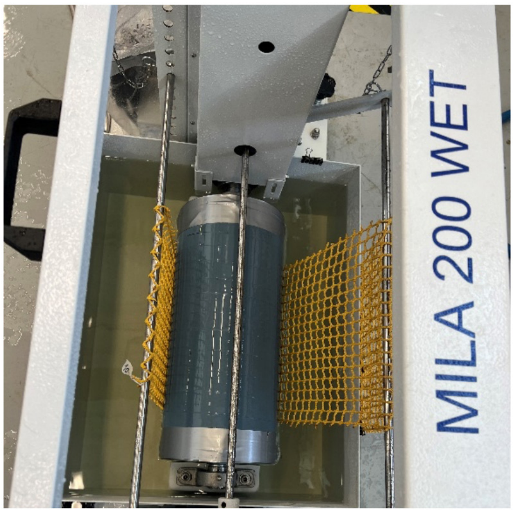

Figure 1: Abrasion test conducted with a coated net using a the Buraschi abrasion simulator system.

EFFECT OF FISH PUMPING ON THE ATLANTIC SALMON (*Salmo salar)*

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Atlantic salmon (*Salmo salar*) is one of the most economically important species of the aquaculture industry, with Nordic countries representing some of the major producers. Given its economic importance, an increased number of companies are implementing the use of recirculated aquaculture systems (RAS) for the production of Atlantic salmon. Throughout a production cycle in RAS, fish are subjected to a variety of stressful events, including transportation. In the case of Atlantic salmon, previous studies have focused on investigating the impact of truck transportation and transfer to sea on the health and stress status of the fish. However, information on the effect of pumping (e.g. from one tank to another) on *S. salar* is scarce. For this reason, the present research has focused on studying the impact of fish pumping on *S. salar* smolts in an industrial RAS, as well as their ability to return to basal levels, investigating the biochemical profile in the plasma, as well as the expression of relevant stress-related and immunoregulatory genes in a wide range of organs.

Salmo salar smolts were collected from an industrial RAS around a fish pumping event, transporting the fish from one tank to another. The sampling points were the following: 1) Three days before transfer; 2) During transfer; 3) 1h post stress (hps); 4) 24hps; 5) 7 days post stress (dps); 6) 14 dps. At each point, 8 individuals were sampled for blood, gills, liver, gut, and head kidney. Plasma was obtained through centrifugation of the blood for the characterization of the biochemical profile, and organs were destined to RT-qPCR analyses. The investigated biochemical parameters included cortisol, glucose, and indicators of hepatic health, whereas the researched genes included gr1, mr, muc2, il1β, and tnfα.

The results displayed a significant increase in cortisol, with a slightly shifted increase in glucose levels, as predicted, indicating that fish suffered from stress during the pumping event, and the detected values of cortisol and glucose returned to basal levels in a matter of days (Figure 1). Furthermore, the expression of some genes related to the stress and immunoregulatory response was significantly affected by the process.

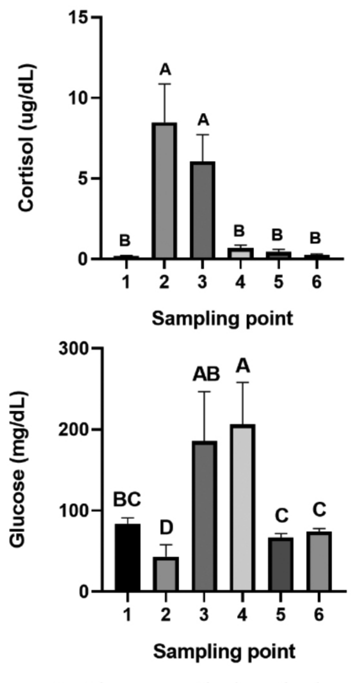

Figure 1: Plasma cortisol and glucose levels determined in *Salmo salar* during a pumping event. Sampling points: 1) Three days before transfer; 2) During transfer; 3)1h post stress (hps); 4) 24hps; 5) 7 days post stress (dps); 6) 14 dps.

A NOVEL METHOD FOR EXTERNAL FERTILISED MARINE FISH PROPAGATION: SPERM INSEMINATION

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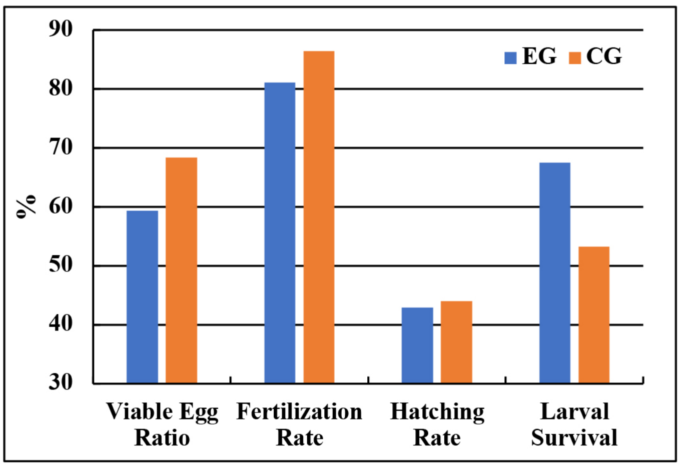
Introduction: In the current practice of marine finfish aquaculture, broodstocks are stocked in tanks at varying ratios of female to male, which could result in use of too much tank area, feed and labour and failure in the mating of targeted female and males in selective breeding programs. In this study it is aimed to understand the success of sperm lavage method on fertilisation, hatching and larval survival rates on marine finfish culture and European seabass *Dicentrarchus labrax*, used as a model organism for investigation.

Method: Females were injected 30 µg/kg BW LHRHa, single shot (600-650 µ oocyte size) in two experimental treatments including a control group representing a routine culture method and the second test group representing the novel method were tested in the study. In the novel examination group, the sperm collected from selected four males was inseminated to two female breeders 56 hours after hormone application (0.5 mL per ovarium per kg) (Müller et al. 2018). The experiment was conducted in three replications. Eggs were collected, weighed and then placed in incubator tanks for hatching. Embryonic developments were checked separately in the cooled incubator at 14.5°C for 4 days (Panini et al 2001). Hatching rates were checked in incubation tanks and larvae were distributed for larval rearing condition. Finally, larval survival rates were calculated after 28 days of larval weaning. This study was funded by TÜBITAK 121N529.

Results: Viable eggs ratio, fertilization rate and hatching rate were higher in CG (68.41%, 81.14% and 42.92%) but not significantly different from EG (59.32%, 86.41% and 43.96%) ($P>0.05$). On the other hand, larval survivor was higher in EG (67.53%) than CG (53.2%) but not significantly ($P>0.05$).

Conclusion: The result from this novel method is promising. A high practicality of the technologies is a great white hope that will open new doors for the future in the aquaculture sector by reducing the costs of broodstock management, fostering much more successful selective breeding programs, solving propagation problems of many candidate freshwater and marine fish species and appearing an unexpected tool for hybridization of fish.

Literature:
Müller et al. 2018. https://doi.org/10.1016/j.aquaculture.2017.09.025
Panini et al. 2001. https://doi.org/10.1023/A:1014261830098



A NEW APPROACH FOR THE HORMONE APPLICATION IN MARINE FISH CULTURE: ALBUMIN AS A HORMONE CARRIER

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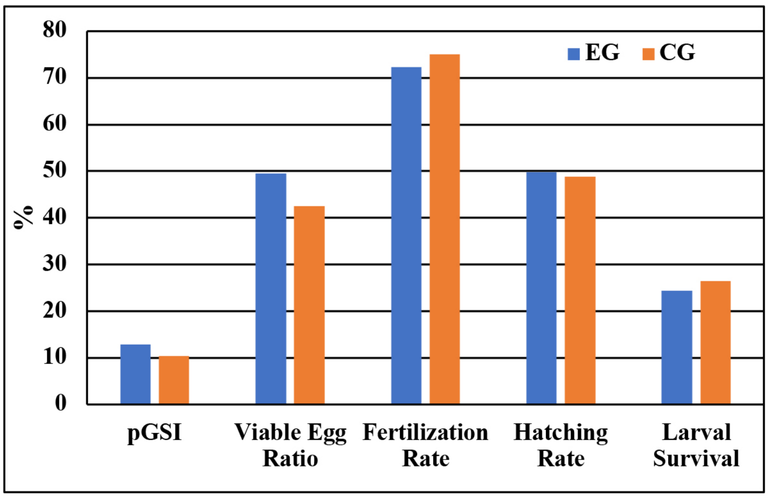
Introduction: Hormone application by intramuscular or intraperitoneal is a standard method in many fish to induce ovulation of eggs as much as equally. However, the success of the hormonal injections depends on many factors like timing, environment, handlings stress. The aims this study are to increase egg quality and received amount of egg per kg female at a synchronized time in marine fish species.

Method: European seabass (*Dicentrarchus labrax*) was used as a model fish. Females were injected intramuscularly by 30 µg/kg BW LHRHa, single shot (600-650 µ oocyte size) in Control Group (CG) and this group representing a routine culture method. Same dose of hormone application to females in examination group (EG) was carried out by ovarian lavage method using poultry egg albumin (as a hormone carrier). In the EG, the sperm collected from selected three males was inseminated (0.5 mL per ovarium per kg) to two female breeders 54 hours after hormone application (Müller et al. 2018). The experiment was tested in triplicate tanks. Eggs were collected, weighed and then placed in incubator tanks for hatching. Embryonic developments were checked separately in the cooled incubator at 14.5°C for 4 days (Panini et al 2001). Hatching rates were checked in incubation tanks and larvae were distributed for larval rearing condition. Finally, pGSI, larval survival rates were calculated after 28 days of larval weaning. This study was funded by TÜBITAK 121N529.

Results: Except the fertilization and larval survival rate all other parameters were better in EG than CG but not significantly different (P>0.05). In EG and CG no significant differences found in fertilisation (72.28% and 75.08% respectively) and larval survival rate (24.36% and 26.38 respectively) either (P>0.05). Latency time of both groups after hormone application to spawning occurrence was similar, between 38-92 hours according to oocyte size and readiness of the female.

Application of ovarian lavage hormone method gave successful results with better results of pGSI and viable egg ratio. This method can be applied to more sensitive fish under anaesthesia.

Literature:
Müller et al. 2018. https://doi.org/10.1016/j.aquaculture.2017.09.025
Panini et al. 2001. https://doi.org/10.1023/A:1014261830098



BLACK SOLDIER FLY LARVAE MEAL IN FEEDS TO ATLANTIC SALMON (*Salmo salar*) IN FRESHWATER AND SEAWATER

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Black soldier fly larvae (BSFL) is a novel raw material in aquafeeds and can be processed similarly to fish meal by fractionation in solids, stickwater, and oil. The fish meal stickwater has a high content of low-weight molecular components that are essential -in a high-quality fish meal- for feed intake, growth, and overall health in Atlantic salmon (*Salmo salar*). For optimal utilization of stickwater, the moisture content of the fraction has to be reduced before being combined with the solid phase to produce a meal that is dried and grained to specifications needed for optimal utilization in feeds and prolonged shelf life. Special equipment and knowledge are needed, but this process will improve the ingredient's sustainability, as well as technical and nutritional quality. BSFL meal produced by Innovafeed (France) was used in two trials with salmon in the Research Council of Norway-funded project Millennial Salmon (#319987). Atlantic salmon parr (26g) reared in freshwater were given five feeds across triplicate tanks. These included a control diet and 4 test diets where 10% of fish meal was substitiuted with BSFL meal, with an increasing ratio of BSFL stickwater : solids ratio. The experiment lasted for 8 weeks to study the growth, digestion, and welfare of fish fed the respective diets. BSFL meal with stickwater was further used in a 3-component mixture design with fish meal and soy protein concentrate. In total, seven feeds were formulated, varying in proportions: 75 – 275 g kg^{-1} fish meal, 23 – 223 g kg^{-1} soy protein concentrate, and 0 – 200 g kg^{-1} BSFL with stickwater. These feeds were given to Atlantic salmon post-smolts (258g) reared in seawater, across triplicate tanks for a duration of12 weeks. Both freshwater and seawater experiments showed that BSFL meal is a potential protein source in aquafeeds that can effectivelly substitute portions of fish meal and soy without affecting feed intake or growth. However, it was observed that high inclusion of BSFL meal could result in reduced growth rates for Atlantic salmon, particularly in feeds with low fish meal content. Extended results from the experiments will be presented at the conference.

MILLENNIAL SALMON – IMPLEMENTATION OF NOVEL INGREDIENTS IN FEEDS FOR A SUSTAINABLE SALMON

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In recent decades there has been a prominent shift in aquafeed research towards finding alternative ingredients to replace fishmeal and fish oil. This trend has resulted in a growing utilization of plant-based ingredients, while other novel ingredients are now mature enough to be considered suitable for widespread adoption by the industry. The balanced composition of fatty acids, amino acids, and micronutrients, makes few alternatives comparable to marine ingredients. In the Research Council of Norway-funded project Millennial Salmon (#319987), we are utilizing novel sources that can contribute to both lipids and proteins in feeds. DHA-rich microalgae biomass is a low trophic sustainable source, already available in large volumes in feed raw material markets, that has been tested by major commercial actors in Norwegian salmon farming at low dietary levels. Similarly, protein-rich insect meal from black soldier fly larvae (BSFL) has shown promising performance as fish meal replacement in Atlantic salmon (*Salmo salar*) feeds. The Millennial salmon project is a strategic partnership among world-leading European organisations spaning the value chain of the salmon farming industry. This includes R&D parteners like Nofima (Norway) and Sintef Ocean (Norway), raw material suppliers like Innovafeed (France) and Corbion (Netherlands), feed producers like EWOS Cargill (Norway), salmon producers like Mowi (Norway), product developers like Labeyrie (France) and a retailer, Auchan (France). The project aims to develope knowledge-based sustainable solutions in salmon farming and produce a responsibly sourced final product. It focuses on studying best practices in downstream processing, and the inclusion of BSFL meal and liquid microalgae biomass into salmon feeds. AlgaPrime™ DHA LS microalgae (*Schizochytrium sp.*) plant oil suspension from Corbion, was tested in feed mixes before extrusion and during the post-drying vacuum coating step. Digestibility was assessed in Atlantic salmon postsmolts. Salmon had similar digestibility regardless of the step where the microalgae suspension was added in the feed. However, the microalge quantity added during coating depended on pellet pore size distribution. Moreover, the microalgae biomass was studied as a fish oil replacement in diets (changing EPA:DHA ratio from 0.8 to <0.1) for salmon post-smolts reared in seacages (Mowi). The BSFL meal from Innovafeed, was tested as protein substitute in salmon (freshwater parr and seawater post-smolt) feeding trials at Nofima's research facilities. A summary of the trial results, coupled with product quality insights (Labyrie), consumer perspectives on Millennial salmon products (Auchan), and insights on go-to-market strategy and sustainability conciderations (Sintef) will be presented at the conference.

BINDERS AND NOVEL INGREDIENTS IN ATLANTIC SALMON (*Salmo salar*) FEEDS TO MODULATE FECAL QUALITY FOR BETTER WATER QUALITY AND FECAL REMOVAL IN AQUACULTURE SYSTEMS

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Although nutrient emissions can be reduced by the selection of highly digestible ingredients and the optimization of physical feed quality, we know surprisingly little about how these changes will affect the physicochemical characteristics of fecal waste. Changing the feed composition can have profound effects on the composition and degradability of fecal waste, which in turn affects nutrient bioavailability and the valorization potential of sludge. Physical properties will determine how much of the fecal waste can be removed from the water as sludge, and how much remains suspended/dissolved in the water. At low fecal recovery rates, the high residual organic load can heavily affect water quality, fish welfare and biofilter performance in recirculating aquaculture systems (RAS).

Binders are included in extruded fish feed to produce pellets with high durability that can be handled in transport and pneumatic feeding systems without crushing. Furthermore, residues of the binders in the feeds that are not digested by the fish can affect the disintegration rate of fecal waste in the water. In addition, the binding properties of protein ingredients can affect feed and fecal quality. We tested several binders and novel protein ingredients in feeds given to Atlantic salmon (*Salmo salar*) postsmolt in flow through tanks. In this trial, we also tested the newly developed "spillbox" for a separate collection of feed spill and feces at the tank outlet. Based on the results from the first trials, diets were selected, and tested with Atlantic salmon in replicated RAS units. The trials lasted from 2 to 6 weeks, with the objective to study digestibility of the feeds, characterize fecal properties, and subsequently study their effect on water quality in RAS. We found that binders and protein ingredients from the same origin of raw materials had similar effects on fecal properties. We observed no correlation between fecal properties and digestibility, thus the amount of certain indigestible compounds is determined for better fecal quality and collection of fecal waste. Detailed information about ingredients used in the trials will be presented at the conference.

LAB SCALE FEASIBILITY STUDY INVESTIGATING THE POTENTIAL USE OF THE SHELLFISH TOWER DESIGN FOR OPEN OCEAN CULTIVATION OF *Ulva compressa* IN DIFFERENT SALINITY ENVIRONMENTS

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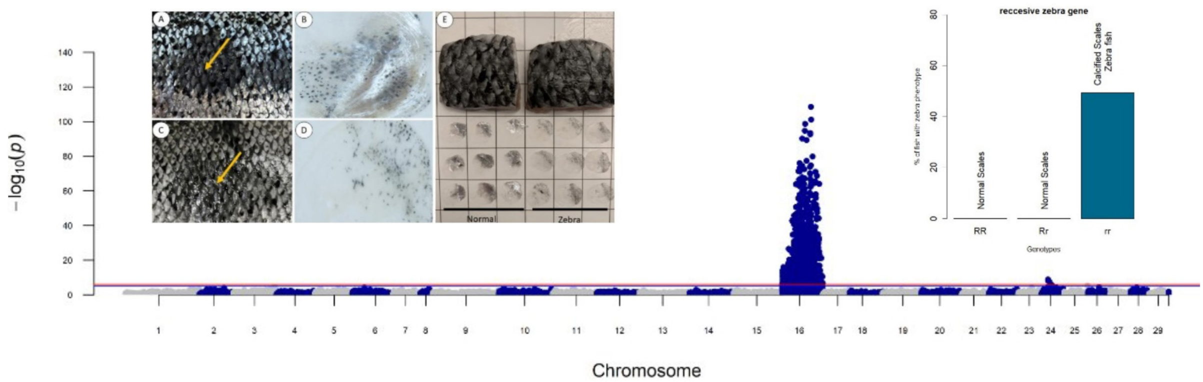
Low trophic aquatic food provides the potential to reduce food and nutrition insecurity and tackle malnutrition, while simultaneously posing little stress on the climate and environment, thereby delivering essential ecosystem services and enabling the achievement of the UN SDGs. The Shellfish Tower is an innovative system design for open ocean aquaculture of bivalves, another low trophic aquatic food. In the current study, we examined the feasibility of using the Shellfish Tower for cultivation of *Ulva compressa. Ulva compressa* is widespread in the Baltic and North Sea and can exhibit two different morphologies: a tubular form, most common in the Baltic, and a foliose form, most common in the North Sea. Therefore, we tested the hypotheses that the Shellfish Tower could be used to cultivate *U. compressa* in different salinity environments, and that the morphology, physiology and biochemical properties of *U. compressa* would differ in the Baltic and North Sea salinity scenarios. Nets were seeded with *U. compressa* gametes after fertility induction and incubated for one month at 15°C, 30 PSU and 100 µmol photons m-2 s-1 light intensity. Once the *U. compressa* reached 2-3 cm in size, the nets were wrapped around the outer frame of 20 miniature Shellfish Towers, which were constructed based on the original structure design. The frames with seeded *U. compressa* were distributed to four different salinities: 10, 15, 30 and 35 PSU. Growth rates, photosynthetic efficiency and cell size were monitored weekly for 5 weeks. Biochemical properties (protein, carbohydrate, fiber, ash and chlorophyll and carotenoid content) were measured at the end of the experiment. Significant differences in growth rates and photosynthetic efficiency were observed between the low and high salinities (Figure 1), but *U. compressa* successfully grew on the Shellfish Tower models at all salinities (Figure 2). Differences in cell size and biochemical properties will be discussed, but we did not observe the foliose morphology of *U. compressa* in any salinity treatment. This study shows that the Shellfish Tower can be used to cultivate *U. compressa* in different salinity environments, but the success in an open ocean environment must still be evaluated, as well as the impact of the vertical distribution of light and its impact on seaweed growth.



ABSENCE OF NORMAL SCALE LUSTER IN ATLANTIC SALMON: PHENOTYPIC SELECTION WILL TAKE TOO LONG TO ERADICATE

Solomon Antwi Boison, Lene Sveen, Elisabeth Ytteborg, Helene Sabjornsen, Muhammed Aslam, Gerrit Timmerhaus, Panya Sae-Lim, Ashie Norris, Werner Brand and Mathew Baranski

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The Atlantic salmon is one of many fish species that shares a characteristic silvery luster of their skin and scales. This is typically attributed to the evolution of biogenic multilayer reflectors composed of stacks of intracellular anhydrous guanine crystals separated by cytoplasm. In rare cases, individuals without this typical luster are observed, and are not favored by consumers due to their appearance deviating from the shiny silver coloration expected of a fresh and healthy product. There is no evidence to date to suggest that this rare phenotype has any detrimental effects to the fish itself or the quality of the flesh, and based on similarities to other scale mutation phenotypes, we hypothesized that it may be the result of a rare, possible recessive allele segregating in the population. A population of 6050 animals from 260 families of the 2020 yearclass from Mowi Norway were genotyped with a 57K SNP array and phenotyped for this analysis. About 2.5% of the fish were lacking the silvery luster phenotype. We estimated the heritability and performed GWAS of silvery luster (binary phenotype indicating the presence and absence of silvery). Heritabilities for the silvery luster phenotypes was estimated as 0.34 ± 0.02. GWAS revealed a highly significant peak on chromosome 16. Furthermore, animals with two copies of the recessive “T” allele lack the silvery luster coating on their scales. We are currently using sequence information to identify and fine map the causative mutation. The QTL identified could be used to eliminate fish without the luster phenotype.

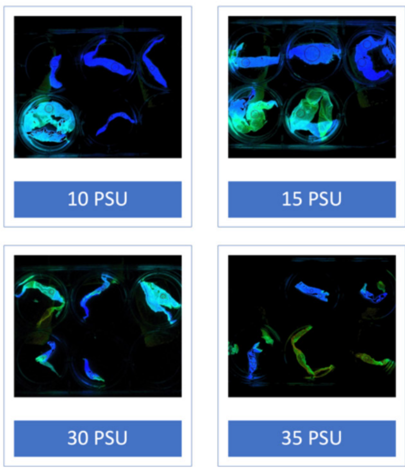

Figure 2 Visualization of maximum photosynthetic efficiency (Fv/Fm) of U. compressa *exposed to different salinities.*

Figure 1 Shellfish Tower model before seeding and directly prior to harvest

THE VISUAL DESIRABILITY OF THE PACIFIC OYSTER *Magallana gigas*: IMPACT OF METABOLOME AND 'CURING' LOCATION

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Aquaculture of oysters is expected to grow from $9 billion to $11 billion by the end of the decade. Desirability of stock oysters drives lease and grow out placement, with three sensory attributes that contribute to an oyster's desirability: visual, smell, and taste (i.e., the meroir, or 'taste of the ocean'). It may be possible to influence these factors simply by moving the 'curing' site to a location with more favourable environmental parameters (i.e., higher salinity, regulated temperature, specific food species). Its visual appeal changes drastically with location, which directly impacts oyster molecular mechanisms and desirable phenotypes.

This study looked at the effects of sites and legacy signals on the metabolome of oysters, which is important for oyster aquaculture. Through this study, metabolites contributing to specific visual characteristics will be identified, allowing industry to select optimal 'curing' sites that are conducive to these metabolic pathways and desirable oyster phenotypes.

To meet this need, oysters were translocated from two leases, Smithton (NW coast) and Boomer Bay (SE coast), to a 'curing' site, Pitt Water, chosen by Tasmania Oyster Co. This move is commonly done to improve the quality of oysters in Tasmania. Individuals (n=20) were randomly selected for analysis, two weeks and four weeks post trans-location. Water samples and environmental parameters were taken at each sampling point in parallel to oyster samples. Selected oysters were photographed and blindly assessed and graded on their mantle, body and shell fullness. Oysters were dissected targeting the mantle, gill, palp and digestive gland. Tissues were analysed for central carbon metabolism (CCM) metabolites using a liquid chromatography mass spectrometry (LC-TQ) targeted metabolomics methodology.

Results indicated the up regulation of 4-Methyl-2-oxovaleric acid in the mantle tissue between high and low-grade oysters with the down regulation of Xanthine and Glyoxylic acid. In contrast, the digestive gland showed significant up regulation of Cytidine 5-Diphosphates, Vanillic acids and Uridine 5-Monophosphate and down regulation of 2-Deoxyadenosine, 2-Deoxyuridines and L-Sorboses. More generally, there was significant up regulation in 25 metabolites between tissues with significant down regulation found in 52.

Understanding the metabolome of oysters is important when determining causes of visual desirability metrics. Changes in the metabolome and microbiome have a direct association with the visual desirability of oysters and can be manipulated by the source oyster's 'curing' location.

Ulva (SEA LETTUCE), THE SEAWEED OF CHOICE FOR LAND-BASED INTEGRATED AQUACULTURE – BUT WHICH *Ulva* SPECIES?

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Ulva (the sea lettuce) is ubiquitous in shallow seas around the world and has been grown in land-based systems for many decades. The aquaculture of *Ulva* is increasing rapidly, globally, with much potential for new products and processes (Bolton et al., 2016). Foliose *Ulva* species are generally the seaweed of choice for integrated land-based systems, grown in the effluent from marine animal aquaculture. In the past, selecting an *Ulva* has generally been done by trial and error. This contribution will discuss the choice of an *Ulva* species for cultivation.

Since 2003 *Ulva* has included both foliose (bladed) and tubular (formerly 'Enteromorpha') forms. Currently over 100 species of *Ulva* are recognised, although many of these are little-known. A major upheaval is taking place in *Ulva* diversity/taxonomy and it is currently very difficult to put an accurate name on *Ulva* without molecular barcoding studies, given their morphological similarities. Material in land-based systems is still mostly recorded as *Ulva lactuca*, usually erroneously, but commercial species recently barcoded include *Ulva lacinulata* (in South Africa and Europe) and *Ulva ohnoi* (in Australia).

There are numerous reasons for cultivating *Ulva*, and thus critical decisions are needed in choosing an *Ulva* species for land-based cultivation. Important characteristics include: high growth rate, ability to grow vegetatively for long periods, chemical composition (depending on product), and palatability, attractiveness and sensory quality if grown for feed/food.

There is currently an explosion of research on *Ulva* cultivation around the world and examples of 'reinventing the wheel', including the spreading of some unfortunate myths. It is critical that the taxonomy of this genus reaches consensus soon, and then that those working to establish *Ulva* in land-based systems try different species/strains and choose their material on carefully selected criteria. A good rule of thumb is to experiment initially with material growing unattached in sheltered bays or lagoons, similar habitats to the environment in land-based aquaculture systems. Alternatively, the more biotechnological approach (being carried out in Europe) is to take large numbers of species/strains and test them for various parameters in the laboratory. It is to be hoped that material which shows promise can be barcoded to give an accurate comparative picture of the best species/strains for specific purposes. Relevant countries/regions should set up a barcoding laboratory for aquaculture organisms and, as the industry develops, seriously consider funding a seaweed biobank.

Reference:

Bolton JJ, Cyrus MD, Brand MJ, Joubert M, Macey BM (2016). Why grow *Ulva*? Its potential in the future of seaweed aquaculture. Perspectives in Phycology 3: 113-120.

This study received funding from the EU Horizon 2020 Research & Innovation Programme ASTRAL Project under Grant Agreement No. 863034

MICROALGAE STRAIN OPTIMIZATION BY CONTINUOUS SELECTION PRESSURE: A FOCUS ON OMEGA 3 AND PIGMENT OVERPRODUCTION

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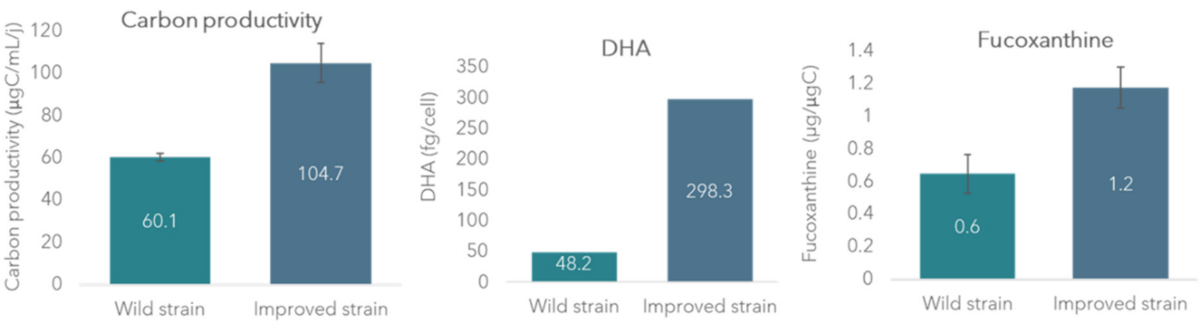
DareWin Evolution offers natural and robust selection methods as a viable and complementary alternatives to genetically modified organism (GMO) techniques for optimizing microalgae strains for the production of molecules of interest in microalgal industry.

In partnership with famous French laboratory (Inria, CNRS and Sorbonne University) and based on 10 years of dedicated works, our team developed new approaches mixing Darwinian selection theory and specific algorithms in a unique photobioreactor to refine new selection methodologies, yielding particularly promising outcomes. In details, Darwinian dynamic selection process involves applying adjustable selection pressure of increasing intensity to a population maintained in a continuous system. In this setup, individuals most able to adapt, with improved traits of interest, gradually emerge, dominate, and persist. Selection pressure is applied using numerical approaches to maximise the selection efficiency.

DareWin Evolution has successfully implemented its selection protocols across a wide range of fresh and marine microalgae species, achieving improvements in various characteristics. After 6 months of selection, our proof-of-concept results demonstrate the feasibility of enhancing temperature tolerance, doubling productivity, increasing the content of certain carotenoids by more than 2, and boosting omega-3 (DHA) content (x6) for both autotrophic and heterotrophic strains, highlighting the versatility and efficacy of our approach (see fig).



STRESS STATUS OF RAINBOW TROUT *Oncorhynchus mykiss* REARED IN AN INNOVATIVE PHOTO-ELECTROCATALYTIC RAS

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The present study is part of the PRIMA project Fish-PhotoCAT, which aims to evaluate the efficiency of a smart purification system in improving water quality, fish production, and environmental sustainability in aquaculture This system utilizes an innovative TiO_2-based photo-electrocatalytic (PEC) filter for the remediation of nitrogen compounds in water. This new-generation, low-cost filter is developed from the traditional filters used in aquaculture, combining the purifying action of the TiO_2 and the biocidal action of the UV. The efficiency of this filter will be tested in both freshwater and seawater RAS systems, where two important Mediterranean farmed fish species are reared: the rainbow trout and the gilthead seabream. Specifically, this preliminary study evaluated the effects of the PEC filter on the stress status of rainbow trout during the grow-out phase, measuring cortisol, the primary stress-related hormone in fish, in a suite of matrices to obtain a comprehensive assessment of both short- and long-term stress response.

The trial was performed at the Spallanzani Institute (Italy) where rainbow trout (IBW: 156±4.5g) were reared for four weeks at a low stocking density (15kg/m^3) in six 500-L tanks equipped with either traditional UV-filter (CTRL; three tanks) or the PEC filter (PEC; three tanks). Prior to sampling, fish were euthanized with an overdose of anesthetic (MS222 Sigma-Aldrich), followed by spinal cord transection. Cortisol levels were measured in plasma, skin mucus, muscle, fin, and scales, using a specific microtitre radioimmunoassay (RIA). Statistical analysis was carried out using the T-test for body weight data and the Mann-Whitney U test for cortisol data with a significance level set at $p < 0.05$.

Fish body weight significantly increased during the trial, with no differences observed between the two systems (Table 1). Similarly, no differences were found in cortisol levels in any of the analyzed matrices, indicating that fish reared in the two systems exhibited a similar stress status. It is noteworthy that the measured cortisol levels were very low (Table 1) and comparable to the basal levels already reported for rainbow trout.

In conclusion, this innovative system was successfully tested in rainbow trout, with effectiveness assessed in terms of fish growth and stress response. Future investigations are planned to test this purification system in on-growing trout at a higher stocking density (30 kg/m^3), during the juvenile phase, along with the selected seawater candidate, the gilthead seabream.

Table 1. All data are expressed as mean ± standard error.

	Body Weight (g)	**Plasma (ng/ml)**	**Mucus (ng/ml)**	**Muscle (ng/g)**	**Fin (ng/g)**	**Scales (ng/g)**
CTRL	218.50 ± 8.69	3.54 ± 0.77	0.24 ± 0.03	0.39 ± 0.05	0.36 ± 0.05	0.61 ± 0.08
PEC	211.83 ± 7.34	4.58 ± 1.55	0.27 ± 0.04	0.87 ± 0.22	0.59 ± 0.14	1.34 ± 0.39

IN-WATER ELECTRIC STUNNING FOR TILAPIA: FROM LABORATORY TO INDUSTRY

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Global tilapia production is estimated at 6 million tonnes (14.5 billion individuals) annually (Mood et al., 2023). The slaughter process commonly comprises live transport, chilling followed by bleeding, descaling and evisceration. Here we report on trials investigating in-water electric stunning as a humane stunning method for tilapia. Laboratory trials were followed by a commercial trial in which an in-water electric stunner was installed in parallel with an existing live-chilling line.

Laboratory trials first focused on establishing successful stunning parameters for tilapia. This was done by establishing whether insensibility was achieved immediately at the onset of a stun using clinical and EEG assessments. Analysis of the EEG power spectrum showed a significant drop in power after the stun, similar to that observed in the same fish under terminal anesthesia. A similar drop in power has been considered indicative of unconsciousness in other species (Beyssen et al., 2004). The second aim of these trials was to determine an exposure duration that ensured unconsciousness persisted long enough for death to occur through bleeding.

Commercial trials were performed using the stunning parameters established in the lab. To this end, a purpose-built stunner was installed in-line in a plant capable of processing 12 tonnes/hr. Fish were assessed for clinical signs of consciousness following the stun, and as they progressed through the processing line. There was no indication of inadequate stunning or returning consciousness. In-house quality assessments showed no negative effects of in-water electric stunning for tilapia.

Conclusions

Tilapia are a hardy species and known for being difficult to kill. Here we have demonstrated that improved welfare through in-water electric stunning is not only possible in a small scale laboratory context, but is commercially feasible as well.

References

Mood, A., Lara, E., Boyland, N. K., & Brooke, P. (2023). Estimating global numbers of farmed fishes killed for food annually from 1990 to 2019. *Animal welfare (South Mimms, England)*, *32*, e12. https://doi.org/10.1017/awf.2023.4

Beyssen, C., Babile, R., & Fernandez, X. (2004). Electrocorticogram spectral analysis and somatosensory evoked potentials as tools to assess electrical stunning efficiency in ducks. *British Poultry Science*, *45*(3), 409–415. https://doi.org/10.1080/0007166041000173092

NITROGEN, PHOSPHORUS, AND CARBON RETENTION USE EFFICIENCY IN BIOFLOC SYSTEM OF NILE TIPALIA (*Oreochromis niloticus*) FED WITH HIGH NON-STARCH POLYSACCHARIDES DIET

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Nile tilapia (*Oreochromis niloticus*) is one of highest produced species in inland aquaculture in South-East Asia with a rapid increase in the last 30 years. One factor causing the production increase is the addition of fish feed. However, feeding enriches the water with nutrients and contaminates surrounding water bodies when discharged without treatment.

Biofloc in water bodies acts as a natural water treatment agent that can decompose organic waste in ponds and transform it into protein biomass. However, commercial feed often produces organic waste with an imbalance C:N ratio to support the growth of microbiota particularly due to the lack of carbon in fish feces. This study aims to investigate the effects of substituting starch-rich ingredients (Control-diet) with non-starch polysaccharides (NSP) rich ingredients (High-NSP-diet) on nitrogen (N), phosphorus (P), and carbon (C) retention use efficiency by fish and biofloc. The NSP ingredients mostly come from palm kernel meal, rice bran, and corn gluten feed. Both diets are equal in protein but the High-NSP-diet contains triple fiber.

There is a trend that the High-NSP-diet results in a lower fish average daily gain compared to the Control-diet (around 11% lower, p-value>0.05). Both treatments result in similar biofloc characteristics (N, P, C concentration). However, nutrients were better used by biofloc under the High-NSP-diet. This was shown by a lower retention efficiency (RE) of N, P, and C in the water of almost 4%, 12%, and 7%, respectively, as compared to the Control-diet (Figure 1). RE of N and C were similar between both diets from week 0 to week 4. Then, from week 4 to week 6, biofloc in the High-NSP-diet shows a statistically higher RE of C and N than the Control-diet (33-43% higher), indicating a richer carbon in the water of the High-NSP-diet pond after four weeks.

Economic calculation shows that the High-NSP-diet can save feed production costs to 3,630 IDR/kg. Therefore, incorporating of High-NSP-diet has the potential to reduce feed costs without sacrificing much on fish production, and to improve nutrient use efficiency.

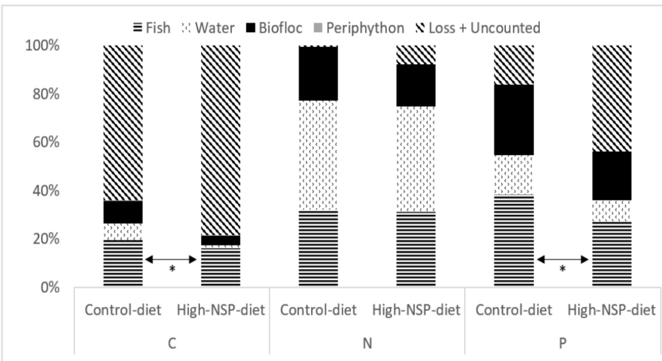

FIGURE 1. N, P, and C retention efficiency at the end of experiment (8th week) in ponds fed with Control-diet or High-NSP-diet. * indicates p-value <0.05 of Control-diet vs High-NSP-diet of the appointed compartment.

HYDROGEN PEROXIDE BATH LEADS TO STRESS AND MODULATES THE PROTEOME AND TRANSCRIPTOME OF ATLANTIC SALMON *Salmo salar* SKIN

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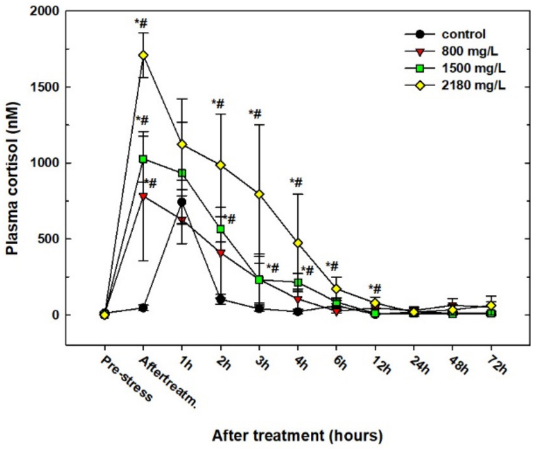
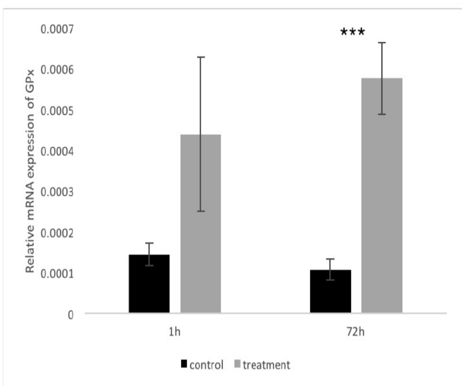
In Norwegian Atlantic salmon farming, sea lice infestation is a significant obstacle, drawing widespread attention from stakeholders, authorities, and media alike. Farmers need to manage sea lice outbreaks effectively and, at the same time, keep the welfare of the fish in focus. Consequently, understanding stress dynamics, particularly concerning common treatments like hydrogen peroxide, has become imperative. Hydrogen peroxide, primarily used to combat sea lice and Amoebic Gill Disease (AGD), is a double-edged sword. While it effectively addresses infestations, its impact on fish stress responses remains less explored. To address this issue, we examined how hydrogen peroxide treatment influences stress responses and the skin proteome and gene expression, shedding light on the underlying mechanisms in Atlantic salmon.

The experiment, spanning four weeks, involved different treatment groups exposed to varying hydrogen peroxide concentrations alongside a control group. Results unveiled a significant increase in plasma cortisol levels across all groups post-treatment, indicative of an acute stress response, particularly at higher concentrations. Notably, the stress response demonstrated a dose-dependent nature, with stronger doses eliciting more pronounced and prolonged responses. Proteomic analysis revealed alterations in protein expression related to structural integrity, wound healing, and immune defence. Concurrently, gene expression profiling highlighted significant increases in genes associated with hydrogen peroxide degradation and immune activation, indicative of an extended stress response lasting beyond 72 hours post-treatment. These findings underscore the interaction between hydrogen peroxide treatment and fish welfare in aquaculture practices. While it proves effective against sea lice, its potential to induce stress necessitates cautious application. Striking a balance between efficacy and welfare considerations remains paramount to ensure the sustainable growth of the Atlantic salmon industry while safeguarding animal well-being.





SEA URCHINS IN IMTA: TWO CASE STUDIES FROM ASTRAL IMTA LAB SOUTH AFRICA

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As part of the EU-funded ASTRAL project, the South African IMTA lab investigated two local sea urchin species (*Tripneustes gratilla* and *Parechinus angulosus*) grown in two different integrated multi-trophic aquaculture (IMTA) scenarios.

The urchin species investigated in the first case study, *T. gratilla*, has been taken from research- to pilot-commercial scale within the framework of the ASTRAL project. Hatchery-produced urchins were successfully grown in an IMTA system at Buffeljags Abalone Farm in South Africa. In this system, the dissolved nutrients released by the urchins were used as an input to grow *Ulva*, whereafter the *Ulva* was used as a feed or feed component for the urchins, thereby improving circularity in the IMTA system. An assessment of sea urchin feeding regimes and nutrient cycling in the IMTA system was conducted to identify best practices and recommendations for future commercial-scale land-based IMTA of this sea urchin species. A key outcome from this case study was that the nutrients in the particulate organic matter (POM) released by the urchins could be captured to further promote circularity in the IMTA.

The second case study was based on the Cape sea urchin *P. angulosus*, as there is a known natural relationship between the Cape urchin and juvenile abalone (*Haliotis midae*) along the South African coast. Juvenile abalone are predominantly found under the sea urchins, where they are likely seeking shelter and food, but there could also be a transfer of beneficial microorganisms from the urchins to the abalone as they have access to the urchin faecal matter and faecal matter-associated microorganisms. Therefore, Cape urchin faecal matter was assessed as a functional feed for juvenile abalone in a hatchery-scale trial. It was found that there are growth, survival and microbiome transfer benefits associated with the use of sea urchin faecal matter as a supplementary feed for juvenile abalone. To assess the Cape urchin as an additional product, a six-month growth trial was conducted using wild-collected *P. angulosus*, where results showed that gonad enhancement was possible in a short amount of time. However, further research is required to better understand the dietary requirements and growth of this species at different life stages.

This contribution will highlight species selection criteria, optimal growing conditions and key findings across the two sea urchin species investigated by the IMTA lab SA. Though *T. gratilla* and *P. angulosus* are in different phases of research and development, both case studies highlight the value of using co-products from one species as an input for another and show that these species have the potential to be cultivated using IMTA technologies.

This study received funding from the EU Horizon 2020 Research & Innovation Programme ASTRAL Project under Grant Agreement No. 863034.

THE EFFECTS OF DIETARY SUPPLEMENTATION WITH SULFATED POLYSACCHARIDES FROM *Nannochloropsis oceanica* ON THE ANTIOXIDANT AND IMMUNE STATUS OF Dicentrarchus labrax INFECTED WITH *Photobacterium damselae* SUBSP. *piscicida*

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European seabass (Dicentrarchus labrax) production suffers significant economic losses caused by Photobacterium damselae subsp. piscicida (Phdp). Using natural and sustainable ingredients to supplement fish diets has been proposed to minimize disease outbreaks in aquaculture. The microalgae Nannochloropsis oceanica (Nanno) is a promising candidate, as it is known to be natural and rich in water-soluble sulfated polysaccharides (SPS), known for their antioxidant and immunostimulatory properties. However, extracting SPS can result in large amounts of residual biomass, whose potential remains unknown. Thus, this study evaluated the potential of dietary supplementation with a SPS extract, or a biomass residue from SPS extraction, on the antioxidant and immune responses of the European seabass.

Nanno was processed using high-pressure homogenization (Nanno HPH) to improve SPS extraction. The residue from the HPH process was hydrolyzed with an alcalase enzyme (Nanno ALC) to increase protein extraction. Fractions obtained from both methods were used to formulate five experimental diets: i) a commercial diet with no supplementation (CTRL); ii) CTRL with a low dose of Nanno HPH; iii) CTRL with a high dose of Nanno HPH; iv) CTRL with a low dose of Nanno ALC; and v) CTRL with a high dose of Nanno ALC. Each diet was tested in triplicates and seabass were fed for 16 days, after which the fish were infected with Phdp (5×10^{5} CFU ml^{-1}). Three fish per tank were sampled before (T0) and after infection (at 24h and 48h) to evaluate dietary effects on antioxidant (reduced/oxidized glutathione (GSH/GSSG) ratio; glutathione peroxidase, GPx; and glutathione reductase, Gr, in liver) and immune parameters (lysozyme, peroxidase, and antiprotease in plasma). Mortality was recorded over the following 15 days.

The results revealed that the low dose of Nanno HPH was able to minimize the negative impact of the infection on the redox status of fish by keeping higher hepatic GSH/GSSG ratio levels compared to the other experimental groups. The redox balance was ensured by the activation of GPx and GR enzymes, whose activity increased in the group fed the low dose of Nanno HPH than in other experimental groups. As expected, the activity of humoral immune parameters in the plasma increased at 24h after infection in all experimental diets. Still, a more pronounced increase in the antiprotease activity of fish fed the low dose of Nanno HPH was noticeable compared to the CTR group. This result was in line with the mortality rate, which tended to be lower, though not statistically significant, in the group fed the low dose of Nanno HPH compared to other experimental diets. This study provided evidence of the potential of a SPS extract supplementation to enhance the antioxidant system of European seabass infected with Phdp. Further studies using molecular techniques are needed to confirm its potential to boost the immune response of European seabass.

IMPROVING THE DELOUSING EFFICACY OF FARMED BALLAN WRASSE *Labrus bergylta* AND LUMPFISH *Cyclopterus lumpus* IN ATLANTIC SALMON *Salmo salar* AQUACULTURE

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Considerable gains in reliable delousing efficacy of farmed cleaner fish (ballan wrasse *Labrus bergylta* and lumpfish *Cyclopterus lumpus*) can be achieved by improving the behavioural quality of hatchery reared cleaner fish. Delousing behaviour is known to be highly variable between individual ballan wrasse and lumpfish within a population. Sea lice foraging is a difficult trait to phenotype, and here fish personality and coping style are used as a proxy for delousing ability that can be used to select good delousers for ongoing domestication programmes.

A tank bioassay was used to predict the behaviour components of ballan wrasse and lumpfish. Individual fish (n = 60 per species) were presented with consecutive stimuli (shelter/open water, novel object, mirror). Fish were video recorded and videos were analysed to predict levels of activity, anxiety, sociality, boldness and aggression for each fish. Then, each fish was moved to a tank containing 3D salmon models with 0, 1 or 6 model sea lice. Manual observations were recorded to test the interactions of cleaner fish with salmon models as a proxy for real salmon and lice. Results show that overall lumpfish are bolder than ballan wrasse as they are quicker to leave the shelter ($P < 0.05$), approach the novel object more often ($P < 0.05$) and engage with the salmon models more than the wrasse ($P < 0.001$). Both species prefer model salmon with more sea lice ($P < 0.001$).

In a similar experiment, individual ballan wrasse and lumpfish (n = 24 per species) were tested using the same behaviour bioassay as previously except videos were analysed using the R package "EBimage". The fish were then individually cohabited in a tank with 10 Atlantic salmon (*Salmo salar*) infected with adult sea lice (*Lepeophtheirus salmonis*, mean n per fish = 10). Sea lice on the salmon were counted at 0h, 48h, 96h and 144h to calculate delousing rates per cleaner fish and allow delousing rates to be correlated with behaviour components. Delousing in both species was highly variable but only 27% of the wrasse and 17% of the lumpfish deloused more than 20% of lice after 144h. Wrasse delousing was positively correlated with approaches to the novel object and negatively correlated with mean velocity, whereas lumpfish delousing was not strongly correlated with behaviour.

Delousing ability between individual ballan wrasse and lumpfish is highly variable, and these results show that a limited number of fish within farmed populations are good delousers. Whether personality and delousing ability are heritable traits is yet to be determined, but simple behaviour tests could be used to predict delousing ability in cleaner fish for selecting potential broodstock for breeding future generations of farmed cleaner fish.

THE EFFECT OF OREGANO ESSENTIAL OIL ON GROWTH PERFORMANCE AND MORTALITY OF SHRIMP (*Liptopenaeus vannamei*) UNDER *Vibrio parahaemolyticus* CHALLENGE

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The increasing need for healthy protein to feed the global population has led to rapid expansion in shrimp production (*Litopenaeus vannamei)*. However, intensification of production has led to increased disease challenges. Oregano essential oil (OEO) has been widely demonstrated to support the health and performance of monogastric livestock, however, to date little research has been performed in aquatic species. To investigate the effect of OEO in shrimp a trial was performed at Nong Lam University in Vietnam under *vibrio parahaemolyticus* challenge.

Pathogen-free shrimp from post-larval stage were raised for 8 weeks in a recirculation system using two treatments (Control and OEO). Shrimp were fed 4 times daily at 5% of mean body weight and uneaten food was removed after 2 hours and dried then deducted from the total feed. Water temperature was 27± 1^{o} C and salinity was 10 ± 5 g l^{-1}. Water changes and bio-filtration kept ammonia below 0.5mg l^{-1} and nitrite-N below 0.15 mg l^{-1}. Shrimp with initial weights of 1.5±0.2g were randomly assigned to 0.5m3 tanks with four tanks per treatment. Basal diets are shown in (Table 1). Orego-Stim Forte (Anpario plc.) was added to one treatment at 3.5kg/t. At 8 weeks, 20 **s**hrimp per treatment were challenged with *vibrio parahaemolyticus at* 10^5 CFU/ml (confirmed via spectrophotometry and serial plate counts) and mortality was assessed over 14 days. Data was statistically analysed in IBM SPSS 2.0 using one-way ANOVA with a Duncan posthoc.

There was no significant difference in growth performance between treatments (Table 2). However, following the bacterial challenge, there was a significant (p=0.05) reduction equal to 17.5% in mortality at the end of study (Figure 1.)

Conclusion: The dietary supplementation of OEO resulted in a substantial reduction in total mortality under *vibrio parahaemolyticus* challenge. This indicates that OEO could be a useful tool for supressing this challenge and reducing mortality in shrimp.

Table 1: Diet formulation

Ingredient	Inclusion (%)
Fish meal 67%	11.195
Shrimp meal 33%	3.0
Poultry meal	10.0
Soybean meal 47%	30.0
Fermented SBM	8.0
Wheat Gluten	7.0
Wheat flour	27.0
Lecithin	2.255
Fish oil	1.0
Stay C	0.05
Premix	0.5

Table 2: Growth performance

	Control	OEO
Initial BW(g/shrimp)	1.57 ± 0.02^a	1.54 ± 0.03^a
Final BW (g/shrimp)	15.1 ± 0.5^a	15.7 ± 1.2^a
Weight gain (g/shrimp)	13.5 ± 0.5^a	14.1 ± 1.2^a
SGR (%/d)	4.0 ± 0.1^a	4.2 ± 0.1^a
FCR	1.22 ± 0.13^a	1.21 ± 0.07^a
Feed Intake (g/shrimp/d)	0.28 ± 0.01^a	0.27 ± 0.00^a

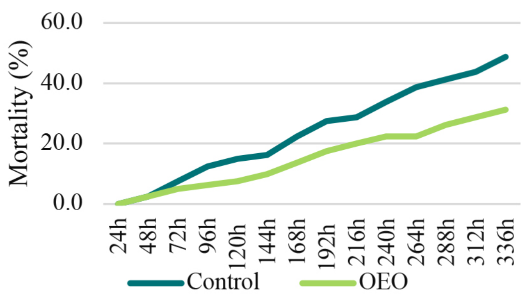

Figure 1: Mortality results under *vibrio parahaemolyticus* challenge

Flavobacterium covae AND CHANNEL CATFISH VIRUS COINFECTION IN CHANNEL CATFISH *Ictalurus punctatus*

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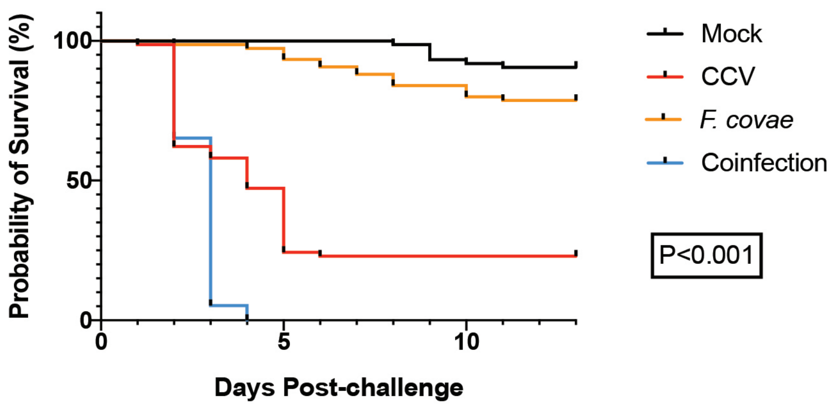
In U.S. channel catfish (*Ictalurus punctatus*) production, bacterial pathogens are primarily responsible for disease within production ponds. Channel catfish virus (CCV) has also played a role in production mortality and mainly impacts fry and fingerling production. In a pond-rearing environment, pathogen coinfections may increase the severity of the constituent pathogens and elevate mortality, thus potentiating economic losses for U.S. producers. A recent study assessed and characterized the effects of bacterial and viral coinfection in juvenile channel catfish. Single immersion infections of *F. covae* (ALG-00-530) and CCV (2013-CCV-DRB), alongside a mixed coinfected treatment group, were incorporated into the experimental design.

The single virulent *F. covae* infection resulted in a total cumulative percent mortality (CPM) of 21.3 ± 6.7 %, while the CCV-only group was 77.0 ± 9.2 %. A coinfection (half-dose combination) of each pathogen demonstrated pronounced mortality (100.0 ± 0.0 %) over 13 days following the initial challenge.

Trial results indicate changes in catfish mortality levels and trends from simultaneous exposure to multiple pathogens (Figure 1). Reducing disease outbreaks in catfish farming is critical to enhancing production yields and quality products, and comprehending infection dynamics of pathogens coinfections will provide more insight into targeted control measures for catfish health. A further understanding of the channel catfish immune response via transcriptomic analyses may also elucidate host factors involved in mixed infection exposures. Reducing disease outbreaks in catfish farming is critical to enhancing production yields and quality products, and comprehending infection dynamics of pathogens coinfections will provide more insight into targeted control measures for catfish health.

Figure 1. Kaplan-Meier survival curve of mortality from the coinfection trial.



EFFECTS OF BACTERIAL SINGLE CELL PROTEINS AND NUCLEOTIDES ON DISEASE TOLERANCE AGAINST EHP *Enterocytozoon hepatopenaei* INFECTION IN WHITE LEG SHRIMP JUVENILES *Penaeus vannamei*

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Enterocytozoon hepatopenaei (EHP) is one of the biggest challenges in shrimp aquaculture. EHP is an intracellular parasitic disease that infects the hepatopancreas and intestine in shrimp and can cause retarded growth, size variation and major economic losses. In a challenge laboratory study at Shrimpvet (Vietnam), the effects of the inclusion of single cell proteins (SCP) and bacterial nucleotides in shrimp feed was investigated on the tolerance against EHP infection.

The trial was carried out for 51 days including 2 days of acclimation, 14 days pre-challenge, 7 days of EHP *per os* challenge, and 28 days of post-challenge. The trial consisted of five groups with five replicates including: group 1 (positive control), group 2 (nucleotides at 400 g/MT inclusion), group 3 (nucleotides at 800 g/MT inclusion), group 4 (SCP strain_1 at 5% inclusion), group 5 (SCP strain_2 at 5% inclusion). Fifty shrimp were stocked into each 350 Liter experimental tank.

Survival rates and growth performance parameters (at day 51) are shown in TABLE 1.

Survival rates and growth performance parameters (at day 51) are shown in TABLE 1.

	survival (%)	initial mean weight (g)	final mean weight (g)	ADG (g/day)	SGR (%/day)	Total feed consumption (g)	FCR	Size CV (%)
control	60.40 ± 8.05^{b}	1.36 ± 0.00^{a}	9.27 ± 0.16^{d}	0.15 ± 0.00^{d}	3.77 ± 0.03^{d}	404.25 ± 3.96^{c}	1.25 ± 0.09^{c}	20.98 ± 1.35^{c}
nucleotides 400 g/MT	66.40 ± 11.26^{ab}	1.36 ± 0.00^{a}	10.06 ± 0.31^{c}	0.17 ± 0.01^{c}	3.93 ± 0.06^{c}	410.30 ± 16.50^{c}	1.13 ± 0.10^{b}	18.80 ± 3.29^{bc}
nucleotides 800 g/MT	70.80 ± 3.03^{a}	1.36 ± 0.00^{a}	10.68 ± 0.30^{b}	0.18 ± 0.01^{b}	4.04 ± 0.06^{b}	417.15 ± 1.05^{ab}	1.02 ± 0.01^{a}	14.16 ± 0.52^{a}
SCP_1 (5%)	74.00 ± 3.74^{a}	1.36 ± 0.00^{a}	11.09 ± 0.22^{a}	0.19 ± 0.00^{a}	4.12 ± 0.04^{a}	437.36 ± 6.74^{a}	1.00 ± 0.04^{a}	14.24 ± 0.27^{a}
SCP_2 (5%)	70.80 ± 5.22^{a}	1.36 ± 0.00^{a}	10.96 ± 0.24^{ab}	0.19 ± 0.00^{ab}	4.09 ± 0.04^{ab}	426.62 ± 6.61^{ab}	1.01 ± 0.04^{a}	16.61 ± 1.60^{ab}

Values are presented as mean ± standard deviation. Different letters on the same column indicate significant differences (P<0.05).

HARNESSING EFFICIENT GENETIC IMPROVEMENT IN SHRIMP BREEDING

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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. In shrimp, breeding programs are currently in use ranging from relatively simple mass selection plans to sophisticated application of genomic selection.

Phenotypes that can be selected directly such as growth are relatively easy to measure and select for in most breeding programs. Indirect or complex traits such as disease resistance, robustness, or selecting for a combination of important traits is more complicated both to measure and to fit into a statistical broodstock selection model. We will briefly discuss the general concepts and common strategies for breeding program management, from the simplest requiring the least amount of investment to the more complex, requiring greater investment but delivering greater genetic gains across more traits. We will present information on mass selection, family selection, and genomic selection as applied to shrimp breeding. More importantly, we will illustrate the use of the phenotypes and genotypes for a breeding program and how the breeding strategy should be designed to maximize economic returns by balancing input costs with the expected genetic and economic gains for a commercial aquaculture producer. We use internal rate of return (IRR) as an economic metric to quantify choices in breeding strategy.

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 design of a genetic improvement program is the consideration of each program's breeding goals, size, and available budget along with selection and availability of appropriate tools to support such a design.

GENOME EDITING IN PACIFIC WHITE SHRIMP *L. vannamei*

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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 shrimp 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.

With Genome Editing, genomic improvements can be accelerated to meet the growing needs of the aquaculture industry. While the agricultural and livestock industries have embraced Genome Editing, the aquaculture industry has fallen behind considerably in the application of these technologies. The complexity of farmed aquaculture species and the necessary support systems present a significant barrier to the application of genome editing. Genome Editing can offer improvements in several areas, such as disease resistance, high growth, high yield, monosex culture, and environmental sustainability.

The implementation of Genome Editing in Pacific White Shrimp (*L. vannamei*), a major seafood product, will be important for the aquaculture industry. Several methods of gene editing have been reported in shrimp, with varying degrees of success. These methods are limited by the issues of the delivery of the gene editing enzymes and the survival of the larvae to adulthood on a commercially relevant scale. The progress in developing genome editing tools for *L. vannamei* remains limited.

Here, we will present our success in creating genome edits in *L. vannamei*, discuss the opportunities for genome editing to improve shrimp aquaculture, and highlight the challenges in the implementation of genome editing technologies in *L. vannamei*.

MULTI-USE OF OFFSHORE WIND FARMS WITH LOW-TROPHIC AQUACULTURE CAN HELP ACHIEVE GLOBAL SUSTAINABILITY GOALS

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The coastal zone is heavily impacted by human activities and the demand for space is continuously increasing. European coastal seas host a wide range of different competitive uses, such as shipping, oil and gas exploitation, wind farms, mining, recreational activities, aquaculture, fishing, and conservation. This exploitation of the coastal zone substantially increases the stress on marine ecosystems, leads to spatial conflicts, and hinders the expansion of many of these uses. Sustainable development of the blue economy requires area-efficient use of marine space and innovative solutions demonstrating net emission capture thereby contributing to mitigating eutrophication and climate change.

Multi-use of offshore wind farms (OWF) with low-trophic aquaculture (LTA) could provide sustainable energy, nutritious seafood, and restorative ecosystem services through nutrients and carbon capture and utilization. This potential was investigated in the projects OLAMUR and Wind@sea. In a transition zone between marine and brackish seas, our models predicted that allocating 10% of projected wind farm areas to blue mussel and sugar kelp aquaculture in the North Sea - Baltic Sea transition zone could yield 18 t-fresh weight ha^{1} yr^{1}. Total carbon captured and harvested from seaweed biomass and mussel shells would equal 40% of the carbon dioxide emissions from the Danish agricultural sector.

Global LTA production is projected to increase by 132% compared to current production in a realistic scenario of OWF development (Table 1). Pessimistic and optimistic projections predicted a 22% and 400% increase, respectively. With technological and regulatory challenges still to be addressed, these findings demonstrate a vast potential of multi-use in offshore areas, which can generate blue biomass with fewer user conflicts, while mitigating eutrophication and climate change, thereby supporting multiple global sustainable development goals.

Region	Current LTA harvest (Kt-FW)	2030 Realistic projection OWF-LTA harvest (Kt-FW)	2030 Pessimistic projection OWF-LTA harvest (Kt-FW)	2030 Optimistic projection OWF-LTA harvest (Kt-FW)
Africa	2.9	22	0	84
Asia	17,732	5,281	1661	15,680
Australia-NZ	2.5	2,594	0	9,148
North America	24.2	2,644	52	18,054
South America	404	3,868	0	8,183
Northern Europe	91.6	8,717	2,135	37,456
Southern Europe	361	1,361	158	4,583
Total	18,618	24,487 (132%)	4,007 (22%)	93,146 (400%)

Table 1. Low trophic aquaculture (LTA) harvest in the different regions currently and in the three future projections of offshore wind farm (OWF) development. Table modified from Maar et al. 2023. Communications Earth & Environment. 4:447. https://www.nature.com/articles/s43247-023-01116-6.

UNPACKING "OFFSHORE AQUACULTURE": THE IMPORTANCE OF DECOUPLING IT FROM "EXPOSED" AND "DISTANCE FROM THE COAST"

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The terms "offshore", "open ocean", and "deep blue" have been used to describe aquacultures development 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 an environment where aquaculture is conducted, usually the geographic distance from shore or infrastructure, or the level of exposure to an extended fetch associated with high waves and strong currents. Other terms are used to describe aquaculture operations either closer to shore, such as "nearshore", "inshore", "onshore", "coastal aquaculture", and "sheltered aquaculture", as well as very far from shore, such as "EEZ aquaculture". The multiplicity of terminologies creates confusion among the many stakeholders involved and risks miscommunication of important properties of the aquaculture activity.

To reduce ambiguity for characterising these types of aquaculture environments the ICES Working Group for Open Ocean Aquaculture (WGOOA) established a clearer definition to: 1) promote a common understanding and avoid misuse for different classifications; 2) enable regulators to identify 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/vessels will operate; 5) provide insurers and investors with better means to assess risk and premiums; and 6) to circumvent the emergence of narratives that root in different cognitive interpretations of the terminology in public discourse arenas. In this presentation, we also discuss the evolution of the use of the term "offshore aquaculture" and the pressing need for a change to a more precise and robust term such as "exposed aquaculture", which is able to convey clearer information. Adopting this clearer definition of "exposed" will allow the user to define a site with more than just distance to shore.

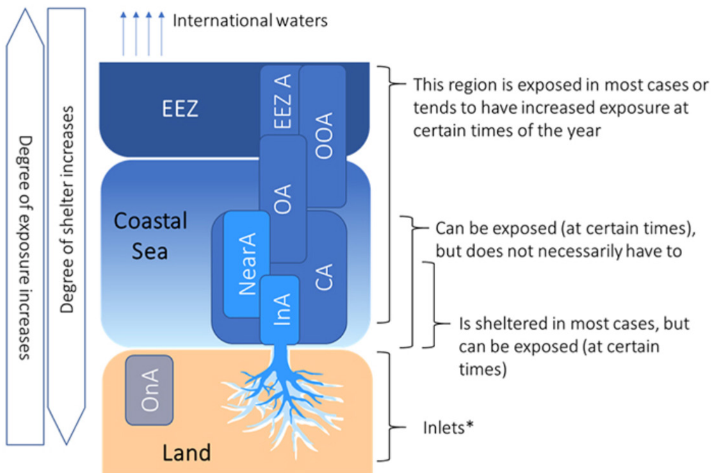

Fig. 1: Illustration of the different aquaculture terms that have to do with a location description. Modified after Buck et al. 2024, Frontiers in Aquaculture.

CONSUMERS' PREFERENCES TOWARDS FARMED SALMON IN CHINA: INTEGRATING SENSORY AND CHOICE EXPERIMENTS

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There is a growing demand for salmon in China and in 2022 the country imported about 199 thousand tonnes of salmon valued at $1.4 billion. In 2018, the Chinese government decided to allow farmed rainbow trout to be labelled and sold domestically as salmon, providing consumers with more options. This controversial decision aimed to offer an alternative that bridged the gap between domestically produced rainbow trout and imported farmed Atlantic salmon, known for its luxury and premium quality. Thus, this study aimed to evaluate whether country-of-origin information affected consumers' sensory perceptions and willingness to pay (WTP) for a salmon product. Using a within-subject design with two evaluation rounds (blind and informed) we combined hedonic liking where 110 consumers rated three salmon samples (one from each country: Norway, Chile, and China) with a discrete choice experiment.

A linear mixed-effects model analysis indicated that the consumer overall liking of Norwegian and Chilean salmon significantly increased after the country-of-origin information was provided, while regarding Chinese salmon no significant difference was noted. Under blind conditions, no significant difference was noted between Chinese, Chilean and Norwegian salmon (China vs Norway: -0.01 ± 0.16, $p = 0.198$) and (China vs Chile: 0.33 ± 0.16, $p = 0.116$). Once informed of provenance, Norwegian salmon was significantly preferred over both Chilean (0.63 ± 0.16, $p = 0.000$), and Chinese salmon (0.59 ± 0.16, $p = 0.001$), between which there was no preference (-0.045 ± 0.16, $p = 0.961$).

A multinomial logit model analysis indicated that willingness to pay for salmon was unaffected by country of production unless provenance was informed when consumer salmon choice became significantly influenced. Ecolabel, price, and overall liking were also found to impact consumer choice.

In summary, these results offer valuable insights for salmon producers/importers/marketers to customize their approaches in response to consumer preferences, refine product positioning, and seize upon opportunities within the competitive salmon market in China.

NON-INVASIVE BIOMARKER DISCOVERY IN FARMED FISH SPECIES: A MULTIOMICS APPROACH

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New knowledge on how farmed animals cope with current aquaculture practices is of utmost importance to improve management and sustainability of the aquaculture sector. Available biomarkers to profile immune and welfare status of farmed animals are still limited and generally require invasive procedures (e.g., blood and tissue collection, biosensor surgical implantation). Therefore, this work aims to discover new non-invasive biomarkers of health and welfare, which will in turn allow the development of a disease prediction tool through machine learning (Figure 1).

Data is continuously gathered from different trials done within the IGNITION project. Focusing on three different species: Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and European seabass (*Dicentrarchus labrax*), water, skin, and epidermic mucus samples are being collected to generate transcriptomic, proteomic, and microbiome data. The data will be analysed and gathered into a centralized database, which, besides the data generated by the IGNITION project, also contains open-source data.

This database will later be integrated using machine learning (ML) methods with the main goal of developing high-performance tools to predict disease resistance and animal robustness. ML will be applied to confirm the strength of *a priori* established markers, but also to identify novel ones with high predictive power.

Current focus is on transcriptomic data analysis, mainly miRNA data, and database updates. Quality control will be done using FASTQC (0.12.1) and Multiqc (1.19) and sequences trimmed using Cutadapt (4.8). Since the focus is on novel biomarker discovery, the pipeline will be based on the miRDeep2 (0.1.3) software package for identification of novel and known miRNAs in deep sequencing data.

Work Co-funded by UKRI and by the European Union's Horizon Europe research and innovation programme (GA No. 101084651 - project IGNITION). TB received funds from FCT through grant 2023.04651.BDANA.

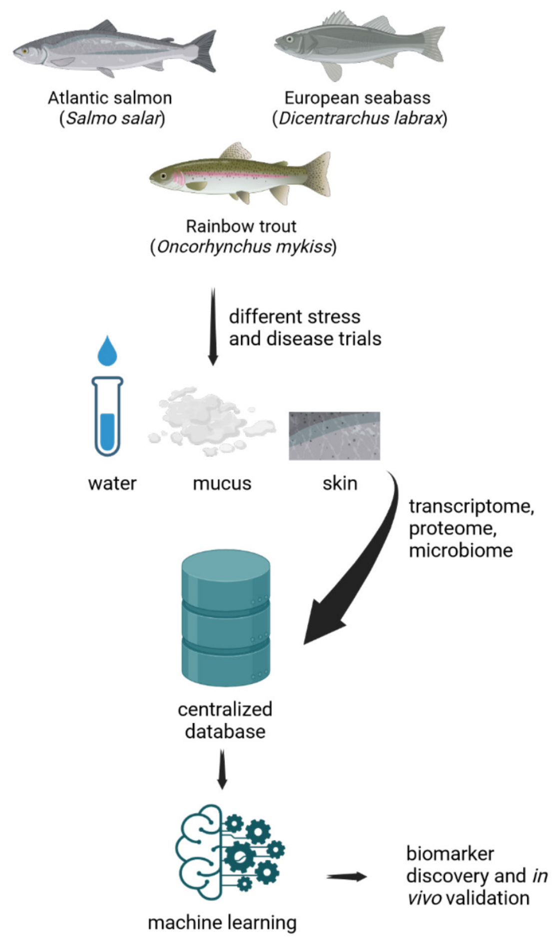

Figure 1. Novel biomarker discovery workflow scheme. Created with BioRender.com.

INVESTIGATING ATLANTIC SALMON BEHAVIOURAL RESPONSE TO GILL HEALTH FOR IMPROVED WELFARE IN COMMERCIAL FARMS

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As the aquaculture industry is growing, more sophisticated technology is required to monitor farms and ensure sustainability and good fish welfare. 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. The goal of this study was to identify changes to farmed Atlantic salmon (*Salmo salar*) behaviour related to fish health status. For this, cameras were deployed within all cages in each of 2 Atlantic salmon marine farms located in the Outer Hebrides, Scotland. One 'study' cage in each farm was equipped with 5 and 4 cameras, for sites A and B, respectively, for higher spatial coverage of fish behaviour throughout the cage. An algorithm was created by Observe Technologies to process video footage from these cameras and transform it into behavioural data, termed 'activity', which encompasses fish abundance, speed, and shoal cohesion. Daily validation occurred for the duration of the study, whereby experts compare the videos to output from the algorithm. Additionally, gill health scores were evaluated weekly at both sites through the visual sampling of 10 fish in each cage (0 = healthy gills, 5 = necrotic tissue). During summer 2023, gill health issues arose at both farms, leading to fish stress which was evident in the behavioural data. Prior to the rise in gill health scores, the average (± standard deviation) fish activity levels were 36.8 ± 11.3 % and 32.5 ± 10.5 % for each study cage in sites A and B, respectively. Following the increase in gill health scores, the fish activity rose significantly with a mean of 57.5 ± 14.6 % and 51.1 ± 19.0 %, respectively ($U = 2\ 10^6$, $p < 0.001$ and $U = 5.9\ 10^5$, $p < 0.001$). Consequently, there was a significant correlation between PGD scores and activity at both farms ($p < 0.001$; $R^2 = 0.42$ and 0.62, respectively; Fig. 1). The observed increase in fish activity was observed in all cages at each farm and corresponded with a shift towards the centre of the cage, indicating shoaling behaviour commonly associated with a stress response. Subsequent investigations will explore the feasibility of an early-warning indicator for compromised fish health, potentially preceding visual detection by farmers.

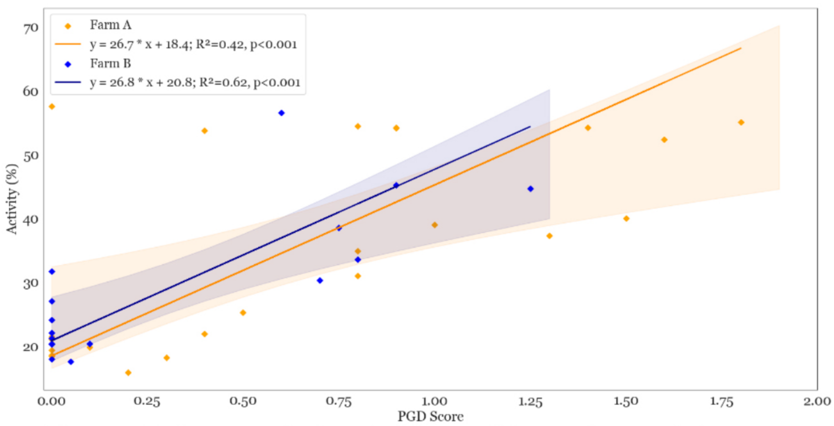

Figure 1 The correlation (with confidence intervals) between fish activity (%) and Proliferative Gill Disease (PGD) scores for two farms in Scotland during the summer and early autumn of 2023.

AQUACULTURE CO-MANAGEMENT FOR SUSTAINABLE AQUATIC FOOD

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Aquaculture is not commonly considered a collective form of aquatic food production in need of collaborative management. Nevertheless, aquaculture in its many forms is dependent on a range of shared public resources derived from inland, coastal and marine ecosystems. Aquaculture producers also face a range of production risks - such as disease and water quality - that also extend beyond the boundaries of their farms and interact with these wider ecosystems. This presentation introduces the concept of 'aquaculture co-management' or collaborative aquaculture management as a means of ensuring socially and environmentally responsible aquatic food production. Defined as a set of strategic and operational collaborative arrangements that enable shared but differentiated responsibility, rights and benefits, aquaculture co-management can assist members states to implement ecosystem approaches to aquaculture, and also achieve many of the wider social and environmental ambitions set out in global aquatic food policy - including the FAO Blue Transformations Roadmap to 2030. The presentation will elaborate four types of co-management – (1) communal, (2) collective, (3) intersectoral and (4) zonal (see Figure 1) - and their potential for implementation in inland, coastal, and marine areas around the world.

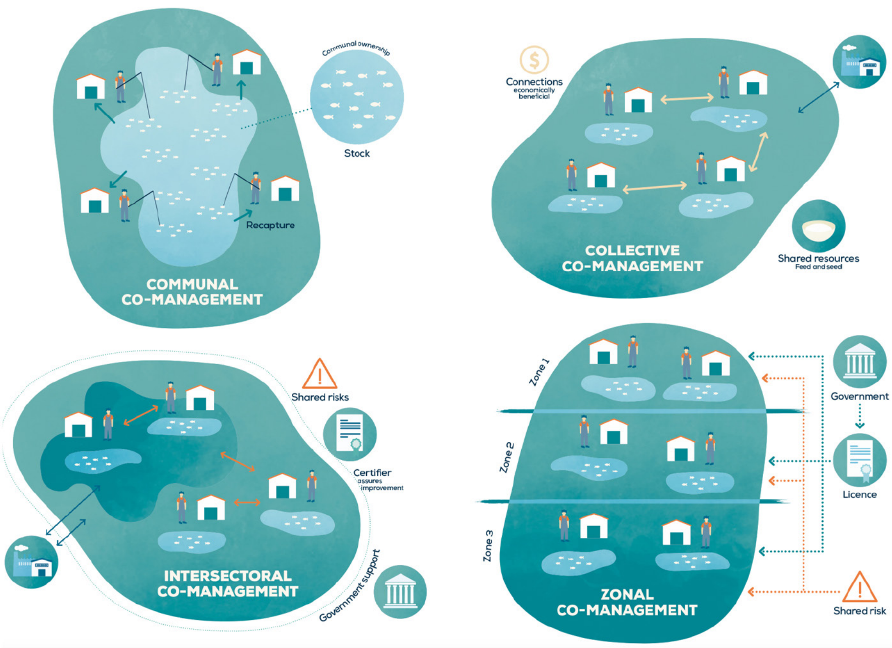

Figure 1. Typology of aquaculture co-management

SUSTAINABLE AQUACULTURE IN AFRICA

Peter Teye Busumprah

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This poster is to address UN Ocean Decade Challenges 2&3 which focuses on restore and protect the biodiversity and ecosystem, and sustainably feed the global population.

It focuses on SDG 1,2 , 12 & 14 which is centred on , No poverty, zero hunger, responsible consumption and production , and life below water .

Africa has achieved impressive double-digit growth in aquaculture, despite recent challenges from COVID-19 and diseases. However, the continent's high fish demand and declining capture fisheries highlight the urgent need for substantial growth in aquaculture production. Currently, most countries heavily rely on fish imports, impacting their national income. Aquaculture in Africa primarily utilizes semi-intensive systems like ponds, with limited intensive cage farms. The expansion of cage culture and availability of commercial feed have contributed to increased aquaculture production. Mariculture, though on a smaller scale, is practiced in countries like Egypt. Except for Egypt, aquaculture's contribution to fish production in Africa remains relatively low.

Aquaculture in Africa continues to experience double-digit growth despite reduced growth rates globally. Despite this, aquaculture production in Africa is still low: contributing less than 3% to the global basket. Apart from Egypt, most African countries rely on capture fisheries and export to meet their fish demand with little contribution from aquaculture.

Since 2000, most African governments have had aquaculture as a national priority enacting various policies to augment the increasing private sector investment to enhance growth. Yet aquaculture on the continent remains challenged in several aspects. Early Career Ocean Professionals Aquaculture team in Africa aims to identify some of these obstacles, propose mitigations and commit to their successful rollout to promote aquaculture development.

QUANTIFYING MALE REPRODUCTIVE PERFORMANCE TO IMPROVE HATCHERY PRODUCTION OF HYBRID CATFISH

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Catfish farming accounts for nearly 70% of total U.S. freshwater aquaculture production, where the channel catfish, *Ictalurus punctatus* □ × blue catfish, *I. furcatus* □ hybrid constitutes >50% of the harvest. Current technology to produce hybrid embryos is labor intensive and requires the sacrifice of males for *in vitro* fertilization. This is time-consuming and costly, as blue catfish do not reach maturity for 4-7 years. Catfish sperm are often of inadequate quality/quantity and do not necessarily give high fertility and offspring viability. As a result, we tested the "Testicular-Mediated Paternal Effects Hypothesis for Aquaculture". In this hypothesis, male reproductive performance indices (i.e., body metrics, testes morphology, sperm quality, sex steroid hormone profiles, and sperm/testes gene expression) are expected to predict crucial industry-relevant performance traits, such as fry growth and survival. These traits are essential during early life stages (i.e., egg to early juveniles) when catfish are prone to high mortality and developmental abnormalities. Throughout this project, we employed a multi-dimensional approach where physiological, cellular, and molecular indices were quantified to comprehensively assess male reproductive performance to improve hybrid catfish production (Fig. 1).

In this presentation, results from the following objectives will be reviewed: (i) investigate how paternal age impacts male reproductive performance and ensuing offspring performance, (ii) measure a series of reproductive performance indices, and then use these indices to forecast the storage potential of sperm for cryo-banking and future production, (iii) evaluate impact of cryopreservation and refrigerated storage on proxies of sperm quality and offspring performance, (iv) determine whether sperm storage/environmental conditions lead to molecular changes affecting breeding success.

Together, our results will lead to a better understanding of the mechanisms underlying reproduction, gonadal function/production, and gamete preservation at cellular, molecular, genetic, organ system, and whole-animal levels. Knowledge gained will reduce production costs and improve reproductive efficiency for a farm animal (catfish).

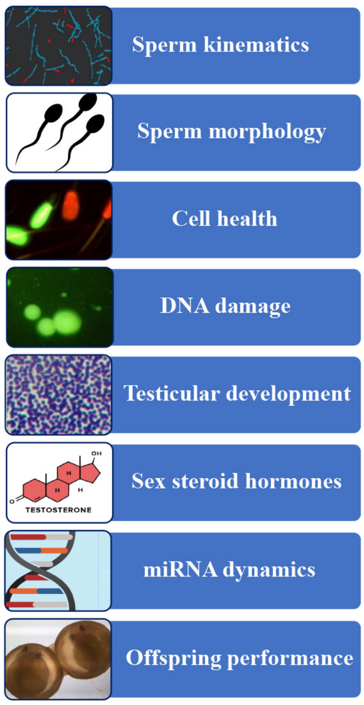

Figure 1. Comprehensive analyses used to test the *Testicular-Mediated Paternal Effects Hypothesis for Aquaculture*

EXERCISE MAY IMPROVE SEAWATER ACCLIMATION IN SALMONIDS

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Many populations of salmonid fishes migrate long distances often against forceful currents. Their seaward and spawning migrations may require days to weeks of sustained swimming interspersed with bouts of burst swimming to move through rapids and over waterfalls. They are truly some of nature's great athletes. The energetic stress of migration and the resulting allocation of energy to swimming should mean that other physiological processes may be compromised. During exercise, trout are known to modify their gills to improve oxygen uptake, but this change has been shown to lead to ion loss from the body. We examined if ionoregulatory mechanisms in the gill are up-regulated to deal with this exercise induced ion disturbance. Rainbow trout were challenged to either a sustained (3 body length (BL)/second) or burst (8 BL/second) swimming exercise regimen. Plasma osmolarity, sodium, and chloride levels initially decreased in response to exercise but returned to normal over a four-day period. The return to normal plasma ion homeostasis suggests either the capacity of the osmoregulatory tissues to regulate internal ions is improved, or more energy is available for osmoregulation following exercise. Gill Na^+K^+-ATPase activity significantly increased in response to both sustained and burst swimming challenges by ~20%, while H^+-ATPase activity was only elevated following burst swimming. Gill Na^+K^+-ATPase isoform a1a was significantly increased in response to exercise. In a follow up study aimed to determine if exercise improved salinity acclimation, chinook salmon were swum at 1.0 BL/second for three weeks and then allowed to acclimate to full strength seawater for seven days. Salinity tolerance indicators were measured to assess osmoregulatory success. Exercised fish exposed to seawater were able to regulate muscle water content, plasma sodium and chloride levels back to control levels slightly faster than non-exercised fish. The exercised fish were also able to upregulate gill Na^+K^+-ATPase activity faster than non-exercised fish. The results of this study expand our understanding of how fish can respond to multiple simultaneous stressors, and the interactions between exercise and whole-body ion and water balance mechanisms. They also suggest there may be some benefit to maintaining salmonid under a light exercise regimen to improve seawater acclimation.

DIET AND IMMUNITY:
ENHANCING HEALTH IN FARMED FISH THROUGH NUTRITION

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Fish health and ability to resist infection, parasitism, and stress are strongly conditioned by diet. Inadequate nutrition leads to poor health status and increased susceptibility to disease. Besides ensuring fish nutritional needs are met, feed formulation also allows for the inclusion of additional nutritional (e.g. omega-3 and omega-6 fatty acids) or non-nutritional (e.g. immunostimulant additives) characteristics that promote health or stronger immune responses in farmed fish.

In the past nine years, my colleagues and I have tested different dietary manipulations on the immune response of Atlantic salmon *Salmo salar* and lumpfish *Cyclopterus lumpus* exposed to actual or simulated infections and co-infections (e.g. sea lice *Lepeophtheirus salmonis*, formalin-killed bacteria). This research was funded by various Canadian agencies, including Genome Canada, NSERC, and the Ocean Frontier Institute, and involved multiple research teams from academia (e.g. Memorial University of Newfoundland, University of Prince Edward Island), government (e.g. Department of Fisheries and Oceans), and industry partners (e.g. Cargill, Cermaq-Canada). Our work explored the immunomodulatory effects of various sustainably produced non-marine ingredients, such as terrestrial animal/vegetable protein and lipid sources, as well as functional additives. Dietary effects on fish immunity were evaluated through the statistical integration of disparate sources of data, e.g. transcriptomics, pathogen/parasite load, clinical scoring, growth performance, and body/tissue chemical composition.

This presentation will review many of this research's key findings, discuss potential applications for future solutions to prevent and remediate fish health-related problems in the industry, and open new research avenues.

Diagram summarising the concept, philosophy, and methods of the fish nutrition and health interaction research reviewed in this presentation. This research bridges two research areas: fish nutrition (1) and immunity (2); while evaluating sustainable ingredients (3); under 4) collaborative research (academia, government, and industry); via integration of transcriptomics (5) growth/health parameters (6), and body/tissue composition data (7).

ANTIBACTERIAL ACTIVITY OF PROPYL-PROPANETHIOSUFINATE (PTS) AND PROPYL-PROPANE-THIOSULFONATE (PTSO) FROM ALLIUM CEPA AGAINST EUROPEAN SEABASS *Dicentrarchus labrax* PATHOGENS. *IN VITRO* AND *IN VIVO* EVALUATION

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The use of phytogenic extracts is considered a sustainable strategy for the prevention of fish diseases, including Alliaceae as a potential option due to their variety of bioactive compounds.

In this study, we analysed the antibacterial potential of propyl-propane-thiosulfinate (PTS) and propyl-propane-thiosulfonate (PTSO) from onions. The *in vitro* activity against *Vibrio anguillarum*, *Tenacibaculum maritimum*, and *Photobacterium damselae* subsp. *piscicida* of both compounds was tested by the determination of the Minimum Bactericidal Concentration (MBC). In addition, a diet that consisted of a blend of PTS/PTSO in a proportion 1:1; w/w (300 mg/kg) was compared against to a control formula with no addition of PTS/PTSO; a total of 240 European seabass (*Dicentrarchus labrax*) juveniles were challenged against *V. anguillarum* after 10 weeks of dietary administration.

All strains were sensitive to both compounds. PTSO showed the highest inhibitory effect against all target strains.

After the 10 weeks of dietary administration, fish fed with PTS/PTSO included in the basal formula showed no differences in fish performance parameters (SGR, FCR and survival) compared to the control group. At termination of the *V. anguillarum* challenge, fish fed with PTS/PTSO included in the formula showed the highest survival, increasing up to 70.0%, whereas in the control group, the survival was 51.2%. These results suggest the inclusion of PTS and PTSO in feed as a natural strategy to prevent vibriosis in European sea bass.

Table 1. Minimum Bactericidal Concentration (MBC) of PTS and PTSO against target strains.

Strain	PTS (mg/L)	PTSO (mg/L)
Vibrio anguillarum	78.12	39.02
Tebacibaculum maritimum	312.50	78.12
P. damselae subsp. piscicida	156.25	156.25

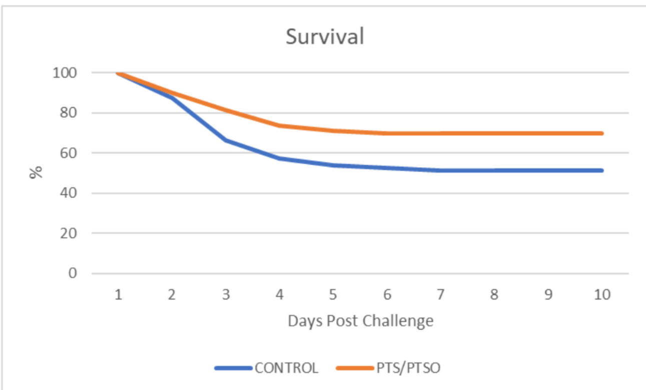

Figure 1. Survival curves after intraperitoneal inoculation of *V. anguillarum*.

IN VIVO EFFECTS OF LIPOPOLYSACCHARIDE (LPS) ON THE METABOLISM AND WELFARE OF GILTHEAD SEA BREAM *Sparus aurata* JUVENILES FED WITH PLANT-BASED DIETS ENRICHED WITH NUTRACEUTICALS

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The present study aimed to evaluate the improvement capacity of a microalgae-based nutraceutical compound in diets formulated with hydrolyzed vegetable protein in the face of a lipopolysaccharide (LPS) injection challenge. To this end, three diets were formulated: i) a control diet (C) based on fish meal (FM) and fish oil (FO); ii) a plant-based diet with the substitution of 75 % FM by hydrolyzed vegetable protein and oils (PP); and iii) PP diet supplemented with 2 % of the GreenGrapeLB nutraceutical compound formulated based on a hydrolysate of microalgae and a polyphenolic extract of white grape bagasse (LB). *Sparus aurata* juveniles (13-14 g initial mean weight) were distributed in nine tanks (3 tanks per experimental diet) and fed *ad libitum* for 84 days. Subsequently, eight animals per experimental diet were intraperitoneally injected with two treatments: i) Saline (control injection) and ii) Lipopolysaccharides (4 mg LPS/kg fish) and sampled after 72 hours.

Plasma and hepatic glucose levels remained constant between diets and treatments (Fig. 1A), which has been observed in similar studies with other species. The reduction of lactate levels in the PP-Saline group (Fig. 1B) could suggest that lactate absorption and elimination processes by some tissues, i.e. liver, are being stimulated, as the levels in this tissue followed an inverse pattern to those observed in plasma (higher hepatic lactate concentrations in PP group, although not significantly). On the other hand, the higher plasma triglyceride levels (TAG) in the LB group (Fig. 1C) may be due to an effect of the diet itself due to the content of fatty acids of different natures present in the microalgae contained in the LB nutraceutical employed. However, the pattern of changes in TAG values after LPS injection between diets was different, increasing in the C and PP groups but decreasing in the LB group, although not significantly so. This suggests a lipolysis stimulation in adipose tissues in the groups fed with these diets (C and PP), as described in other studies, and that the LB compound may act as an inhibitor of this response induced by an LPS challenge. Finally, no differences were observed between diets and treatments concerning plasma cortisol concentrations (data not shown).

Acknowledgments:
The authors wish to thank the support of the Experimental Feeds Service of the University of Almería. The GreenGrape product has been developed by *I-Grape* and *Lifebioencapsulation* company within the H2020 NeoGiant project (# 101036768). Anyell Caderno is supported by a Ph.D. fellowship from the Regional Government of Andalusia 2021 (PREDOC_02015).

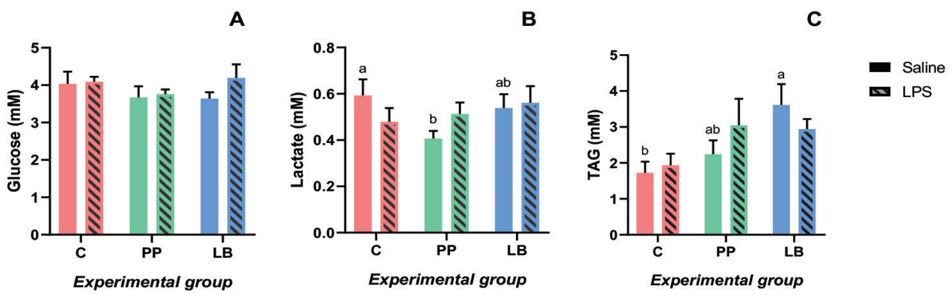

Figure 1. Plasma metabolites in *Sparus aurata*: A. glucose; B. lactate; and C. triglycerides.

GENOME SEQUENCING AND GENOME-WIDE COPY NUMBER ANALYSES REVEAL POTENTIAL CANDIDATE REGIONS AND GENES LINKED TO WSSV RESISTANCE IN THE PHILIPPINE BLACK TIGER SHRIMP *Penaeus monodon*

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Penaeus monodon (black tiger shrimp) is one of the most commercially important aquaculture species worldwide and in the Philippines. Genetic improvement of *P. monodon* stocks with desirable production traits, such as enhanced disease resistance is an important approach to improving production. The sequencing, assembly, and annotation of its genome are considered crucial steps for establishing the groundwork for the development of "omics" resources, enabling the effective and efficient breeding of *P. monodon* for the selection of superior stocks. In this study, a genome sequence was generated and assembled for the black tiger shrimp which was utilized to scan genome-wide copy number variation for the identification of potential regions and genes associated with WSSV resistance. Copy number variations (CNV) were detected in the pool sequencing of susceptible and resistant groups utilizing the read depth and the read pair signature methods. Structural variations associated with WSSV resistance were identified. Most of these variations were nestled within intergenic regions, indicating that gene expression is likely affected by the majority of these variations. Additionally, genes known to be responsible for the innate immune response were also found within a 1-Mb radius of the variants. Together with the genome assembly, the identified candidate markers constitute a vital resource for refining breeding strategies for Penaeid species.

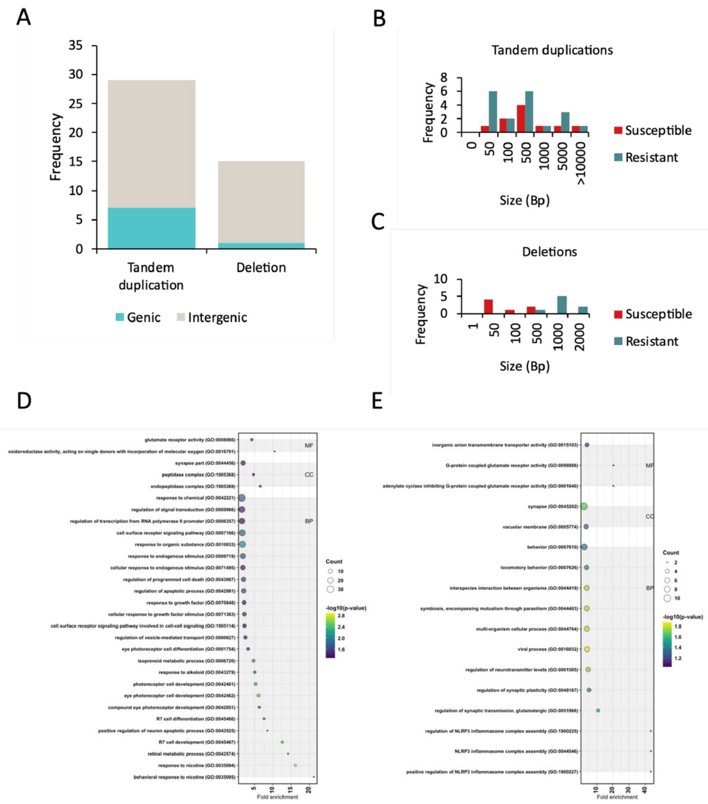

FIGURE 1. CNV-trait association analysis for WSSV resistance. **A** Frequency and proportion of tandem duplications and deletions in intergenic and genic regions. **B** Frequency and size distribution of tandem duplications and deletions in susceptible and resistant pools. Gene Ontology enrichment analysis of genes within a 1-Mbp radius from intergenic tandem duplications (**D**) and deletions (**E**) in the resistant pools.

INDICATORS FOR THE MONITORING OF ANCHOVY DURING THE FISHING SEASONS

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The Peruvian ecosystem is characterized by the great productivity of fish. Despite representing only 0.1% of the world's ocean surface, it produces around 10% of the world's catches.

Peru has the world's largest single-species (anchoveta) fishery, supporting the industry in terms of fishmeal and fish oil production. Being the first fishing country in unloading volumes of a single species over 3 decades. The anchovy fishery in the north-central zone has two fishing seasons (April-July and November-January).

Within the fishing industry, we find companies that represent around 60% of the global share. The Diamante fishing company has more than 30 years in the fishing sector. At the beginning of the anchovy quota law (2009), Diamante had around 30 fishing vessels which for various reasons has been reduced to this year 2023 with 22 vessels.

During the anchovy fishing seasons, a large volume of data is generated; from the departure of the boat (crew, fuel stock, hour meters), arrives at the fishing area (Date and time), makes coves (Biometry, number of coves, distribution of fishing in holds), Departure from fishing area (Date estimated arrival), Arrival (Stock of fuel, news upon arrival). Unloading (flat berthing, hopper unloading) and set sail again).

Diamante with support in SAP developed a web application in FIORI to be able to support this volume of data that is not only of interest to the fleet but also to the company. The recorded data is complementary for the management of indicators such as the degree of freshness (volatile nitrogen rate), capture times, % of juveniles, among others. With the calculation of these values we generate statistics for decision-making such as extraction unit cost, performance of fishing captains, fishing purchase behavior. In addition to SAP, we have sources of information such as fleet positioning, which we attach to SAP to determine indicators such as Gallon/mile traveled, theoretical gallon consumption according to speeds and number of stalls.

We not only use SAP for data storage, we use less sophisticated means such as the office suite to quickly communicate the progress of our own fleet and how we are doing with the competition.

In addition, with the pool of information stored in the diamond database, we can use them to manage projections with POWER BI (Moving Average), fleet behavior scenario, thus making decisions based on statistical data.

USE OF RAINBOW TROUT *Oncorhynchus mykiss* INTESTINAL IN VITRO PLATFORMS TO EVALUATE THE NUTRITIONAL AND HEALTH VALUE OF DIFFERENT DIETS

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Feed has a predominant weight in determining the production costs and the sustainability of aquaculture. In vivo feeding trials are fundamental for developing new feeds, but also have some limitations, due to their length, costs, and the need to sacrifice many animals. Therefore, an in vitro platform could provide an effective screening tool to test feed nutritional and health values. Here we tested bicameral platforms recently developed[1] in our laboratory, based on cell lines derived respectively from the proximal (RTpiMI) and distal (RTdiMI) intestine. We evaluated the effects of a prolonged exposure (21 days) to: a reference diet (RD), rich in fish meal and fish oil; a diet in which animal proteins were replaced with soy-derived proteins (SD); an experimental diet in which fish components were partially substituted by feather meal obtained from by-products of the poultry industries (FD); L-15 medium with no feed (Control). Barrier integrity was evaluated measuring transepithelial electrical resistance (TEER), cell morphology and alanine aminopeptidase enzyme activity. Results showed that SD disrupted epithelial barrier integrity of the proximal but not the distal intestine platform after 3 days of exposure (fig. 1). However, the effect was reversible since, barrier integrity was fully restored after SD was removed. On the other hand, RD and FD stimulated a progressive cell proliferation response along the 21 days of exposure in both platforms.

We conclude that proximal intestine cells are more sensitive than those of distal intestine, particularly to SD. The other diets had a milder effect and caused cell proliferation, rather than epithelial barrier disruption. We interpret this reaction as a sign of inflammation, probably due to the lack of mucus protection, similar to the one observed *in vivo* when challenging diets induce an intestinal folds branching[2] increase. In addition, RTpiMI cells ability to fully recover from the initial damage caused by SD is of great interest, since it suggests the possibility to use this platform to identify target molecules that can mitigate the effects of anti-nutritional factors. We are currently studying cell ability to absorb single amino acids as a possible way to discriminate the effects of different diets more subtly. Overall, our data indicate that the use of feed pellets digested in vitro with fish enzymes, combined with their exposure to intestinal cell platforms, provides useful indication on the nutritional and health value of different diets.

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References

Verdile, N. et al., *Cells,* 2023. https://doi.org/10.3390/cells12141843
Verdile, N. et al., *Aquaculture*, 2023. https://doi.org/10.1016/j.aquaculture.2022.739031

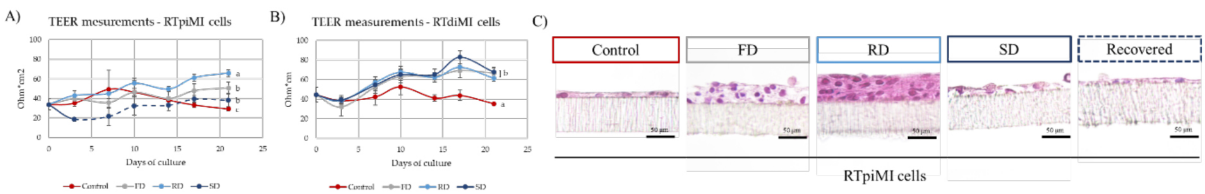

Figure 1: TEER values during 21 days of exposure to different diets in the RTpiMI (A) and RTdiMI (B) platforms. Values are expressed as mean ± standard deviation. Superscripts represent statistically significant values ($p<0.05$). Sections showing RTpiMI cells morphological changes (C) after exposure to different diets.

LIPID FORMULATIONS TO ENHANCE ERYTHROMYCIN'S ANTIMICROBIAL EFFICACY IN AQUACULTURE

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Erythromycin (ERY) is a wide-spectrum macrolide antibiotic that acts by interfering with protein synthesis. ERY is active against gram-positive and gram-negative pathogens, and it is among the antibiotics used by the major aquaculture producers to treat infectious diseases. It is commonly used for controlling lactococcosis (*Lactococcus garviae*), bacterial kidney disease (*Renibacterium salmoninarum*), and streptococcosis (*Streptococcus* spp.), and it is also prescribed for extra-label use. Nonetheless, the treatment can be challenging due to ERY's hydrophobic characteristics, poor aqueous solubility, instability in an acidic medium, low bioavailability, and bitter taste. This study aimed to encapsulate ERY in lipid formulations to overcome these disadvantages, enhancing ERY's antimicrobial efficacy. The nanostructured lipid carrier systems (NLCs) loaded with ERY were prepared by hot emulsification using high-speed homogenization followed by ultrasonication. The lipid matrix, composed of solid and liquid lipids, is biocompatible and biodegradable. The study investigated the use of various surfactants, including Whey protein concentrate, Poloxamer, Quillaja saponins, and Gellucire, on NLC properties and product stability throughout 28 days. The resulting NLCs base formulations are presented in Table 1.

The NLCs were characterized through determination of the pH, electrical conductivity, particle size, polydispersity index (PdI), and zeta potential (n=3), and the results are shown in Table 2.

The particle size can be linked to the surfactant used. Zeta potential values (>|30| mV) indicated the physical stability of the NLCs, maintained during 28 days. F1 and F2 formulations showed lower polydispersity, while F3 and F4 exhibited medium polydispersity. The bactericidal activity of F1, F2, and F3 was assessed by the microdilution broth method against *Aeromonas spp*. isolated from fish, revealing that the lipid formulations, particularly those with natural surfactants (Quillaja saponins and whey protein concentrate), demonstrated enhanced efficacy. Thus, the lipid formulations have significant potential to improve ERY's solubility, stability, and bioavailability, with the added advantage of being biocompatible and biodegradable. Given that microbial disease outbreaks are a major challenge to sustainable aquaculture, this research can lead to the development of new antibiotic delivery systems with improved efficacy, promoting the safer use of antimicrobials within the One Health concept.

Table 1. Composition of NLC base formulations containing erythromycin.

Formulation	Solid lipid	Liquid lipid	Surfactant
F1	Compritol 5%	Oleic acid 2%	Whey protein concentrate 4%
F2	Compritol 5%	Oleic acid 2%	Poloxamer 4%
F3	Compritol 5%	Oleic acid 2%	Quillaja saponins 5%
F4	Compritol 5%	Oleic acid 2%	Gellucire 4%

Table 2. NLCs loaded ERY physicochemical characterization.

Lipid formulation	pH	Conductivity (μS cm^{-1})	Size (nm)	PdI	Zeta Potential (mV)
F1	5.53 ± 0.01	101.52 ± 0.13	521.7 ± 12.8	0.14 ± 0.04	-37.2 ± 0.40
F2	6.04 ± 0.06	143.61 ± 3.04	160.3 ± 2.0	0.17 ± 0.02	-27.7 ± 0.15
F3	4.98 ± 0.03	101.34 ± 4.97	427.2 ± 4	0.47 ± 0.09	-35.9 ± 1.30
F4	6.48 ± 0.03	104.56 ± 0.04	383.2 ± 1.97	0.54 ± 0.01	-38.4 ± 0.36

PROBIOTICS EFFECTS ON MITOCHONDRIAL PLASTICITY FROM HEAD-KIDNEY LEUCOCYTES COMPROMISED BY FATTY LIVER DISEASE DEVELOPED IN *Sparus aurata*

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A diet high in lipids can result in an increase in lipid accumulation, particularly, in the liver of fish, ultimately leading to the development of fatty liver disease (FLD). This issue has been documented globally in aquaculture settings involving various fish species, including rainbow trout, medaka, tilapia, and grass-fish. Various research studies have indicated that the use of probiotics in mice fed high-fat diets can potentially mitigate some of the symptoms of FLD, such as hepatic steatosis. Proposed mechanisms include the reduction of lipid deposition in the liver, decreased oxidative stress, and anti-inflammatory properties, among others. *Shewanella putrefaciens* Pdp11, identified as a probiotic strain from farmed gilthead seabream [1], exhibits several probiotic traits, including the ability to enhance and regulate the levels of fatty acids like linoleic acid in fish [2].

With the aim of studying the impact of probiotic supplementation on fatty acid disease, 180 specimens of gilthead seabream were disposed into 12 aquaria and fed for 3 months with the following diets: control diet, control diet+Pdp11, diet supplemented diet with 10% fish oil extra and supplemented diet with 10% fish oil extra+Pdp11. Each month and at the end of the fed trial, 6 animals from each treatment were sacrificed, and the head kidney leucocytes were isolated and adjusted to 10^6 cells per well. A mitochondrial stress test was then performed to assess the bioenergetic capacities of the cells. Proton leak, the maximal respiration, spare respiratory capacity, ATP production, and coupling efficiency from head kidney leucocytes were significantly affected on fish fed the 10% fish oil extra diet. Besides, leucocytes from fish fed with 10% fish oil extra+Pdp11 showed values similar to control in almost all analyzed parameters. Our findings suggest that the addition of Pdp11 may play a role in managing the metabolic syndrome resulting from an imbalanced diet. This probiotic can also influence the mitochondrial plasticity from head-kidney leucocytes, being a key parameter for cellular response.

This study forms part of the ThinkInAzul programme and was supported by MCIN with funding from European Union NextGeneration EU (PRTR-C17.I01) and by *Comunidad Autónoma de la Región de Murcia - Fundación Séneca.*

[1] M. Cámara-Ruiz, M.C. Balebona, M.Á. Moriñigo, M.Á. Esteban, Probiotic *Shewanella putrefaciens* (SpPdp11) as a Fish Health Modulator: A Review., Microorganisms. 8 (2020).

[2] I. García de la Banda, C. Lobo, J. León-Rubio, E. Al, Influence of two closely related probiotics on juvenile Senegalese sole (*Solea senegalensis*, Kaup 1858) performance and protection against *Photobacterium damselae* subsp. piscicida. Aquaculture. 306(2010)281-288.

[3] S.T. Tapia-Paniagua, S. Vidal, C. Lobo, et al., The treatment with the probiotic *Shewanella putrefaciens* Pdp11 of specimens of *Solea senegalensis* exposed to high stocking densities to enhance their resistance to disease, Fish Shellfish Immunol. 41 (2014) 209-221.

EFFECT OF PROBIOTICS ON THE IMMUNE SYSTEM COMPROMISED BY FATTY LIVER DISEASE DEVELOPED IN *Sparus aurata*

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A diet rich in lipids can increase lipid deposition and lead to a significant accumulation of fatty acids in the liver of fish, promoting fatty liver disease (FLD). This problem has been reported worldwide in the aquaculture of a wide range of fish species, such as rainbow trout, medaka, tilapia, and grass-fish. Diverse experimental trials have reported that the administration of probiotics to mice fed high-fat-diet may prevent some of the FLD sings, such as hepatic steatosis. Mechanisms such as the reduction of hepatic lipid deposition, reduction of oxidative stress and anti-inflammatory effects, among others, have been proposed. *Shewanella putrefaciens* Pdp11, a probiotic strain isolated from farmed gilthead seabream [1] exhibits several probiotic characteristics, including the ability to increase and modulate the content of fatty acids such as linoleic acid in fish [2].

Aiming to study the impact of probiotic supplementation on fatty acid disease, 180 specimens of gilthead seabream were disposed into 12 aquaria and fed for 3 months with the following diets: control diet, control diet+Pdp11, diet supplemented diet with 10% fish oil extra and supplemented diet with 10% fish oil extra+Pdp11. Each month and at the end of the fed trial, 6 animals from each treatment were sacrificed, and the head kidney leucocytes were isolated. The phagocytic activity, respiratory burst and peroxidase activity were analysed. The fish fed with the 10% fish oil extra diet showed signs of hepatic steatosis. No changes were observed in peroxidase activity in the two first months, but the respiratory burst and phagocytosis were significantly affected in the fish fed the diet supplemented diet with 10% fish oil extra. These results suggest that the unbalanced diet is producing a metabolic disorder, affecting the cellular response. On the other hand, fish fed the diet with 10% of fish oil and probiotic did not show these alterations on the cellular response. It has been previously reported the ability of Pdp11 to mediate in stress response, intestinal microbiota and fish immunity [3]. Our results seem to indicate the Pdp11 supplementation could mediate in the metabolic syndrome caused by the unbalanced diet, as well as modulate the cellular response.

This study forms part of the ThinkInAzul programme and was supported by MCIN with funding from European Union NextGeneration EU (PRTR-C17.I01) and by *Comunidad Autónoma de la Región de Murcia - Fundación Séneca*.

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PHAGOCYTIC MECHANISMS OF EUROPEAN SEABASS (*Dicentrarchus labrax*) ERYTHROCYTES

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In the field of vertebrate innate immunity, phagocytosis is a crucial defence process by which a cell is able to engulf a particle of diverse nature (*e.g.,* cellular debris, microorganisms, etc.). This process is predominantly executed by leucocytes (macrophages, granulocytes and B lymphocytes), although it has been shown that in fish, other cells named non-professional phagocytes, such as fibroblasts, skin mucus-secreting cells, thrombocytes and endothelial cells, are able of develop phagocytic mechanisms. In this context, although fish erythrocytes have been implicated in immune activities, their role in phagocytosis has received little attention. Therefore, this study aims to elucidate whether erythrocytes from European seabass (*Dicentrarchus labrax*) exhibit phagocytic activity.

In this study, ten specimens of European seabass (41.75 g ± 4.93 g, 19.55 cm ± 1.08 cm) obtained from a local farm (Murcia, Spain), and maintained in the Marine Fish Facilities at the University of Murcia (Spain), were anesthetized, and blood samples were collected. Erythrocytes from of seabass specimens were isolated from systemic blood with a 51 % Percoll density gradient (Pharmacia). Subsequently, erythrocytes were incubated for 20, 40, 60, 60, 80, 100 and 120 minutes with heat-inactivated yeast (*Saccharomyces cerevisiae*) labelled with fluorescein isothiocyanate. Then, the phagocytic activities of erythrocytes were studied by flow cytometry and electron microscopy.

Our results revealed an increase in the phagocytic ability at 40, 60 and 80 min of incubation. Similarly, the phagocytic capacity also increased at 40 min of incubation. In addition, the results obtained by flow cytometry were corroborated by transmission electron microscopy. For instance, erythrocytes showed characteristic invaginations and evaginations (pseudopod emission), and vesicle formation that could be identified with phagosomes and phagolysosomes. Furthermore, field emission scanning electron microscopy revealed evaginations of the cytoplasmic membrane of seabass erythrocytes.

These results demonstrate for the first time the phagocytic process developed by European seabass erythrocytes, which could be of interest for basic research in haematology and immunology, and also for possible application in the aquaculture sector.

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USE OF SERUM PROTEINOGRAM TO DETECT INFLAMMATION IN GILTHEAD SEABREAM (*Sparus aurata*)

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The proteinogram is a semiquantitative analytical method that separates proteins into distinct bands using electrophoresis. This technique has been widely used in clinic to identify abnormal or elevated proteins in serum or other fluids, indicating possible liver, inflammatory, and immune diseases. However, this method has not been studied in fish for diagnostic use.

In our study, gilthead seabream specimens (100.38 g ± 1.49 g, 16.76 cm ± 0.12 cm) obtained from a local farm (Murcia, Spain), and maintained in the Marine Fish Facilities at the University of Murcia (Spain), were injected intramuscularly with 500 μL of phosphate buffered saline (PBS, as control), or carrageenan solution (1%, Sigma) in PBS. Fish were injected again at 7 and 14 days with 250 μL of λ-carrageenan. At 15 days post-injection, fish were anesthetized, and blood samples were collected. Proteins were quantified with the Direct Detect Spectrometer (Merck-Millipore) and serum proteins were then separated using a bioanalyzer (with Agilent Protein230 kit) from which electropherograms were obtained. Finally, proteins from the diluted serum samples were identified by HPLC-mass spectrometry. Protein concentrations from electropherograms were averaged by molecular weight, normalized to 100%, and ranges were determined based on major protein limits. Our results were expressed as mean ± standard error of the mean (SEM) and data were analysed by Student’s t-test to determine variations between experimental groups ($p<0.05$).

The results of electropherograms showed four major peaks of proteins with 19.1, 76.3, 104.4, and 156.7 kDa, respectively. Matching the molecular weight of the proteins found by HPLC-mass spectrometry and by electropherograms, the four major proteins were identified as apolipoprotein A-II (20.0 kDa), serotransferrin (74.3 kDa), inter-α-trypsin inhibitor heavy chain H3-like (102.2 kDa), and α-2-macroglobulin-like (160.7 kDa). These proteins were represented as Apo, β, α1 and α2 fractions, respectively. The Apo fraction decreased in the group of fish injected with λ-carrageenan compared to the control group, while the α1 and α2 fractions increased in the serum of fish injected with λ-carrageenan.

These results obtained with serum proteinogram provide a new approach to detect inflammation in fish, providing a basis in the rapid diagnose of health disorders or diseases in farmed fish.

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DETERMINATION OF THE SERUM PROTEINOGRAM PROFILE OF EUROPEAN SEABASS (*Dicentrarchus labrax*) FOR THE DIAGNOSIS OF FISH DISEASES

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A proteinogram is a semiquantitative analysis method that allows the separation of proteins by electrophoresis into different bands. This methodology has been widely used in clinical practice to detect the presence of abnormal or excessive proteins in serum, or other body fluids, which can be indicative of liver, inflammatory and immune disorders. Specifically, electropherograms have recently emerged as a specialization of protein electrophoresis in which the fluorescence intensities of proteins are measured as a function of their migration times. This method, widely used in humans, has not been studied in fish for diagnostic purposes.

In this study, ten specimens of European seabass (*Dicentrarchus labrax*) (40.40 g ± 3.07 g, 15.67 cm ± 0.38 cm) obtained from a local farm (Murcia, Spain), and maintained in the Marine Fish Facilities at the University of Murcia (Spain), were anesthetized with clove oil (20 mg L^{-1}, Guinama®), and blood samples were collected. Proteins were quantified with the Direct Detect Spectrometer (Merck-Millipore) and adjusted to the same concentration in PBS. Serum proteins were then separated by SDS-PAGE and using a bioanalyzer (with Agilent Protein80 and Protein230 kits) from which electropherograms were obtained. Finally, proteins from the diluted serum samples were identified by HPLC-mass spectrometry. Protein concentrations from electropherograms were averaged by molecular weight, normalized to 100%, and ranges were determined based on major protein limits.

The results obtained with SDS-PAGE analysis revealed four main protein bands around 11, 25, 70 and 100 kDa in the serum of the European seabass specimens, and several non-well-defined bands of proteins. Similarly, the electropherograms also showed four major peaks of proteins with the same molecular weight, representing, 13.2%, 14.5%, 29.1%, and 10.8% of the total serum proteins, respectively. The rest of minor proteins comprised the remaining percentage. The results obtained with HPLC-mass spectrometry showed 119 proteins in the serum of European seabass. Matching the molecular weight of the proteins found by HPLC-mass spectrometry and by electropherograms, the four major proteins were identified as apolipoprotein C-I, Si:ch1073-126c3.2 protein, serotransferrin and Inter-alpha-trypsin inhibitor heavy chain 2.

These results could contribute to establish the normal reference values for the serum proteinogram of European seabass, providing a basis for addressing new approaches in the rapid diagnose of health disorders or diseases in farmed fish.

Acknowledgements
This research was supported by the "*Programa para la recualificación del sistema universitario español para* 2021-2023 - Margarita Salas Modality (R-1593/2022)". This work forms part of the ThinkInAzul programme and was supported by MCIN with funding from European Union Next Generation EU (PRTR-C17.I01) and by *Comunidad Autónoma de la Región de Murcia - Fundación Séneca*.

FINDING SYNERGIES BETWEEN FISH FARMERS AND FISH FEED MANUFACTURERS TO FACILITATE MORE SUSTAINABLE CHOICES

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Norway is the world's largest producer of farmed Atlantic salmon, with a production of around 1.2 million tonnes in 2021. Still, expansion is expected to continue, and the plan is to produce approximately 5 million tonnes of salmon by 2050. With this expansion, as well as with the rising importance of sustainability in aquaculture, there is a need to find more sustainable feeds. The present study aims to understand the perception of fish farmers and fish feed manufacturers for the most important criteria related to fish feeds and their sustainability. The analysis focuses on understanding which criteria or information enhances the level of importance assigned by fish farmers to each sustainability dimension (environmental, economic, social), and how this can be used for feed manufacturers to increase sustainable choices along the value chain.

We conducted semi-structured interviews with the most important feed manufacturers in Norway to understand better the important criteria considered in their feeds. Meanwhile, surveys were used to obtain the perspectives of fish farmers. Data from surveys were used to understand better which criteria enhance the level of importance assigned by fish farmers to each of the sustainability dimensions. By using a hybrid-fuzzy TOPSIS methodology, synthetic indicators were estimated, one for each sustainability dimension (ENVSI: environmental; SOSI: social; ECOSI: economic), representing their importance in each segment of analysis.

It is possible to conclude that more sustainable feeds can only be achieved without trade-offs on performance. Furthermore, some strategies to increase overall sustainability choice amongst dimensions would be to focus more on certified feeds and highlighting information to farmers on proper conditions given by employees in the industry. Moreover, marketing oriented to emphasizing local products can enhance more environmental choices by some segments of the population. Along the same line, drawing attention to indicators measuring environmental and biodiversity impact on marketing strategies might enhance environmental choices by farmers.

TABLE 1. Synthetic indicators after Hybrid-fuzzy TOPSIS

Category	Subcategory	ENVSI	SOSI	ECOSI
Location of farm	Trøndelag	0.62	0.60	0.51
	Vestlandet	0.53	0.35	0.52
	Nord-Norge	0.61	0.61	0.49
	Other	0.50	0.57	0.44
The company has certifications	Yes	0.58	0.50	0.61
	No or nor answer	0.53	0.49	0.27
Number of employees	Micro (1-10)	0.31	0.45	0.06
	Small (11-50)	0.48	0.48	0.44
	Medium or higher (>50)	0.74	0.53	0.71
Type of feed used	Commercial	0.49	0.42	0.48
	Tailor-made feed	0.72	0.61	0.62
Aquaculture system used by the company	RAS	0.60	0.52	0.39
	Sea cages	0.55	0.45	0.54
Feeding system	Automatic	0.59	0.53	0.50
	Manual	0.42	0.38	0.42
Agreement to the idea of using more local products in your feed formulation.	Disagree (2)	0.24	0.24	0.00
	Neutral (3)	0.50	0.50	0.57
	Agree (4)	0.56	0.40	0.38
	Highly agree (5)	0.89	0.84	0.72
Willingness to pay more for feed formulations with a higher proportion of local products	Yes	0.91	0.91	0.54
	Maybe	0.42	0.31	0.42
	No	0.56	0.53	0.56
Importance of certified/organic ingredients in feed formulation	Low importance or Neutral (2-3)	0.42	0.37	0.46
	Important (4)	0.58	0.44	0.52
	Very Important (5)	0.83	0.75	0.62
Importance of the use of products offering competitive advantage that can be translated to the consumer (additional willingness to pay)	Low importance or Neutral (2-3)	0.30	0.47	0.50
	Important (4)	0.71	0.57	0.51
	Very Important (5)	0.69	0.25	0.60
Importance of price of the product	Neutral (3)	0.48	0.34	0.06
	Important (4)	0.30	0.28	0.37
	Very Important (5)	0.83	0.71	0.78
Importance on the land-use change generated by the ingredients included in the formulation.	None or Low importance (1-2)	0.26	0.24	0.65
	Neutral (3)	0.43	0.36	0.43
	Important or very important (4-5)	0.79	0.67	0.59
Importance of the negative impacts on biodiversity (e.g., by-catch generated by marine resources, etc.) of the ingredients of the formulation	Low importance or Neutral (2-3)	0.27	0.24	0.50
	Important (4)	0.64	0.70	0.42
	Very Important (5)	0.84	0.49	0.67
Importance of the emissions of the feed formulation	Low importance (2)	0.00	0.00	0.31
	Neutral (3)	0.41	0.31	0.52
	Important (4)	0.55	0.50	0.36
	Very Important (5)	0.93	0.75	0.91
Importance of the social contributions of the company offering the feed (social campaigns, etc)	Low importance (2)	0.33	0.24	0.77
	Neutral (3)	0.41	0.28	0.47
	Important (4)	0.69	0.63	0.46
	Very Important (5)	1.00	0.93	0.77
Importance of knowing that feed provider guarantees proper conditions to their employees	Neutral (3)	0.35	0.29	0.38
	Important (4)	0.66	0.59	0.56
	Very Important (5)	0.75	0.59	0.67
Importance of the contribution of the company to the local economy (employment generated)	Low importance (2)	1.00	0.73	0.65
	Neutral (3)	0.41	0.36	0.47
	Important (4)	0.74	0.57	0.49
	Very Important (5)	0.74	1.00	1.00

Funding: This investigation is part of an industry-academic collaborative research project (CalaFeed) funded by the Norwegian Research Council (NRC).

Calanus Finmarchicus AS AN ALTERNATIVE FEED INGREDIENT FOR NORWEGIAN SALMON AQUACULTURE: ENVIRONMENTAL SUSTAINABILITY AND FUTURE CHALLENGES AND BARRIERS

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Norway is the world's leading producer of Atlantic salmon, with an estimated 1.2 million tonnes produced in 2021. However, growth in the Norwegian market is expected to continue, with plans aiming to produce approximately 5 million tonnes of salmon by 2050, implying a significant increase in the market for aquafeeds. Considering this, the aquaculture industry faces a substantial challenge in sourcing sustainable and reasonably priced feed raw materials.

Future challenges for alternative feed ingredients for aquaculture include sustainably developing oceans while reducing reliance on the human food chain for seafood production. Many ocean species, especially on lower trophic levels, are either not harvested or are only marginally utilized. One of these species is *Calanus finmarchicus*, a protein and lipid-rich zooplankton in large amounts in the North Atlantic Ocean. Calanus processing currently produces three primary by-products: oil, powder, and hydrolysate. Although the production volume is currently relatively small, they have the potential (estimated biomass of 290 million tonnes) to support the sustainable, local expansion of Norwegian salmon aquaculture.

In this study, we use life cycle assessment (LCA) to evaluate the environmental effects of Calanus by-products for use in the Norwegian aquafeed market. The results (**Table 1**) show that regarding economic allocation, Calanus oil has the most significant impact on the various categories, primarily due to the combustion of fossil fuels required for harvesting, freezing on board, and transportation to the port. The findings highlight the need to improve the efficiency of Calanus harvesting, as literary evidence suggests that Calanus fisheries lag far behind other fishing in terms of efficiency. Furthermore, the results show that packaging-related processes significantly impact the hydrolysate and powder by-products.

Furthermore, the scenario analysis revealed that the allocation method and electricity mix assumption (hydropower vs fossil fuel) significantly impact the analysis's results. In the electricity mix assumption, the geographic location of the processing facilities is an important consideration. Finally, it was found that Calanus by-products could be a more environmentally sustainable alternative protein and oil than other traditional feeds derived from soy or similar krill by-products for the Norwegian market.

However, being an environmentally friendly alternative does not automatically make Calanus a preferred alternative feed ingredient. Based on the data from regional feed producers and experts, we further elaborate on possible barriers in using *Calanus finmarchicus* as a resource in Norwegian salmon aquaculture.

Funding: This investigation is part of an industry-academic collaborative research project (CalaFeed) funded by the Norwegian Research Council (NRC).

Table 1. Greenhouse emissions using economic allocation

Greenhouse gas emissions			
Product	1 kg of oil	1 kg of hydrolysate	1 kg of powder
Total emissions (kg CO2 eq)	**6.78**	**0.58**	**1.12**
Harvesting	73.8 %	28.8 %	41.8 %
Initial packaging	7.0 %	2.7 %	4.0 %
Storage of frozen product	1.4 %	0.5 %	0.8 %
Machines operating	2.6 %	1.0 %	1.5 %
Initial processing	7.1 %	2.8 %	4.0 %
Centrifugation	0.6 %	0.2 %	0.3 %
Stick water to hydrolysate	4.3 %	26.8 %	28.3 %
Distribution in Norway	3.2 %	37.2 %	19.3 %

A META-ANALYSIS REVEALING THE TECHNICAL, ENVIRONMENTAL, AND HOST-ASSOCIATED FACTORS THAT SHAPE THE GUT MICROBIOTA OF ATLANTIC SALMON AND RAINBOW TROUT

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Salmonids, specifically Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), are commonly farmed and their gut microbiota plays important roles for optimal growth, health, and physiology. However, differences in experimental design, technical factors and bioinformatics make it challenging to compare the results from different studies and draw general conclusions about their influence on the fish gut microbiota. For a more comprehensive understanding of the gut microbiota, we collected all the publicly accessible 16S rRNA gene sequencing data with clearly stated sample metadata from freshwater Atlantic salmon and rainbow trout intestinal contents and mucosa sequenced on the Illumina MiSeq platform. A total of 783 samples from 19 published studies were included in this meta-analysis to test the impact of the technical, environmental, and host-associated factors. This meta-analysis revealed that all the tested factors significantly influenced the alpha and beta diversities of the gut microbiota of salmon and trout. Permutational multivariate analysis of variance (PERMANOVA) was applied to investigate the influence of the factors on beta diversity. According to the statictical results (Table 1), technical factors, especially target region and DNA extraction kit, affected the beta diversity to a larger extent, while host-associated and environmental factors, especially diet and initial fish weight, had a higher impact on the alpha diversity. Salmon had a higher alpha diversity and higher abundance of *Enterococcus* and *Staphylococcus* than trout, which by contrast had higher abundance of *Weissella* and *Mycoplasma*. The results of this meta-analysis fill in a critical knowledge gap that demonstrate technical methodologies must be standardized and factors associated with host and environment need to be accounted for in the future design of salmonid gut microbiota experiments.

Table 1. The impact of the influencing factors on the beta diversity of gut microbiota in freshwater salmonid fishes using weighted UniFrac and PERMANOVA

Factor	Factor type	Sample size	p-value	R squared	Pseudo-F
Target hypervariable region	Technical	783	<0.001	0.244	125.90
DNA extraction kit	Technical	783	<0.001	0.191	46.00
Diet	Environmental	745	<0.001	0.187	29.75
DNA polymerase	Technical	713	<0.001	0.173	32.46
Initial weight	Host-associated	706	<0.001	0.160	37.02
Rearing system	Environmental	572	<0.001	0.152	46.41
Flow rate	Environmental	406	<0.001	0.141	64.26
Daylight	Environmental	549	<0.001	0.123	54.48
Intestinal region	Host-associated	783	<0.001	0.116	51.26
Specific growth rate	Host-associated	356	<0.001	0.110	48.10
Feed conversion ratio	Host-associated	315	<0.001	0.090	38.16
Species	Host-associated	783	<0.001	0.081	68.45
Temperature	Environmental	680	<0.001	0.075	20.98
Weight gain	Host-associated	193	<0.001	0.061	25.51

Note: 9999 permutations were performed in each of the PERMANOVA tests to obtain the p value, R square, and pseudo-F values.

DETERMINING THE APPROPRIATE SAMPLING TIMEFRAME FOR MEASURING FECAL CORTISOL AND ITS METABOLITES IN FARMED ATLANTIC SALMON

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Stress assessment is crucial in aquaculture to ensure and improve the health and well-being of production animals, including fish. Assessing fecal cortisol and its metabolites (FCMs) is a less invasive and more labor-efficient method for monitoring the overall health and welfare of salmonids compared to plasma cortisol (PC) assessment. Determining the appropriate sampling timeframe is essential for accurate measurement. The primary objective of this study was to compare the results of our serial studies to further investigate the optimal sampling times for FCMs in farmed Atlantic salmon of different sizes. Blood, intestinal contents, and feces samples were collected before, during, and after a stress-inducing event, including net cleaning, sea lice counting and chasing. The PC and its metabolite levels in the mid-intestinal contents (MICMs) and FCMs were assessed by an enzyme-linked immunosorbent assay (ELISA) and LC-MS/MS techniques.

In a group of Atlantic salmon (1 kg), blood, intestinal contents, or feces samples were collected before, during, and at 2 and 20 hours after a net cleaning event. The results showed a sequential increase in PC levels and FCMs following net cleaning. The elevation of FCMs was <u>not</u> observed at <u>2 hours</u>, with a peak occurring at 20 hours post-net cleaning. There was no strong correlation between PC and FCM due to different time lag after acute stress.

Next, a fish group with an average weight of 438 grams was sampled before and at 1, 2, 4, 6, 12, 24, 48, and 72 hours after netting and relocation. The results showed that peak feces levels were observed 24 hours post-netting and relocation.

Both results underscore the importance of considering delayed and extended sampling patterns when evaluating acute stress with FCMs in aquatic environments. They suggest that 24 hours post-handling is an optimal time window for FCMs sampling in fish over 400 grams.

Conversely, in a fish group averaging 82 grams, an elevation in FCM levels were detected <u>40 minutes</u> after chasing. FCM levels showed a significant correlation with PC levels in these fish. In another study, a fish group averaging 188 grams was subjected to suspected extra strain during a one-hour period of capture, sedation, and transport. After two hours of sampling, simultaneous elevations in PC and FCMs were observed in the same fish.

The most notable finding from these studies is that the secretion of FCMs exhibits a significant size-dependent pattern. In Atlantic salmon over 400 grams, a 24-hour delay for feces sampling was recommended for FCMs testing following acute stress. For fish under 400 grams, especially those under 200 grams, sampling might need to start earlier, and the sampling window appears narrower. Further investigation is required, considering fish size, temperature, and feeding regime.

MEASURING CORTISOL LEVELS IN FISH TO DEVELOP MORE ANIMAL FRIENDLY TECHNOLOGY IN AQUACULTURE PRACTICES

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Several pump systems are currently in use during management operations at aquaculture facilities. A new pump system; The new (MMC) RID pump system was compared to traditional pump systems to assess whether the RID system produced lower stress responses in Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss).

The stress response of fish was measured by analyzing circulating cortisol levels in blood plasma at different stages during the pumping process. The following samples were taken:
1. Before the fish enters the pump system
2. Fish arriving in the first tank
3. Fish arriving at the next tank after pumping for 1 hour
4. The fish arriving in the last tank.

Cortisol levels were generally higher in salmon than in rainbow trout. The analyzes showed a lower concentration of the stress hormone cortisol in both salmon and rainbow trout after pumping with the RID pump compared to vacuum pump and centrifugal pump. The RID pump contributed to a lower cortisol level throughout the pumping process, especially in Atlantic salmon, where a significantly lower cortisol concentration was observed in the last part of the process.

This study is part of our effort to develop user-friendly tools for identifying the fish's stress response to their surroundings and to various farm operations. These methods makes it possible for farmers, authorities and technology suppliers to test various events in fish farming, without the fish being killed. This opens new possibilities of identifying fish stressors and the extent of stress the event or environmental conditions is causing. This also opens the possibility of developing and identifying operating and technology systems that contribute to improving welfare of farmed fish.

ASSESSMENT OF AQUACULTURE POLICY RESPONSES TO THE ORGANIC FARM-TO-FORK TARGETS

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In 2019, the EU's Green Deal policy framework for climate change was published, leading to the Farm to Fork and Biodiversity Strategies, which set the ambitious targets for reaching 25% of EU agricultural land managed organically and a significant increase in organic aquaculture by 2030.

The first European regulation defining organic aquaculture (Reg. EC 710/2009) came into effect in July 2010 providing the legal basis for market development and the inclusion of organic aquaculture into the Common Fishery Policy (CFP), through the European Maritime Fisheries and Aquaculture Fund and the Multi-annual National Strategic Plans for Aquaculture, covering the periods of 2014-2020 and 2021-2027. In this study, we assessed if and how organic aquaculture is supported in the multi-annual plans of Austria, Croatia, Denmark, France, Germany, Greece, Ireland, Italy and Spain, whose organic aquaculture production represents about 80% of the whole European production.

In general, the analysis of the state of organic aquaculture is poorly developed in the Multi-annual National Strategic Plans for Aquaculture and the European Maritime, Fisheries and Aquaculture Fund (2021-2027) of the countries considered. Consequently, even the identification of objectives and activities aimed at promoting the development of organic aquaculture is rather marginal in comparison to the effort spent on analyzing and promoting key actions for developing *sustainable* conventional aquaculture. The increase in funding in the current period (2021-2027) compared with the previous period (2014-2020) provides scope for increased support for organic aquaculture, but no ring-fenced funding was identified and the uptake of funding will depend on project submissions by organic companies. In addition, despite the policy support, we found a scarcity of statistical data to quantify outputs. Indeed, it seems questionable whether enough is being done to meet the challenge of reaching the EU's ambitious strategy.

A recent review of the organic aquaculture literature highlights several factors still hindering the development of the organic aquaculture in the EU. It was mostly reported the *perceived feasibility of organic aquaculture* from farmers and the *unavailability and costs of organically produced inputs* are major constraining factors for the development of organic aquaculture. The price relation between organic and conventional products was also evaluated as still insufficient to support the organic aquaculture extra-costs. Thus, accessibility to communication, as well as marketing strategies, might help to support *consumer demand/willingness to buy*. It would appear that the full application of the regulation on organic aquaculture before a greater diffusion and consolidation of production and before the main technical problems have been solved, has not fostered the hoped-for growth of organic aquaculture.

The combination of uncertainty regarding policy commitments, financial and market challenges, and input availability constraints would appear to be currently hampering the growth of organic aquaculture in Europe.

INVESTIGATING THE EFFECTS OF AN ENVIRONMENTALLY SUSTAINABLE DIET FORMULATED USING NOVEL INGREDIENTS ON THE GROWTH, HEALTH AND WELFARE OF GILTHEAD SEABREAM (*Sparus aurata*)

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The use of traditional aquafeed ingredients, such as fish meal and oil, remains a concern for the environmental sustainability of aquaculture. Therefore, significant research efforts are currently focused on identifying alternative protein sources using cost-effective and sustainable ingredients. Various alternative sources, such as plant or single-cell proteins, have been proposed as suitable substitutes. However, the utilization of new innovative feeds must prioritize fish health and welfare, as they directly impact production and consumer perception. This study evaluated the effects of a cost-effective and environmentally friendly innovative diet, which involved reducing fish meal content by 60% and replacing it with a blend of plant, yeast, and krill meal ingredients, on seabream growth, health, and welfare over a 135-day experiment. Various parameters were investigated, including growth, hematological, and biochemical indicators, along with monitoring of energy expenditure using accelerometer tags.

The results revealed no adverse effects of the innovative diet on survival and indicated slight improvements in growth performance among fish fed the innovative diet compared to those fed a commercial control diet (Fig. 1a). Data from accelerometer tags demonstrated higher energy expenditure in fish fed the innovative diet, potentially offset by the diet's composition to maintain growth (Fig. 1b). Blood parameters associated with stress, immunity, and health showed no significant negative impacts from the innovative diet. On the contrary, fish fed the innovative diet exhibited higher levels of immunoglobulin M (Fig. 1c), potentially reflecting enhanced immune defenses. Overall, the innovative diet shows promise for seabream aquaculture, but further long-term studies are necessary.

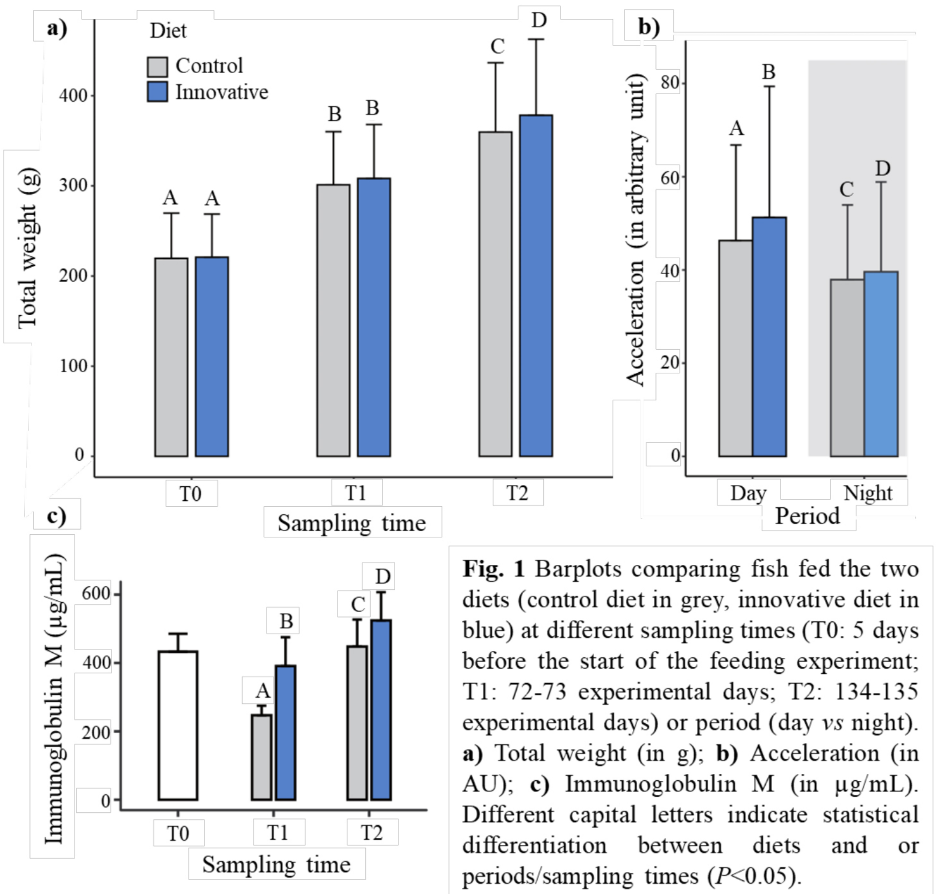

Fig. 1 Barplots comparing fish fed the two diets (control diet in grey, innovative diet in blue) at different sampling times (T0: 5 days before the start of the feeding experiment; T1: 72-73 experimental days; T2: 134-135 experimental days) or period (day *vs* night). **a)** Total weight (in g); **b)** Acceleration (in AU); **c)** Immunoglobulin M (in µg/mL). Different capital letters indicate statistical differentiation between diets and or periods/sampling times (P<0.05).

INVESTIGATING THE EFFECTS OF AN ORGANIC ENVIRONMENTALLY SUSTAINABLE AND COST-EFFECTIVE INNOVATIVE FEED ON GILTHEAD SEABREAM (*Sparus aurata*) GROWTH, HEALTH AND WELFARE THROUGH A MULTIPARAMETRIC APPROACH

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Organic aquaculture is gaining significance worldwide due to the increasing demand for sustainable aquatic products but its development encounters various challenges, such as technical difficulties, limited profitability, and bureaucratic complexities. A critical aspect revolves around fish nutrition, particularly addressing consumer and environmental concerns. Although extensive research exists on sustainable fish feeds in conventional aquaculture, there is a notable gap in research specific to organic aquaculture. The development of innovative feeds for organic aquaculture faces challenges and costs related to the search for novel ingredients, in adequation with organic regulations. This study assessed the potential of an environmentally-friendly and cost-effective organic feed, with 51% fish meal substitution using a blend of animal and plant protein sources (i.e., fermented soy, pea protein, yeast, krill and squid meals) for Gilthead seabream (*Sparus aurata*), a key species in European aquaculture. The development of new aquafeeds must ensure the fulfillment of developmental, physiological, and behavioral needs of animals, with a focus on achieving sustainable production performance. In a 134-day experiment, a multi-function and multi-parameter approach comprehensively assessed the effects of the novel innovative organic feed on the growth, health, and welfare of Gilthead seabream. Innovative technological tools, specifically accelerometer tags, were also utilized for continuous monitoring of energy expenditure.

Results indicated that the innovative diet had no adverse effects on the growth performance and survival of Gilthead seabream. Hematological and biochemical blood indicators showed minimal alterations, suggesting overall that welfare was not compromised. Immunological parameters suggested potential enhancement in immune defenses in fish fed the innovative diet. Finally, swimming activity, recorded by tags, showed some slight diet-related differences in acceleration values, indicating potential variations in energy metabolism. Fish fed the innovative diet showed a lower utilization of the anaerobic metabolism. A complementary multiparametric approach was applied and provided a comprehensive analysis of the global diet effects on fish health and welfare. This approach revealed a differential impact of the two diets on fish health and welfare (Fig. 1). A multicriteria decision analysis showed that the best health/welfare condition was achieved for fish fed the innovative diet.

Overall, the innovative diet shows promising results for Gilthead seabream organic aquaculture, but further investigations are essential, for instance to understand the underlying causes of observed changes in immune parameters.

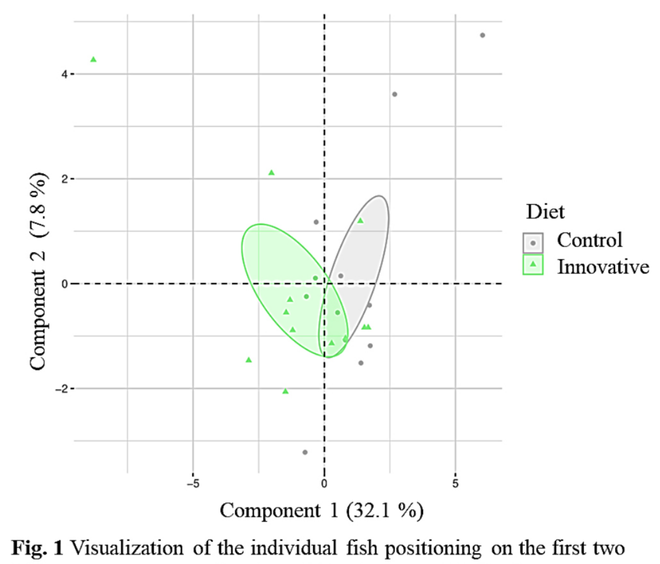

Fig. 1 Visualization of the individual fish positioning on the first two PCA components as a function of diet (commercial diet in grey, innovative diet in green) based on growth, health and welfare traits; confidence ellipses were drawn around diet groups with a confidence level of 0.95.

SALMON AQUACULTURE IN CHILE: PRODUCTION GROWTH AND SOCIO-ECONOMIC IMPACT

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Global aquaculture has presented significant growth rates over the last decades and has contributed to the food security agenda and SDG's objectives. However, has been considered a controversial activity raising concerns about environmental impacts, sociocultural and economic issues, and health. Chilean salmon aquaculture has shown impressive development in production growth, but controversies are not exempt in this industry. Despite having created employment at the local level, it is not particularly welcome in the localities where it operates. There is a widespread opinion that local communities have remained relatively excluded from the economic benefits associated with the industry. This may condition the development of an important industrial activity such as salmon aquaculture. In this paper, we discuss the socio-economic impacts that the salmon industry delivered in southern Chile. The focus is primarily on variables such as employment, salaries, poverty, unionism, income distribution, and migration. Although the socioeconomic effects that the industry initially triggered are widely criticized in the literature, the performance of recent decades indicates that these conditions have improved steadily over time, specifically significantly contributing to the country's economic growth and development, particularly in rural areas. The industry has created well-paying jobs, increased female labor participation, mitigated poverty rates, reduced income inequality, and provided an alternative for people with limited employment opportunities.

POSTPRANDIAL KINETICS OF DIGESTIVE FUNCTION IN RAINBOW TROUT: GENES EXPRESSION, ENZYMATIC ACTIVITY AND BLOOD BIOCHEMISTRY AS A PRACTICAL TOOL FOR NUTRITIONAL STUDIES

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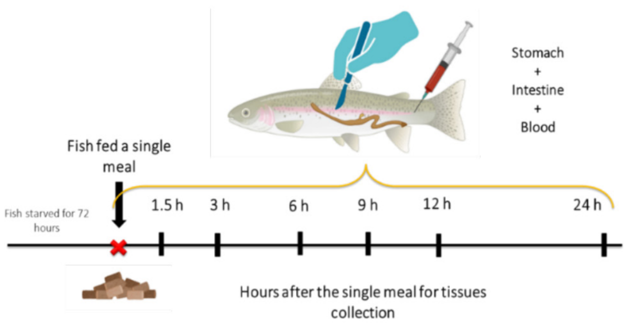
Postprandial kinetics of gastric (chitinase, pepsinogen) and intestinal (alkaline phosphatase, maltase) digestive enzymes and nutrient transporters (peptide transporter 1, sodium-glucose transporter 1) gene expression, brush border membrane (BBM) enzymes activity (alkaline phosphatase, leucine aminopeptidase, maltase, saccharase) and blood biochemistry (triglycerides, cholesterol, protein, albumin, glucose, amino acids) through NMR spectroscopy, were investigated in rainbow trout (*Oncorhynchus mykiss*) fed a commercial aquafeed. For this purpose, fish were starved 72 hours and digestive tracts, and blood were sampled before the meal and at 1.5, 3, 6, 9, 12, and 24 hours post prandial (T0, T1.5, T3, T6, T9, T12 and T24).

The expression of most genes involved in nutrient absorption and digestion was the first process to be activated around 3 hours after feed ingestion, while the expression of pepsinogen in the stomach and maltase in distal intestine peaked at T9 and T12, respectively. Once expressed, BBM enzymes were active in digesting nutrients into monomers, from 3 to 6 hours after meal. Finally, the plasma proteins level increased from T1.5 until T9, while the other blood parameters did not exhibit significant variation during the postprandial period. Although the postprandial kinetics showed that the expression of the genes involved in digestion and nutrient transport, the BBM enzymes activity, and the presence of metabolites in blood were stimulated in different ways by the presence of feed in the digestive tract, data resulted in a sequential path.

This study supplies useful information on the physiological metabolic pattern after a single meal and represent a useful tool for planning nutritional studies involving the digestive and absorptive functions in a model species like rainbow trout.



LOW LEVEL OF ARACHIDONIC ACID IMPROVES REPRODUCTIVE PERFORMANCES AND EGG QUALITY IN RAINBOW TROUT

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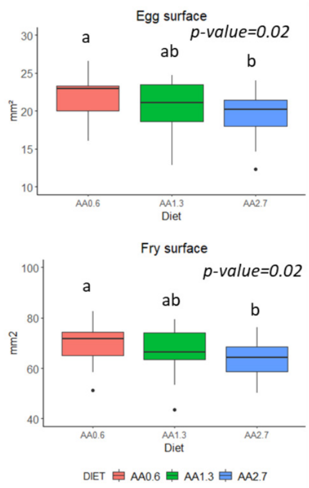
Arachidonic acid (C20:4n-6, AA) is a key component of fish physiology, affecting growth, survival and reproduction. However, imbalances in dietary AA can adversely affect fish health and performance. Despite its importance, the optimal AA requirement for rainbow trout remains unclear. The aim of this study was to investigate the effects of varying dietary AA levels on reproductive performance and egg quality in female rainbow trout broodstock.



Female rainbow trout broodstock were fed diets containing different levels of AA (0.6%, 1.3% or 2.7% of total fatty acids) over a ten-month period. Throughout the spawning season, their spawning performance was closely monitored and the quality of both unfertilised and fertilised eggs was evaluated and their fatty acid composition assessed.

Our results indicate that dietary AA levels have a significant effect on the reproductive performances of rainbow trout. Female broodstock fed the low-AA diet (0.6% AA) had a significant higher body weight than those fed the high-AA diet (2.7% AA). Moreover, the low-AA diet positively influenced reproductive performance, resulting in larger eggs and improved post-resorption fry surface compared to the high-AA diet. In addition, elevated dietary AA levels was associated with increased production of AA-derived pro-inflammatory oxylipins, which may contribute to reduced egg and fry quality.

In conclusion, this study highlights the importance of providing adequate dietary AA levels to female rainbow trout broodstock. Indeed, excessive AA intake has been shown to be detrimental to the quality of eggs and fry, underlining the need for further research to determine optimal AA requirements for farmed fish.

Acknowledgements: This work was carried out as part of a project funded by the Nouvelle-Aquitaine Region (French local authorities) and AquaExcel-TNA 3.0 project (2024– PID21817).

THE EFFECTS OF TOTAL SUSPENDED SOLIDS AND PARTICLE CONTROL TECHNOLOGIES IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

Kurt Carlsen & Henrik Mortensen

founders of CM Aqua Technologies

Introduction
Recirculating aquaculture systems (RAS) are closed-loop systems that reuse most of the water for fish production, with minimal exchange of new water. RAS have many advantages over traditional open systems, such as water conservation, land use efficiency, biosecurity, waste management, and environmental control. However, RAS also face many challenges, such as the accumulation of waste products, the maintenance of water quality, the prevention of disease outbreaks, and the high capital and operational costs.

This presentation address one of the key factors that affect the performance and sustainability of RAS - The level of total suspended solids (TSS) in the water. TSS are the particles that are not dissolved in the water, and they can originate from various sources, such as fish feed, feces, biofilm, algae, and external inputs. TSS can have detrimental effects on fish welfare, the biochemical processes in biofilters, and growth rate of fish, the increased turbidity will also affect light regimes for maturation control.

Therefore, the removal of TSS is essential for the optimal operation of RAS.

The impact of particles for RAS
Particles represent the opportunity to remove the majority of the organic load in the water in RAS. Control of your particles will make the RAS stable and cost-effective.

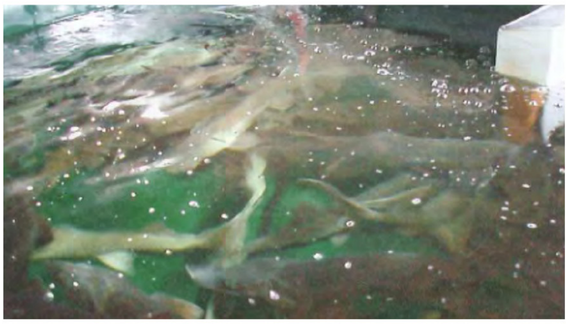
The table fig. 1 show us the potential of effective particle removal in RAS as the leading factor for the water quality, having over 80% effect on the total P removal and up to 80% on the BOD_5 removal. This is the key for balance reliable RAS.

Photo 1 and 2 is from the same sturgeon farm on the same day, but photo 1is form a RAS loop with mechanical filtration for fast removal of the particle, photo 2 has sedimentation filtration for removal of the particles.

Fig. 1 Alexander Brinker and Roland Rosch.

Parameter	Particle bound %	Max potential Removal efficiency, %
Tot-P	Up to 90	84
Tot-N	Up to 32	32
BOD_5	Up to 90	80
TSS	100	91

Photo 1 and 2 by Kurt Carlsen, CM Aqua.



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The approach for Particle Control
Drum filters, are the most common and cost-effective tool for particle removal in RAS, they can remove up to 90% of the particle-bound waste. The patented CM Aqua Beecell micro screen panel can lift and remove the particles gently without leaching back into the water.

Other technologies for fine particle control include intelligent control of drum filters(**IC)**, protein skimmers, and submerged contact filters.

The factors that influence the particle control efficiency:
- Tank design
- Water velocity
- The piping and restrictions
- Fish feed
- Level weirs
- Filter method and opening size
- Turbulence and sedimentation of particles before filtration should be avoided, as they will cause disintegration and erosion of the particles.

The size and shape of particles in RAS is the key for the cost-effective removal, allowing us to create the water for best fish welfare.

Discussion
The growth of RAS worldwide show a lot of innovation and seeking for best cost-effective solutions. Especially the salmonids farmed in RAS are in focus, as they have a high value and are well accepted by the consumers.
Does the design optimize for particle control sufficiently, considering the extreme impact is has?
Is common knowledge on particle control forgotten in the fast growth of the industry?

Reference:
Fig.1: Factors determining the size of suspended solids in a flow-through fish farm Alexander Brinker a,b,c, *, Roland Rosch a Fisheries Research Station of Baden-Wurttemberg, Untere Seestrasse 81, 88085 Langenargen, Germany b Limnological Institute, University of Constance, 78457 Konstanz, Germany c Institute for Lake Research of the Environmental Protection Agency Baden-Wurttemberg, Argenweg 50/1, 88085 Langenargen, Germany Received 14 May 2004; accepted 28 October 2004.

HIGHLY EFFICIENT PRODUCTION OF MARBLED CRAYFISH IN A NOVEL CLOSED AQUACULTURE SYSTEM

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The marbled crayfish (*Procambarus virginalis*) is a unique freshwater crayfish species known for its reproduction through apomictic parthenogenesis, resulting in a monoclonal, all-female population. Widely distributed via the aquarium trade, these crayfish have established stable wild populations through human-induced releases, particularly in Madagascar, where they serve as a popular source of nutritional protein. In our previous work, we showed that that marbled crayfish meat can be readily integrated into Western diets as a healthy and nutritious protein component. Additionally, our analysis of marbled crayfish shells revealed high levels of chitin, a valuable raw material for bioplastics, which can be extracted through our proprietary biorefinery process.

With freshwater crayfish aquaculture in open systems posing substantial ecological challenges, there is a pressing need for alternatives. We have now developed a radically streamlined closed-system aquaculture setup that is specifically tailored to the robustness and resilience of marbled crayfish. It combines low water columns with biofloc culture to substantially reduce the requirements for water, energy, and traditional aquaculture feeds, while also minimizing the risk of scape into natural waterways. Our findings show that marbled crayfish can reach harvest size within 3 months in closed systems, making them a promising candidate for environmentally responsible aquaculture, even in regions with limited resources.

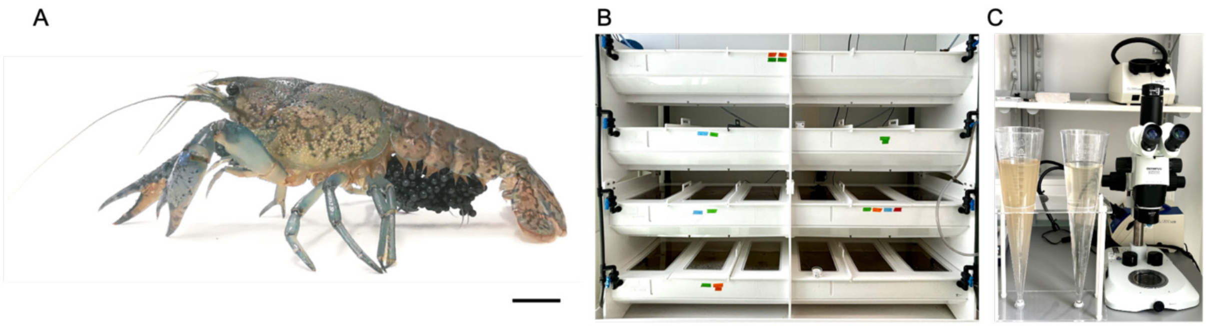

Figure 1: (A) Marbled crayfish (*Procambarus virginalis*, Lyko 2017) carrying approximately 300 eggs. Scale: 1cm. (B)Vertical closed-system aquaculture for crayfish farming. (C) Imhoff cones for monitoring biofloc growth and stability suitable for marbled crayfish healthy growth.

HOW DO ADOLESCENTS FROM RURAL SCHOOLS PERCEIVE AQUAPONICS SYSTEM? CASE STUDY IN THE LARGEST SETTLEMENT IN BRAZIL

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The Itamarati Rural Development Centre has introduced aquaponics systems in schools in the Itamarati settlement, Central-West of Brazil, to promote social technologies to the vulnerable. This study was conducted to understand issues that encourage or discourage rural youth from practicing aquaponics. A questionnaire was designed to rank constraints and opportunities, based on 9 categorical variables, and 36 statements, equally divided into 12 themes (V) (Table 1). Each V was baptised a priori according to the content of statements. The scale (0=disagree; 1=slightly agree; 2=agree; 3=strongly agree) allowed a sum for each V (maximum score per V=9). Interviews were carried out in 2023 with 87 adolescents (aged ~15 to 17), fairly distributed in 3 public schools. A significant part or most of their family income (40%; 38%) came from agricultural activities. 53% were enthusiastic about agricultural activities, 37% were unsure. 39% were uncertain about their desire to work with agriculture/livestock as a profession, 31% did not want to and 30% wanted to. 55% had a fair idea about the functioning and usefulness of aquaponics, 41% had little idea and 4% were sure. 68% visited the system in their school at least ten times. 89% did not have an aquaponics system at home. 62% of respondents were female. V1 and V9 presented the lowest scores. V6 was ranked first (the highest concern), likely due to the small size (500 L), number (~10) of Nile tilapia and vegetables, and because short supply chains are incipient. V4 ranked second, associated with the male/female categorical variable ($P<0.05$), suggesting that female youth perceived a greater need for technical assistance or aversion to risk. Overall, Mood´s median test and discriminant analyses revealed week associations of categorical variables with V, and scores of V1 and V9 were highly convergent among adolescents. Several findings were supported by the literature. Cost, income, advice, aspects for acquisition, maintenance, infrastructure and time available deserve attention in educational initiatives aimed at rural youth and aquaponics.

Table 1 – Constraints perceived for aquaponics.

	Sum*	Mean	Rank
V1- Environmental impact	30.0	0.3	12
V9- Gender	82.0	0.9	11
V11- Sustainability (inputs and innovation)	92.0	1.0	10
V2- Social aspect (people & community)	102.0	1.1	9
V8- Food security	111.0	1.2	8
V10- Age group	120.0	1.3	7
V12- Educational potential	137.0	1.5	6
V7- Economy and market	138.0	1.6	5
V5- Family's rural property	237.0	2.7	4
V3- Technology (inputs + maintenance)	259.0	2.9	3
V4- Rural advisory services	268.0	3.1	2
V6- Household economy and finances	271.0	3.1	1

CAN NATURAL ANTIOXIDANTS SUPPLEMENTED IN THE DIET MITIGATE CHRONIC STRESS IN THE EUROPEAN SEABASS (*Dicentrarchus labrax*)?

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The high stocking densities are normally used to reduce production costs in the aquaculture industry. However, if it is not appropriate for the reared species, it can become a stressor that often triggers chronic responses and negatively affects fish growth, feed intake, or immune functions. Thus, finding methods to reduce stress and improve fish immune and antioxidant status is essential to increase stocking densities. The use of nutraceutics is presented as a solution to improve fish welfare in aquaculture activity. This study assesses the effectiveness of a phytogenic additive with antioxidant properties (AquaNeox) in the diet of the European seabass (*Dicentrarchus labrax*) juveniles at different stocking conditions.

Seabass juveniles (n = 480; w_i = 105 ± 30 g) were placed into 200 L-tanks and distributed in four different experimental groups in triplicate, defined as the combination of feeding (with or without AquaNeox supplementation) and stocking densities. Initial low density (LD) was defined as 5 kg/m^3 and high density (HD) as 37 kg/m^3. Fish were maintained for two months under these rearing conditions. Biometric samplings were performed periodically to determine growth parameters and adjust feeding. At the end of experimental time target tissues were sampled to assess physiological status.

The use of the additive did not negatively affect fish growth. The histomorphometrical parameters determined in liver showed that the use of the additive significantly reduced the hepatocytes area. However, the additive tended to increase triglycerides and cholesterol content in liver. Additionally, the additive helped to maintain the hepatosomatic index in fish kept in HD conditions. Furthermore, other plasma parameters assessed related to the stress response remained constant in animals fed with AquaNeox and reared in HD conditions, showing no significant differences with the LD group. In conclusion, the use of AquaNeox seems to generally reduce the negative effects derived from a high stocking density production, especially in those metabolic responses related with lipid metabolism.

Table 1. Plasma and liver parameters related to lipid metabolism. Different lowercase letters represent significant differences among LD conditions. Different capital letters represent significant differences among HD conditions ($p < 0.05$).

	LD CTRL	LD AquaNeox	HD CTRL	HD AquaNeox
Plasma				
TAG (mg/dL)	874.5 ± 138.6	663.0 ± 102.2	590.4 ± 60.0	695.7 ± 86.1
Cholesterol (mg/dL)	213.0 ± 23.4	243.3 ± 19.3	197.9 ± 9.6	215.8 ± 13.2
Liver				
TAG (mg/g fw)	50.3 ± 2.2	57.8 ± 2.6	50.0 ± 2.8	58.3 ± 2.8
Cholesterol (mg/g fw)	38.2 ± 2.3	39.8 ± 2.0	34.5 ± 1.3	36.1 ± 1.4
Hepatocytes area (µm^2)	144.7 ± 4.2 a	121.1 ± 4.8 b	134.9 ± 3.5 A	120.3 ± 3.1 B

A PHYTOGENIC ADDITIVE TO AMELIORATE THE HIGH STOCKING DENSITY STRESS RESPONSE IN THE EUROPEAN SEABASS (*Dicentrarchus labrax*)

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The improvement of the aquaculture industry goes necessarily by the increase of fish welfare. This can be threatened due to high stocking conditions which trigger stress responses that can negatively affect growth, feed intake and the general health status. To reduce these negative effects, some dietary additives have been reported to ameliorate stress response induced by the stocking conditions for different aquaculture species. This study assesses the effectiveness of a phytogenic additive (TecV) in the diet of the European seabass (*Dicentrarchus labrax*) juveniles grow at different stocking conditions.

Seabass juveniles (n = 438; w_i = 74 ± 18 g) were placed into 240 L-tanks and distributed in four different experimental groups in triplicate, defined as the combination of feeding (with or without TecV supplementation) and the initial stocking density. Initial low density (LD) was defined as 4 kg/m^3 and high density (HD) as 18 kg/m^3. Fish were maintained for 70 days under these rearing conditions and afterward were sampled.

After 70 days, no significant differences on growth parameters were observed and the use of the additive did not induce any negative consequences on fish. However, HD condition induced negative consequences related to the chronic stress situation. For example, HD-fish fed with the control diet decreased intestine length and hepatosomatic index, which was in correspondence with a lower glycogen content in the liver. Additionally, a downregulation of carbohydrates metabolism because of the substrate depletion was observed in HD-fish fed with control diet. These effects were modulated by the addition of TecV in the diet, especially in those parameters related with lipid metabolism, as it is shown by the multivariable analysis performed (Fig. 1). Even both HD groups are overlapped, there seems to be a differentiation between HD control group and animals maintained at LD, whereas this differentiation is not existent in the HD TecV group. In conclusion, HD increased lipid catabolism, but this effect was attenuated by supplementation with the TecV additive.

Acknowledgments: Project PISCIBIEN. Next Generation-EU. Spanish Ministry of Agriculture, Fisheries and Food (*Plan de Recuperación, Transformación y Resiliencia*, RD685/2021). Thanks are also given to TECNOVIT-FARMFAES to provide the prototype.

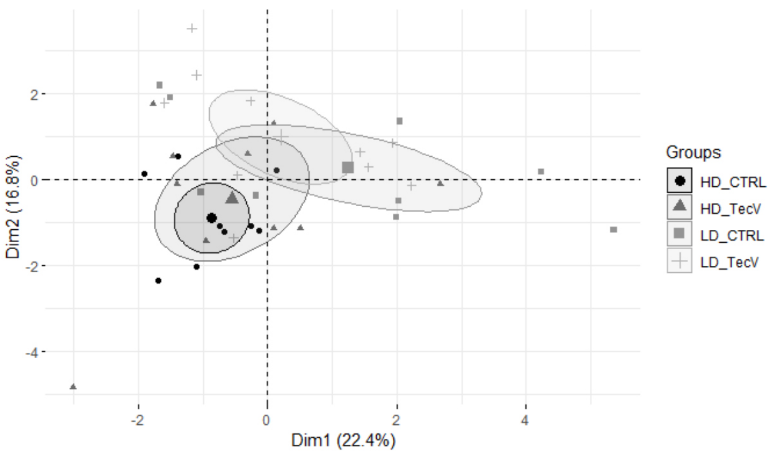

Figure 1. Results of the Principal Components Analysis in variables related to the lipid metabolism.

THE BLUE ECONOMY COOPERATIVE RESEARCH CENTRE PATH TO SUSTAINABLE INTEGRATED SYSTEMS FOR OFFSHORE AQUACULTURE: R&D BUILDING BLOCKS

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Two expanding and critical parts of the blue economy are the use of marine based renewable energy sources and seafood production from marine aquaculture. Established in July 2019, Australia's Blue Economy Cooperative Research Centre (Blue Economy CRC) is aimed at unlocking the potential of the nation's ocean resources through sustainable development. The Blue Economy CRC brings together 44 partners from 10 countries to generate opportunity by using established and new practices to move renewable energy and seafood production offshore safely, economically and sustainably. To facilitate this, the Blue Economy CRC contributes to building effective pathways for offshore development by investing in international and regionally relevant R&D. This activity explores potential synergistic benefits that include shared resources, efficient use of ocean space, less competition amongst other user groups of marine space, reduced operational and maintenance costs from possible shared activities. Key impacts are to increase sustainability and build community trust in blue economy industries. The aim of this presentation is to overview the Blue Economy CRC approach, including R&D building blocks, to realising the opportunities for offshore co-location and/or integration of both renewable energy and aquaculture production systems. Examples will be drawn from our activity in Australia and New Zealand: from salmon aquaculture and from our approach to developing multispecies seafood systems which emphasises meeting seafood market needs.

BOTANICAL BLEND INCLUSION IN FEED STIMULATES *Salmo salar* CELLULAR MEDIATED IMMUNITY IN ex *vivo* STUDY

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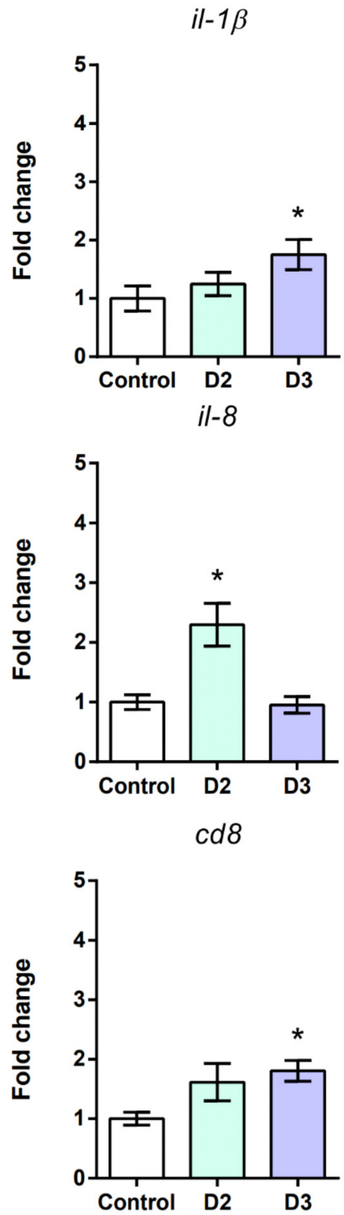
Aquaculture emerges as the fastest-growing sector in animal production, yet it grapples with significant challenges, particularly disease outbreaks which pose formidable obstacles for numerous aquatic species. A considerable amount of attention is thus directed towards substances exhibiting antibacterial properties, essential for bolstering disease resistance and promoting sustainable aquaculture practices. In this study, the impact of incorporating a thymol-based Botanical Blend Formula (BBF), previously validated *in vitro* against *Piscirickettsia salmonis*, on the modulation of genes governing cellular-mediated immunity in Atlantic salmon (*Salmo salar*) is evaluated.



One hundred and eighty-nine Atlantic salmon specimens, with a mean weight of 14g, were randomly assigned to 9 tanks (21 fish per tank) at Universidad Católica de Temuco in Chile. Three distinct diets were administered in triplicate: i) Control, ii) D2 (BBF 1000ppm), and iii) D3 (BBF 2000ppm). Over a period of 43 days, the fish were fed until they doubled in size. Following a 48-hour fasting period, fish selected for tissue sampling were randomly chosen and euthanized using a lethal dose of anesthetic (BZ-20). Six individuals per tank were utilized to obtain anterior kidney samples, which were subsequently dispatched to Universidad Austral de Chile. The transcript levels of il-1β, il-8, and cd8 were then analyzed using RT-qPCR.%.

Graphs depict the expression levels for each diet as the average ± SEM (n=18), relative to the control diet, calculated using the ΔΔCt method with the ef-1α transcript serving as a normalizer. Asterisks highlight statistically significant differences in expression levels relative to the control diet, determined through one-way ANOVA followed by Dunnett's multiple comparisons test (p-value<0.05).

In conclusion, the results indicate that D3 positively modulated markers of immunity, resulting in elevated levels of il-1β and cd8. Although D2 significantly influenced il-8 transcription, it also displayed favourable trends across all analyses. Both diets demonstrated immune-stimulating properties, particularly in enhancing cellular-mediated immunity, suggesting potential for a faster and more efficient response against pathogens.

THYMOL, VANILLIN AND ORGANIC ACIDS PROMOTE PROMOTES GUT HEALTH MODULATING MUCOSAL MICROBIOTA IN *Salmo salar*

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Aquaculture nutrition is at a cross-road: the usage of finite marine resources is a major sustainability challenge while high dietary inclusion of plant proteins has been associated to detrimental effects on growth, promoting inflammation and intestinal dysbiosis. Feed additives enhancing digestive and metabolic processes are of paramount interest and for this reason this study aims to evaluate the effect of a microencapsulated blend of thymol, vanillin and organic acids (AviPlus®Aqua) on the Atlantic salmon (*Salmo salar* L.) mucosal microbiota.

Three hundred Atlantic salmon parrs (average weight of 5.4 g) were distributed into 6 tanks at SPAROS Lda (Portugal). Two diets were manufactured and one of them was enriched with the test additive. Then they were tested in triplicate as Control and D1500. Fish were fed for 13 weeks and then 3 fish per tank were sampled (n=9 per diet) and DNA extracted from the mucosa using QIAmp® Fast DNA Stool Mini Kit. The DNA was sent to an external laboratory for generation of the raw sequence data through Next-generation Sequencing of DNA molecules. All means are described in table 1 and comparison was performed using Student's-Newman-Keuls test (p value <0.05) in IBM SPSS Statistics V18 software.

Results strongly highlight that, in the intestinal mucosa at the genus level, D1500 influenced marked changes on the microbiome profile. Fish fed the treated diet showed the appearance of several genera (*Nostocaceae, Acutedesmus, Leuconostoc, Bradyrhizobiaceae, Gammaproteobacteria)* affecting biodiversity of the bacterial population. It is particularly interesting the appearance of *Leuconostoc*, since several species of this genera have been associated to beneficial effects towards intestinal dysbiosis. Moreover, the intestinal mucosa of fish fed treated diet showed a significantly lower relative abundance of *Sphingomonas*, *Enterobacteriaceae* and *Fusobacteriaceae*. An increased relative abundance of both genera *Sphingomonas* and *Enterobacteriaceae* have been associated to intestinal inflammatory events.

In conclusion this study proved, according with literature on functional additives, that the addition of the botanical based additive (AviPlus® Aqua) at 1.5 kg/MT (D1500) modulated the adherent microbiota in the mucosa of distal intestine, with several of these changes being associated to a preventive role towards dysbiosis.

Table 1 - Genus microbiome profile and statistical analysis.

Genus	CTRL		D1500		P- value
Acutodesmus	0.00 ± 0.00	a	0.46 ± 0.19	b	0.001
Bacillaceae	0.53 ± 0.05	b	0.00 ± 0.00	a	<0.001
Leuconostoc	0.00 ± 0.00	a	0.15 ± 0.06	b	0.001
Bradyrhizobiaceae	0.00 ± 0.00	a	0.26 ± 0.16	b	0.005
Sphingomonas	2.97 ± 0.37	b	1.78 ± 0.37	a	0.019
Betaproteobacteria	0.40 ± 0.18	b	0.00 ± 0.00	a	0.002
Gammaproteobacteria	0.00 ± 0.00	a	0.34 ± 0.12	b	0.001
Aeromonas	0.55 ± 0.20	b	0.00 ± 0.00	a	0.001
Enterobacteriaceae	2.03 ± 0.81	b	0.53 ± 0.13	a	0.015
Brevinemataceae	44.52 ± 5.38	a	57.24 ± 4.21	b	0.032
Fusobacteriaceae	0.75 ± 0.21	b	0.10 ± 0.02	a	0.002

TRANSCRIPTOMIC ANALYSIS OF SKIN RESPONSE TO *Tenacibaculum maritimum* INFECTION IN EUROPEAN SEABASS FED DIETARY METHIONINE SUPPLEMENTATION

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The essential amino acid methionine plays pivotal roles in several physiological processes, from growth to immune function. While dietary methionine supplementation has been shown to improve cell immunity and disease resistance in fish, its impact on mucosal immune processes remains poorly explored. Therefore, the present study aims to characterize the effects of graded levels of methionine surplus on the skin tissue transcriptomes of European seabass (*Dicentrarchus labrax*) upon infection with *Tenacibaculum maritimum*. European seabass juveniles were divided into three groups and fed diets containing DL-methionine at 0.9 %, 1.9 % or 2.9 % of feed (CTRL, MET2 and MET3, respectively). After four weeks of feeding on experimental diets, skin tissue samples were collected. The remaining fish were bath challenged with *T. maritimum* and skin tissue was sampled at 24 and 48 h post-infection (hpi). Mortality was recorded daily for seven days. RNA was extracted and RNA-seq libraries were prepared by Novogene and sequenced using Illumina PE150. Differential gene expression analyses between the different infected dietary groups and the CTRL group before infection were determined with DESeq2 (p-value < 0.01, absolute Log2 Fold Change ≥ 2). Gene Ontology (GO) enrichment analysis was performed in g:Profiler. The exposure to *T. maritimum* induced a local response in the skin of all dietary groups. However, the CTRL group exhibited a stronger pro-inflammatory response at 24 hpi, as evidenced by the higher number of differentially expressed genes and immune-related enriched GO terms. The initially attenuated immune response in the MET2 group was replaced by a more intense one compared to the CTRL fish at 48 hpi. Conversely, the MET3 group appeared to prioritize homeostatic and iron-related pathways over immunological pathways. Furthermore, at 48 hpi, the downregulation of transcripts associated with the immune response suggested an impaired response to the infection in this dietary group. Although statistically non-significant, cumulative mortality was higher in the MET3 group (95%) compared to the other dietary groups (85%). Despite the benefits of consuming suitable levels of methionine, high intake levels above its physiological requirements could switch positive effects into deleterious ones. The delayed pro-inflammatory response of MET2 and the downregulation of immune-related transcripts in the MET3 group, coupled with higher mortality, underscore the need for further investigation into potential adverse effects associated with elevated methionine intake.

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ASSESSING CAPTURE AND TRANSPORT STRESS IN COMMON OCTOPUS USING A NON-INVASIVE METHOD

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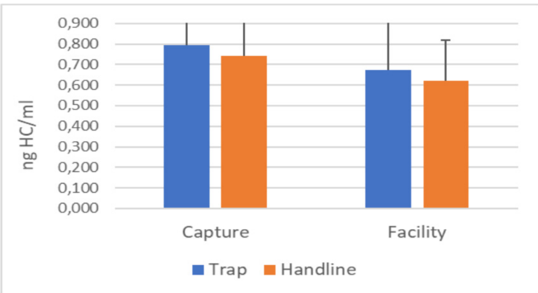
Octopus vulgaris (Cuvier, 1797) is a highly valued species worldwide and its demand has increased significantly in recent years (Iglesias et al., 2014). Specimens are caught in industrial and artisanal fisheries and have been overexploited in recent decades (Sauer et al., 2021). All steps involved in the capture of octopus should be able to avoid any form of PSDLH and should best reduce the stress associated with the severity of the capture technique (Pieroni et al., 2022). Similarly, the transport system (type of tank, isolation, number of individuals, water quality, temperature), timing and inter-species variability (e.g. body size) must be considered when preparing the specimen for the journey. Hence, continuous monitoring of hormones that respond to a stressor throughout can assist with interpretation of octopus behaviour observations. Developing techniques to measure hormones and repeat them on the same individual in a non-invasive way is essential to protect the welfare of the octopus (Chancellor et al., 2021). The aim of this study was to develop a non-invasive method to collect and quantify cortisol levels from octopuses caught using two different fishing techniques (trap and handline) and transported to a facility.

Wild sub-adults of *O. vulgaris* were caught using a traditional handline ("polpara") capture system (n.7: 735.2±35.7g) and traps (n.7: 855.4±84.1g) in the Ionian Sea. Each animal was placed in a PVC cylinder, which was netted to avoid aggression, and transported in an insulated tank (300 L) to the laboratory in Cesenatico. Animals were swabbed on capture and on arrival at the facility (after 10 h). The swab (Puritan Sterile Polyester Tipped Applicators) was applied to the dorsal part of the mantle and cut to fit into in a 2 mL tube containing 1 mL of 70% ethanol. Tubes with swabs were then placed in a -20°C freezer for storage.

Skin swabs have been used previously in fish (Santymire et al., 2022; De Mercado et al., 2018; Schultz et al., 2005) and amphibians (Santymire et al., 2018). The results show that cortisol levels at the time of capture were not statistically different in trapped compared to handline captured subjects (0.793±0.204 vs. 0.675±0.249). After transport and arrival at the facility, cortisol levels decreased for both capture techniques. This shows that the mode of transport did not affect the stress level of the octopus, but contributed to a reduction in cortisol level. The values obtained in this study are in line with those found by Chancellor et al. (2021) on other cephalopod species. In the future, more days of monitoring will be needed after the move to a new habitat in order to have evidence of full acclimatisation of the octopuses.



ASC SUSTAINABLE AQUACULTURE: LEADING HEALTH AND WELFARE CERTIFICATION IN THE GLOBAL MARKET

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Aquaculture represents a significant component of the international seafood trade, contributing substantial value. Over the years, the industry has experienced substantial growth and is projected to continue expanding in the future. The sustainability and longevity of aquaculture operations depend on the adoption of responsible and sustainable farming practices that prioritize the health and welfare of aquatic species throughout their lifecycle.

In this context, the Aquaculture Stewardship Council (ASC) plays a crucial role, offering a globally recognized certification and labelling program for responsibly farmed seafood. ASC's certification program empowers farmers by enabling them to differentiate their products, expand market access, and build consumer trust.

ASC is putting fish health and welfare at the forefront with a newly created principle dedicated to animal health and fish welfare. The principle will include new criteria on health and welfare related to the daily operations at the farm, handling and slaughter requirements.

The presentation will focus on discussing the health and welfare principle as a means to elevate aquaculture practices to a higher standard, emphasizing the production of seafood with a primary focus on animal health and welfare. Specifically, this principle is expected to lead to decreased levels of animal stress and improved welfare conditions, ultimately resulting in enhanced product quality, increased survival rates, and reduced expenses related to disease prevention.

Moreover, the inclusion of this principle adds value to products bearing the ASC label, making them more appealing to prominent seafood markets and conscientious consumers who prioritize certified and ethically produced goods.

BIOMETRIC, BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF THE PACIFIC PYGMY OCTOPUS *Paroctopus digueti* UNDER CONTROLLED CONDITIONS

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Paroctopus digueti is the smallest octopus (15 cm) that inhabits benthic-muddy environments within the Gulf of California. Its life cycle is short (6 months), with direct development, produces up to 300 6-8 mm long eggs sheltered by the female until hatching. These characteristics make *P. digueti* an ideal candidate for culture as a research model.

The present study establishes baseline values of metabolism, biometric and biochemical parameters of *P. digueti*, which allow determining the optimal conditions for development and growth in a short time.

Standard metabolic rate was determined through a closed respirometry system under normal conditions of temperature (26°C) and salinity (38 ups) in three size ranges (15-24 g, 25-33 g, > 34 g). Biometric measurements were taken at the beginning and end of the trial, tissue samples were taken for biochemical analysis (-80°C). Growth was evaluated for a period of 38 days.

The overall mean value of the standard metabolic rate (SMR) was 0.068+0.04 mg O2 g-1h-1 indicative of efficient metabolism, and this showed an inverse relationship with octopus' size (Fig. 1).

All morphophysiological variables indicated a good physiological state (Table 1) and can be considered a baseline of healthy octopuses.

Total protein is the major tissue component (80%), followed by lipids (14%) and carbohydrates (6%).

Information on physiological behavior is still incipient for this species but this work is a good approach.

Acknowledgments: CONAHCYT FORDECYT-PRONACES/3266032 fund. National Commission for Aquaculture and Fisheries (PPF/ DEGOPA-013/23).

TABLE 1. Biometric data, growth rates, and condition indexes.

Variable	Mean ± Std dev
Initial weight, g	31.70 ± 13.85
Final weight, g	37.40 ± 16.27
Total length, mm	162.67 ± 31.29
Mantle length, mm	47.33 ± 14.09
Relative growth rate, g d^{-1}	0.15 ± 0.12
Specific growth rate, %W d^{-1}	0.45 ± 0.30
Fulton, $K_{TW\text{-}TL}$	0.00087 ± 0.00026
Fulton, $K_{TW\text{-}ML}$	0.03953 ± 0.01620

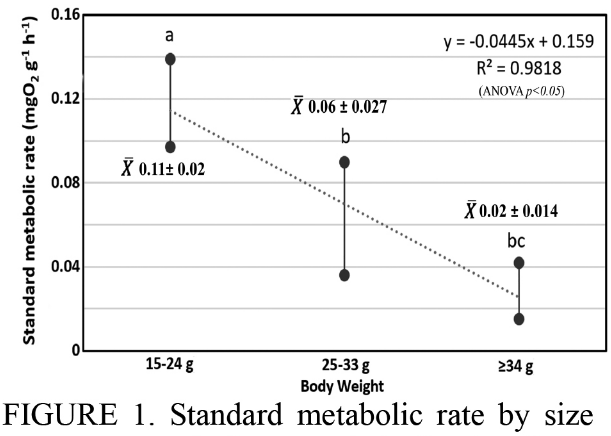

FIGURE 1. Standard metabolic rate by size group. Letters indicate statistically significant differences between groups.

EXPERIENCES ON CAPTIVE BREEDING OF THE PACIFIC PYGMY OCTOPUS P*aroctopus digueti*

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Paroctopus digueti (Perrier & Rochebrune, 1894) is a good candidate to keep in the laboratory to develop the culture as a research model, with a relative fast growth, direct development, a short life cycle and it is feasible adapt it to live in captivity (DeRusha et al., 1987, Jereb et al., 2016, García-Flores, 2017). However, it is important to develop the appropriate conditions for their breeding, considering their habitat and behavior. The present study evaluated different culture conditions with open or recirculating water flow; individual or in group maintenance of juvenile, and using different types of food, that included live and inert diets.

Adult females with "clutches" inside empty shells of *Anadara multicostata* were collected at Ensenada de La Paz, B.C.S., México (24°06'N-24°48'N-110°44'W). They were placed in a 140 L tank, placing each female inside a 500 μm mesh container during the incubation period. After hatching, juveniles were kept as a group for 30 days in tanks with shelters made of bivalve shells, in an open water flow system, at 28±1°C and fed with live Artemia or Mysidaceans or both (Fig. 1).

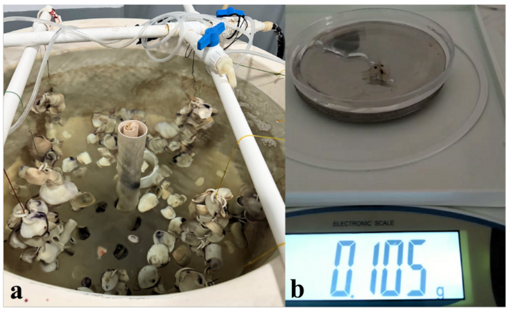

FIGURE 1. a) In group cultivation system, b) Juvenile being weighed.

Thirty-day-old juveniles (0.33 g ± 0.15 SD) were kept in ponds with open and constant flow of seawater, with shells as shelter, inert fresh food (crab, shrimp, and clam), and daily cleaning.

Two treatments were implemented, one with juveniles in groups and the other keeping them individually in 500μm mesh jars. Growth was evaluated in terms of weight gain at the end of experiment.

TABLE 1. Weight gain and survival of juvenile *Paroctopus digueti* by treatment.

Treatment	Weight gain		Survival
	%	per day (g)	%
In group	86.50	0.04	11.54
Individual	58.75	0.01	80

As can be seen in Table 1, when octopuses were kept in groups, they showed higher weight gain. However, survival was much higher when kept individually.

Mortality in the group treatment was mainly due to cannibalism, which may explain the higher weight gain. By keeping them individually, cannibalism was avoided; however, in this case it was difficult to maintain good water quality inside the containers where bacteria and protozoos proliferated.

We concluded the necessity to improve the water quality inside the individual containers and supplementing more shelters and food in the group treatment to obtain better results.

Acknowledgments: CONAHCYT FORDECYT-PRONACES/3266032 fund. National Commission for Aquaculture and Fisheries (PPF/ DEGOPA-013/23).

DEVELOPMENT OF A CHLAMYDOMONAS-BASED VACCINE AGAINST SALMONID ALPHAVIRUS (SAV2) TO IMPROVE THE SUSTAINABILITY OF RAINBOW TROUT AQUACULTURE

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Aquaculture is the cornerstone for meeting the world's growing demand for seafood. However, the sustainable expansion of aquaculture faces persistent obstacles posed by infectious diseases. Among these, infections caused by Salmonid Alphavirus (SAV), commonly known as pancreatic disease (PD) or sleeping disease (SD), have become a major concern in salmonid fish farming. SAV profoundly affects all stages of salmonid production, leading to significant economic losses (1). In response to the threat posed by SAV infections in salmonid aquaculture, the development of new vaccination strategies has become a necessity.

Here, we describe the design, administration and evaluation of a new vaccine against SAV2 produced in the microalgae *Chlamydomonas reinhartii*. We targeted the antigenic regions of the E2 glycoprotein based on knowledge of the mammalian alphavirus analogue Chikungunya (2), as well as previous studies in SAV by Bermont (3). The sequences were meticulously designed for heterologous expression in *Chlamydomonas*, incorporating codon optimization and intron insertions to improve expression efficiency. These sequences were cloned using the Modular Cloning system and nuclear-transformed by glass beads. By western blot analysis, we identified the most efficient vaccine production colony for further amplification. Subsequently, the vaccine was formulated into fish feed and administered orally in rainbow trout for follow-up analyses related to innate and adaptative immune response. Vaccine protection efficacy was evaluated by challenging with SAV2, and analyzing mortality and viral load by qPCR.

References

1. Herath, T. K., & Thompson, K. D. (2022). Salmonid alphavirus and pancreas disease. In *Aquaculture Pathophysiology* (pp. 225-246). Academic Press.
2. Weber, C., Büchner, S. M., & Schnierle, B. S. (2015). A small antigenic determinant of the Chikungunya virus E2 protein is sufficient to induce neutralizing antibodies which are partially protective in mice. *PLoS neglected tropical diseases*, 9(4), e0003684.
3. Merour, E., Lamoureux, A., Biacchesi, S., & Bremont, M. (2016). Fine mapping of a salmonid E2 alphavirus neutralizing epitope. *Journal of General Virology*, 97(4), 893-900.

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GLOBAL WARMING AND NANOPLASTIC TOXICITY

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Plastics have low density, longevity, excellent barrier properties and relatively low cost, due to these features they are ideal materials for a wide range of manufacturing and packaging applications. According to records, a continuously increasing global annual production of plastics have around 300 million t and this total continues to grow at about 4 % per year. Due to the chemically linked by weak secondary bonds or by physical interaction of polymers, eventually, nano-sized particles from any size of the plastic would spread to the transient environment. The worrying part is that 'smaller particles are generally more toxic than the corresponding bulk material at the same mass concentration'. On the other hand, increasing nanoplastics (NPs) pollution may lead to unknown environmental risks when considered together with climate change, which has the potential to become an increasingly important environmental issue in the coming decades.

In vivo studies have shown that nano-sized plastic exposure has resulted in bioaccumulation within the body even brain, leading to oxidative DNA damage in the brain regions where it bioaccumulates, compromising immune responses, induction liver lesions and ultimately affecting behavior, physiology and metabolism [1]. Moreover, according to the results of our recent studies, these toxic effects can become more dramatic with low temperature increases [2–5].

References

1. Sökmen TÖ, Sulukan E, Türkoğlu M, Baran A, Özkaraca M, Ceyhun SB. Polystyrene nanoplastics (20 nm) are able to bioaccumulate and cause oxidative DNA damages in the brain tissue of zebrafish embryo (Danio rerio). Neurotoxicology [Internet]. 2020; Available from: https://www.mendeley.com/catalogue/a09ee920-16af-3052-a1db-511076679277/
2. Sulukan E, Baran A, Şenol O, Yildirim S, Mavi A, Ceyhun HA, et al. The synergic toxicity of temperature increases and nanopolystrene on zebrafish brain implies that global warming may worsen the current risk based on plastic debris. Science of The Total Environment. Elsevier; 2022;808:152092.
3. Sulukan E, Şenol O, Baran A, Kankaynar M, Yıldırım S, Kızıltan T, et al. Nano-sized polystyrene plastic particles affect many cancer-related biological processes even in the next generations; zebrafish modeling. Science of The Total Environment [Internet]. Elsevier; 2022 [cited 2022 Jun 13];838:156391. Available from: https://linkinghub.elsevier.com/retrieve/pii/S004896972203488X
4. Sulukan E, Baran A, Kankaynar M, Kiziltan T, Bolat İ, Yildirim S, et al. Global warming and glyphosate toxicity (II): Offspring zebrafish modelling with behavioral, morphological and immunohistochemical approaches. Science of The Total Environment [Internet]. Elsevier; 2022 [cited 2022 Sep 30];158903. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0048969722060028
5. Sulukan E, Baran A, Şenol O, Kankaynar M, Yıldırım S, Bolat İ, et al. Global warming and glyphosate toxicity (I): Adult zebrafish modelling with behavioural, immunohistochemical and metabolomic approaches. Science of The Total Environment. Elsevier; 2023;858:160086.

A PLANT-BASED SOLUTION TO REDUCE THE IMPACT OF ACUTE STRESS ON *Salmo salar* DURING HANDLING

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Most salmon farming systems involve practices that expose animals to acute handling stressors, which have negative consequences on behavior, growth, performance and survivability. Sedatives or anesthetics are commonly administered to fish to mitigate these consequences (Aydin & Barbas, 2020). Nevertheless, these products can cause mortality and damage to fish. The present experiment aimed to study the effect of a botanical antistress solution (BAS) mainly based on proprietary blend of essential oil bioactives, on the stress induced by handling and crowding subsequent stages.

500 Atlantic salmon (*Salmo salar*) with an average weight of 196.74 ± 2.88 grams were equally divided into 5 tanks at a stocking density of 30.27 kg/m^3. The fish received a standard pelleted diet and after a 14-day acclimatation period, each tank labeled from A to E was exposed to the following in-water concentration of BAS; A-0 ppm (negative control), B-20 ppm, C-40 ppm, D-60 ppm and E-20 ppm at time 0 min + 20 ppm at 2 hrs. The fish were transferred to external containers 35 min after the addition of the BAS and crowded to a density of 85Kg/m^3 to reproduce typical conditions of salmon transportation. These containers contained the same concentration of BAS as the original tanks. Fish stress responses were measured through behavioral observations, cortisol levels at 5 different times following the BAS addition (0 min, 35 min, 2 hrs., 4 hrs. and 48 hrs.) and time to reach a 30% oxygen depletion after transfer. Finally, feed resumption was measured from the transfer on each batch. Data were analyzed using ANOVA (General Linear Model) when appropriate.

Regardless of the essential oil bioactive concentration, salmon's body orientation wasn't affected through the study and was similar to control fish. However, at 4 hours, fish in treatments C and D were excited while a decrease of swimming activity was observed in treatments B and E, compared to the control one. The oxygen depletion time was significantly the longest for fish in treatment E and the lowest for control fish (17.4 *vs.* 11 min; p=0,054). One day after the transfer, treated fish from treatment E displayed a quicker resumption of feed intake compared to the control fish (2.05 g *vs.* 0.69 g). The cortisol level observed in each group was increased from 35 min ($P<0,001$), which indicates a stress response to the experimental transfer model. It became significantly lower ($P<0.05$) in treated groups 4 hours post-treatment, and once again Group E stood out displaying the lowest cortisol level at this time compared to the control fish (204 *vs.* 547 nmol/l; $P<0,01$).

These results suggest the potential of using a BAS for fish relaxation at 20-ppm concentration repeated every 2 hours.

ASSESMENT OF RESIDUAL DEPLETION PROFILE OF ERYTHROMYCIN IN PACU *Piaractus mesopotamicus*

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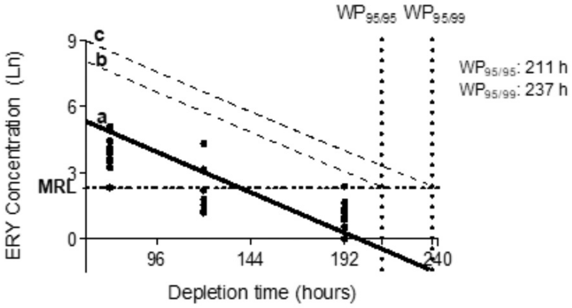
In aquaculture, Erythromycin (ERY) is used to control *Lactococcus garvieae*; *Renibacterium salmoninarum*, infections caused by *Streptococcus* spp., and to treat pre-reproductive fish diseases. Although ERY is not regulated for use in Brazilian aquaculture, this study considers its potential as an alternative to treat bacterial diseases that affect fish production. An experimental study was conducted to evaluate the residual depletion profile of ERY to estimate a minimum safe withdrawal period after oral administration in pacu *Piaractus mesopotamicus*, a native fish species of economic importance in Brazil, considering 100 µg kg^{-1} as maximum residue limit (muscle and skin in natural proportions). This study was approved by the Ethics Committee on the Use of Animals (CEUA) of the School of Agricultural and Veterinary Sciences of UNESP, Jaboticabal, SP, Brazil, where it was performed (Protocol number 016795/19), and it was assessed based on VICH GL48 (2015) and VICH GL57 (2019).

Healthy pacu fish (0.250 kg average weight) were distributed in water tanks, were they received the medicated feed containing ERY at a daily dose of 100 mg (kg BW)$^{-1}$ for seven consecutive days. The average water temperature was 30°C. Eleven fish were randomly sampled at predetermined times 1, 3, 5, 8, 11, and 15 days after the end of the treatment, euthanized, and weighed each time. The muscle and the skin in natural proportions were collected, packed in plastic bags, identified, and stored in a freezer (-20°C) until analysis through LC-MS/MS system. The linear regression graph obtained of the residual depletion curve considering the ERY residuals is presented in Figure 1.

It was possible to estimate a withdrawal time of 240°-day (8 days) for eliminating ERY residues at concentration levels below the maximum residue limit (MRL) considered.

Figure 1. Linear regression graph of the residual depletion curve considering the ERY residuals. MRL: Maximum Residue Limit (100 µg kg^{-1}). Regression: straight line obtained from linear regression of the residual depletion curve. 0.95/95: straight line obtained with 95 % confidence considering the 95 % tolerance limit. 0.99/95: straight line obtained with 95 % confidence considering the tolerance limit of 99 %. 0.99/0.99: straight line obtained with 99 % confidence considering the tolerance limit of 99 %.

This study was published: Campanharo et al. Food Chemistry 405 (2023) 134852 (doi: 10.1016/j.foodchem.2022.134852).



LIPID FORMULATIONS TO ENHANCE ERYTHROMYCIN'S ANTIMICROBIAL EFFICACY IN AQUACULTURE

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Erythromycin (ERY) is a wide-spectrum macrolide antibiotic that acts by interfering with protein synthesis. ERY is active against gram-positive and gram-negative pathogens, and it is among the antibiotics used by the major aquaculture producers to treat infectious diseases. It is commonly used for controlling lactococcosis (*Lactococcus garviae*), bacterial kidney disease (*Renibacterium salmoninarum*), and streptococcosis (*Streptococcus* spp.), and it is also prescribed for extra-label use. Nonetheless, the treatment can be challenging due to ERY's hydrophobic characteristics, poor aqueous solubility, instability in an acidic medium, low bioavailability, and bitter taste. This study aimed to encapsulate ERY in lipid formulations to overcome these disadvantages, enhancing ERY's antimicrobial efficacy. The nanostructured lipid carrier systems (NLCs) loaded with ERY were prepared by hot emulsification using high-speed homogenization followed by ultrasonication. The lipid matrix, composed of solid and liquid lipids, is biocompatible and biodegradable. The study investigated the use of various surfactants, including Whey protein concentrate, Poloxamer, Quillaja saponins, and Gellucire, on NLC properties and product stability throughout 28 days. The resulting NLCs formulations are presented in Table 1.

The characterization of NLCs was evaluated through pH and conductivity analysis, particle size, polydispersity index (PdI), and zeta potential (n=3), and the results are shown in Table 2.

The pH and conductivity contribute to emulsion stability. The particle size can be related to the surfactant used. Zeta potential values (>|30| mV) demonstrate physical stability. Formulations F1 and F2 showed lower polydispersity, while F3 and F4 showed medium polydispersity. The bactericidal activity of F1, F2, and F3 was assessed using the microdilution broth method against *Aeromonas* spp. isolated from fish, revealing that these lipid formulations, especially those with natural surfactants (quillaja saponins and whey protein concentrate), demonstrated enhanced efficacy. Lipid formulations have a significant potential to improve ERY's solubility, stability, and bioavailability, and they also have the advantage of being biocompatible and biodegradable systems. Considering that disease outbreaks are one of the main challenges to the sustainable development of aquaculture, it is important to develop effective alternatives that promote the safe use of antimicrobials, considering animal, human, and environmental health.

Table 1. Composition of NLC formulations containing erythromycin.

Formulation	Solid lipid	Liquid lipid	Surfactant
F1	Compritol 5%	Oleic acid 2%	Whey protein concentrate 4%
F2	Compritol 5%	Oleic acid 2%	Poloxamer 4%
F3	Compritol 5%	Oleic acid 2%	Quillaja saponins 5%
F4	Compritol 5%	Oleic acid 2%	Gellucire 4%

Table 2. NLCs loaded ERY characterization.

Lipid formulation	pH	Conductivity (µS cm^{-1})	Size (nm)	PdI	Zeta Potential (mV)
F1	5.53 ± 0.01	101.52 ± 0.13	521.7 ± 12.8	0.14 ± 0.04	-37.2 ± 0.40
F2	6.04 ± 0.06	143.61 ± 3.04	160.3 ± 2.0	0.17 ± 0.02	-27.7 ± 0.15
F3	4.98 ± 0.03	101.34 ± 4.97	427.2 ± 4	0.47 ± 0.09	-35.9 ± 1.30
F4	6.48 ± 0.03	104.56 ± 0.04	383.2 ± 1.97	0.54 ± 0.01	-38.4 ± 0.36

BIOFLOC AQUACULTURE TECHNOLOGY – A GREEN SOLUTION IN BLUE FOOD PRODUCTION

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The rapid growth of the global population, projected to reach 10 billion by 2050, poses significant challenges in terms of land scarcity and protein shortages, compelling nations to intensify all sectors of food production, including aquaculture. It is crucial to maintain the ecological balance in the food production cycle by managing the carbon and nitrogen cycles effectively, as unchecked emissions can lead to soil degradation, water pollution, loss of biodiversity, and contribute to climate change. Balance is maintained by ensuring that carbon emissions in one system are offset by another and that nitrogen emissions are similarly neutralized.

Biofloc Aquaculture Technology (BFT) can offer a comprehensive solution to these challenges, enabling intensified fish production of 1000-3000 tons per annum on just 3 hectares of land, with minimal water usage, negative carbon footprint, and low nitrogen emissions. BFT transforms organic waste into microbial bioflocs that serve as a natural, protein-rich food source for species grown within an enclosed aquaculture system, decreasing the reliance on external inputs like feed and chemicals. Additionally, BFT can enhance yields and economic performance while promoting the efficient use of resources such as water and energy. In a BFT system, nitrogen generated during fish production, is neutralized using carbon sources like molasses, a byproduct of the sugarcane industry, and rice bran, a waste product from rice polishing. The fish then utilise the bioflocs as a feed source. Additionally, nutrient-rich water from biofloc systems can serve as a valuable input for aquaponics.

Our study utilized tanks of 60, 100, 110, and 250 cubic meters to produce 2, 3, 3.3, and 8 tons of tilapia, respectively, with a feed conversion ratio (FCR) of 1:1. Molasses, calculated at containing 24% carbon, was applied to the tanks at a carbon-to-nitrogen (C:N) ratio of 20:1. The results demonstrate a land footprint of 0.0025 square meters per kilogram of fish produced over a 10 year period of production, and a water footprint of 22 litres per kilogram of fish. The positive carbon footprint of 1025 kg CO_2e from electricity consumption, is offset by the carbon in the molasses used to neutralize nitrogen emissions. By incorporating solar energy to eliminate the carbon footprint from electricity consumption, this project could generate carbon credits for use in carbon transition budgeting.

The commercial potential for biofloc technology in Ghana is significant. Ghana's aquaculture development plan aims to significantly increase fish farming output by 136% by 2027 which will take fish farming output from approximately 89,400 tonnes to 211,600 tonnes by the end of 2027. The plan includes measures to enhance the performance of fish farms, improve environmental standards and address challenges such as the high cost of fish feed. The application of biofloc technology in tilapia production in Ghana has the potential to allow for intensification of fish production within the same or smaller farming footprint, with a reduced carbon impact, reduced water usage and reduced farm gate production costs / higher profitability.

DEVELOPMENT OF POTENTIAL INTEGRATED MULTITROPHIC AQUACULTURE IN ARGENTINA

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Introduction

Marine aquaculture in Argentina has been characterized by the development of experimental and pilot-scale projects that have yet to be transformed into commercial ventures. Therefore, holistic planning is needed to impulse sustainable and profitable development for this activity. Under this framework, we set out to generate data for marine aquaculture planning in Argentina, focused on the development of IMTA.

Methodology

Crucial data about the historical SWOT of aquaculture in Argentina, environmental data, geospatial information, native species biology, and productive cycles were gathered and analyzed.

Results

Two potential IMTA configurations were detected. One comprises the whitebait fish *Galaxias maculatus* (fed aquaculture) and the halophyte glasswort *Sarcocornia magellanica* (extractive aquaculture) cultivated in land-based facilities. The other option includes the blue mussel *Mytilus chilensis* (extractive aquaculture) grown in longlines with the red sea urchin *Loxechinus albus* (fed aquaculture) cultivated in lanterns. The figure shows the productive cycle of proposed IMTA. One of the most significant limitations detected to impulse this activity was the need for connection and joint work between decision-makers and the scientific sector.

Acknowledgements

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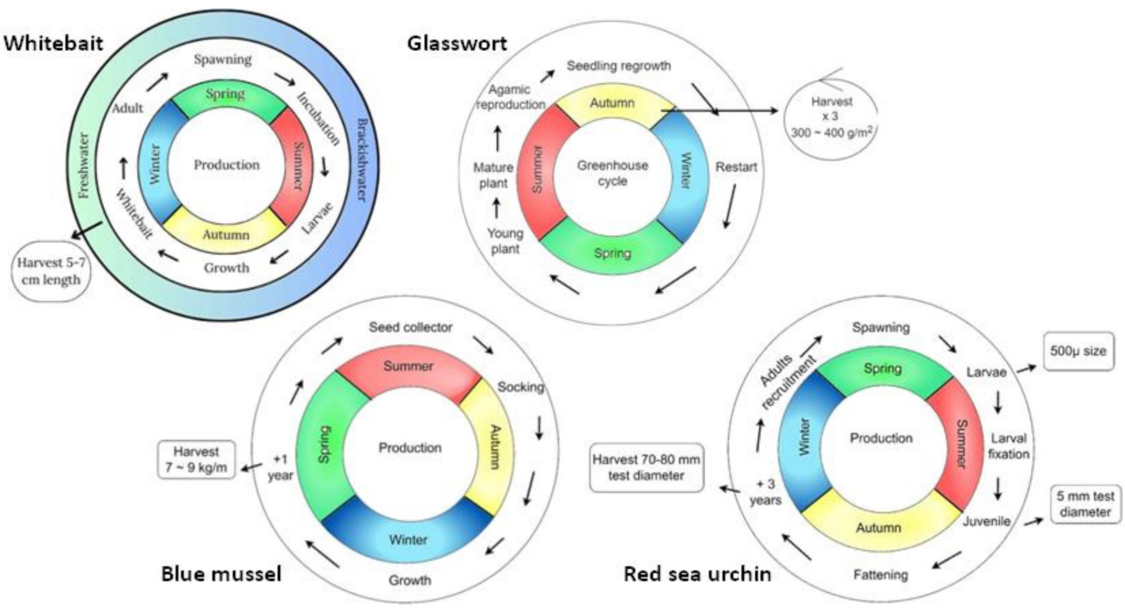

AN OVERVIEW OF THE UNIVERSITY OF NEW HAMPSHIRE'S OFFSHORE AQUACULTURE ACTIVITIES IN THE GULF OF MAINE

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The University of New Hampshire (UNH) has a rich history developing and evaluating offshore farming systems, culturing marine finfish, shellfish and macroalgae species and working with industry. They have established world class facilities including the Jere Chase Ocean Engineering Lab (https://marine.unh.edu/research-centers/facilities/jere-chase-ocean-engineering-laboratory), the Atlantic Marine Energy Center (https://www.amec-us.org/), and the Judd Gregg Marine Research Complex and pier (https://marine.unh.edu/facility/judd-gregg-marine-research-complex). They maintain six, fully permitted aquaculture farms including a 53-ha site located 4.4 km offshore Rye Harbor, NH in 35 m water depth. This site is totally exposed to Northeast storms with seas over 9 m in height and currents up to 0.65 m/sec.

Recent research at UNH has focused on 1. integrated multi-trophic aquaculture of steelhead trout (*Oncorhynchus mykiss*), blue mussels (*Mytilus edulis*) and sugar kelp (*Saccharina latissima*), Fig. 1. This system is used for aquaculture education, training and research, 2. Whale friendly kelp farming systems that reduce the risk of entanglement, and 3. sea scallop aquaculture in bottom condos (*Placopecten magellanicus*). These three research areas will be presented.

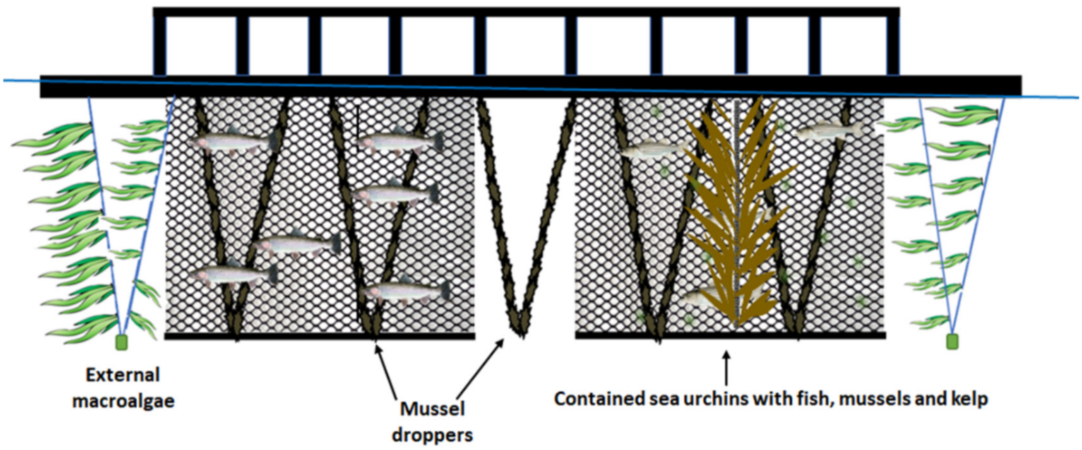

Figure 1. AquaFort, a floating platform that cultures steelhead trout, blue mussels and sugar kelp. The lower trophic species extract organic and inorganic nutrients produced by the fish thus reducing nitrogen inputs to the environment.

SHRIMPGUARD, A SOLUTION FOR WHITE SPOT SYNDROME VIRRUS, DESIGNED USING SOLAQ PLATFORM, DEMONSTRATES 80% EFFICACY AGAINST VIRAL INFECTION IN THE POND

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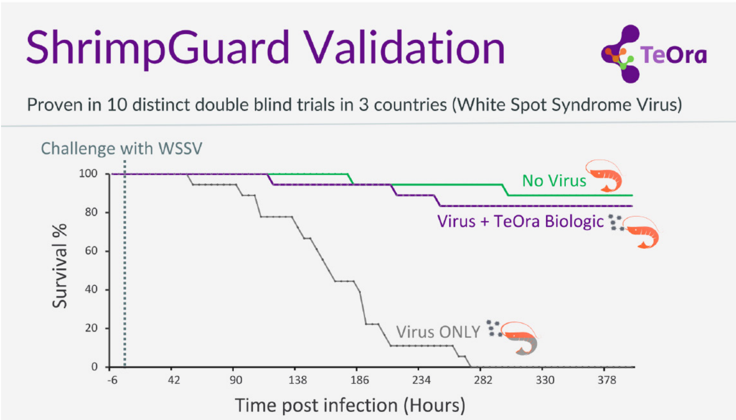
One in three calories in aquaculture are lost to disease. However, disease management solutions in hatcheries and farms are limited, where limited vaccination, antibiotics, pesticides, and biological control remain unable to prevent losses that can reach up to 90% of the grown fish or shrimp. TeOra aims to revolutionize disease management in aquaculture, using cutting-edge synthetic biology and bioinformatics. Our programmable platform can biomanufacture prophylactic and therapeutic solutions, customized to individual disease for multiple fishes and shrimps. We offer a solution that is easy to store, simple in use, and cost-competitive. SOLAQ platform combines a multidisciplinary approach to disease management. It has 3 pillars, the bioinformatics platform, synthetic biology and material science to deliver safe and effective products orally. The bioinformatics platform has a curated aquaculture database and combines it with machines learning and structural prediction models to next generation disease management and vaccine solutions for aquaculture.

SOLAQ platform has been validated in shrimps, targeting the White Spot Syndrome Virus. We have delivered the prophylactic peptides to shrimps in animal challenge trials and have observed an increase in survival from 0% in untreated shrimps to 83% in treated shrimps. The trials also confirmed the safety of our product in shrimps (no adverse health effects, no weight loss). These are orally delivered by inclusion in the feed as a top coat. We have other solutions in development for shrimp and fish diseases.

We aim our products bring cutting edge biotechnology to managing disease challenges with a wide spectrum of customizable solutions. Our solutions are natural, sustainable and safe, with which, we aim to replace/reduce harmful chemicals.



EXPLORING GENETIC DIVERSITY AND HYBRIDISATION PATTERNS OF MUSSELS IN NORTHERN SCOTLAND

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Understanding genetic diversity and gene flow within and between populations of mussels grown in aquaculture informs population fitness and contributes towards sector sustainability. Shetland, in the north of Scotland, is responsible for >50% of UK blue mussel production. As such, we aimed to investigate the genetic diversity and population structure of mussels grown in this important location compared with populations in the north of Scotland. DNA was isolated from samples taken from four populations, each containing 30 animals: one in the northeast of Scotland (Cromarty Firth); the northwest of Scotland (Western Isles); and two populations of locally adapted Shetland mussels. Single Nucleotide Polymorphisms (SNPs) analysis was performed using a medium density multi-species *Mytilus* array developed by Jenny Nascimento-Shulze et al 2023. To analyse subspecies hybridisation, we also included reference samples of *Mytilus* subspecies: *M. galloprovincialis, M. edulis* and *M. trossulus.* The initial analysis reveals that the population in Cromarty appears to be a predominantly *M. edulis* background. On the other hand, mussels from Shetland and the Western Isles display levels of introgression with *M. galloprovincialis.* Higher levels of genetic conservation were seen in the Western Isles compared with the relatively diverse genetics observed in both Shetland populations. Taken together, these data demonstrate that this SNP array provided a robust platform for consistent genotyping of individuals and may be used to further investigate appropriate growing environments for background genetics to enhance mussel health and productivity.

AQUACULTURE MONITORING WITH THE BQI-FAMILY INDEX: A RULE OF THUMB FOR UNDERSTANDING SOURCES OF VARIATION IN ECOLOGICAL STATUS CLASSIFICATION

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Benthic monitoring of macrofaunal communities is a fundamental tool for understanding and mitigating the repercussions of aquaculture in coastal environments. In the Eastern Mediterranean Sea, adhering to the Pearson & Rosenberg model of macrofaunal succession in response to organic enrichment, several benthic indices are employed for monitoring within the European Water Framework Directive (WFD) framework. One such index is the BQI-Family, which offers a cost-effective, taxonomically sufficient approach with Ecological Quality boundaries fine-tuned for the Eastern Mediterranean region. The assessment of the ecological status in Greek aquaculture via the BQI-Family index can serve as a measure for the extent of organic enrichment and the aquaculture's impact on the surrounding ecosystems. Nevertheless, the magnitude of this impact is subject to various factors, including the geographical placement and depth of the fish farming site, the type of the habitat and substrate, and spatial planning aspects of the fish farm, such as the arrangement of cage structure, etc. The cumulative influence of these parameters can lead to fluctuations in the composition of macrobenthic communities, thereby potentially influencing the outcomes of ecological status evaluation.

Under this concept, benthic macrofaunal samples collected from multiple fish farming sites across Greece during the years of 2020 to 2023, were analyzed. These sampling efforts were conducted at a distance of 50 meters from the fish farm cages, adhering to the maximum Allowable Zone of Effect (AZE) as commonly stipulated by Mediterranean regulatory practices. Sampling was carried out using a Van Veen grab with a surface area of $0.1m^2$. Subsequently, the collected samples were sieved using a 1mm sieve, followed by fixation in 4% formalin. Taxonomic analysis was performed at the family level. In addition to the biological samples, various environmental parameters were measured including sampling depth, Secchi depth, substrate type and geochemical parameters. Furthermore, data on habitat type, fish farm structure and distance from shore were recorded. To evaluate the ecological status of each fish farm, the BQI-Family index was calculated and the ecological status of each farm was determined. Subsequently, a comparative analysis of relative ecological status classifications was conducted. This comparison aimed at identifying the optimal set of parameters explaining the observed variation in the ecological assessment.

EFFECTS OF DIETARY ISOFLAVONES ON VITELLOGENIN LEVELS OF GILTHEAD SEA BREAM *Sparus aurata*

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Vitellogenin (VTG) is a precursor of egg yolk proteins and like in all oviparous species, it is present also in fish. It is synthesized in the liver and is transported to the ovary via the bloodstream. VTG suggests the maturity level of female individuals but its gene is also present in male fish. Exogenous factors such as isoflavones (ISFs) present in the plant-derived raw materials used for aquafeeds, can exert estrogenic effects on vitellogenesis. The aim of the present study was to estimate the effects of dietary isoflavones on the vitellogenin levels of gilthead sea bream (*Sparus aurata*), one of the most commercially important species in the Mediterranean aquaculture. This work was part of "Estrofish" project, that was co-financed by Greece and EU, under the "Operational Programme Competitiveness, Entrepreneurship and Innovation - EPAnEK 2014-2020" (MIS: 5052097, Code: T6YBP-00536).

Two isonitrogenous and isoenergetic diets, A and B, with similar fatty acid profile but different ISF content, were used for sea bream rearing. The ISF content of feeds was rich in isoflavones such as genistein (diet A: 9.45 μg/g, diet B: 2.47 μg/g) and daidzein (diet A: 3.41 μg/g, diet B: 2.25 μg/g). Two fish groups, L (initial size 101.4±0.8 g) and S (initial size: 55.58±0,55g) were fed on both diets (4 treatments: LA, LB, SA, SB) at satiation in Recirculating Aquaculture Systems (3 tanks per treatment). After 12 weeks, blood samples were collected. For the determination of plasma VTG a semi-quantitative ELISA method and the polyclonal anti-Rabbit Sole VTG were used. Blood parameters were measured by colorimetric assays. The fatty acid profile of fish (liver and fillet) and diets were estimated by gas chromatography (GC-FID).

OD values of VTG, were significantly higher ($P<0.05$) in LA than in LB treatment. No significant differences were observed in the VTG levels between S treatments. Both fish groups showed significantly higher n-3 LC PUFA levels (% of total fatty acids) in liver, while the opposite in fillet, when fed on diet A compared to B. On the contrary, saturated fatty acids of fillet were significantly higher in fish fed on diet A compared to B, either in group S (mainly 16:0) or group L (mainly very long-chain saturates). In addition, the levels of 18:1n-9 were lower in LA than in LB treatment, especially in the liver. Moreover, in group L the levels of liver lipid content and hepatosomatic index, as well as plasma LDH, GPT, TG, LDL and total cholesterol were lower in LA compared to LB treatment.

Measurement of vitellogenin levels is an important biomarker of estrogenic activity in farmed fish. In this study, it has been shown that ISFs, have the potential to induce hepatic VTG production combined with antilipogenic effect on the liver of sea bream. The increased content of diet A in isoflavones and especially in genistein seems to have estrogenic effect only on larger individuals, whereas smaller individuals remain unaffected. This could be attributed to the degree of sexual maturation of larger individuals, the higher consumption of ISFs via feed or the activation of biochemical pathways related to lipid metabolism. To elucidate this hypothesis further study on mRNA levels is needed.

MATERIAL CIRCULARITY INDICATOR FOR INFRASTRUCTURE IN IMTA SYSTEMS IN THE CONTEXT OF THE ASTRAL PROJECT

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The EU funded project ASTRAL aims at developing and providing innovative techniques and species combinations to improve integrated multi-trophic aquaculture (IMTA) operations. A circularity assessment was carried out to provide evidence-based metrics on how the new aquaculture systems performed in the context of the circular economy. Particularly, for the specific case study in Scotland performing co-culture of seaweeds and bivalves, we identified the major drivers to increase the circularity in infrastructure components.

The study aimed to provide an assessment of circularity at Port-a-Bhuiltin, an experimental low-trophic aquaculture (LTA) site on the west coast of Scotland, in accordance with the following scenarios (Table 1):

Table 1. Study scenarios.

Scenario IMTA Lab Scotland	Species	Circularity Strategy
Scenario 1 (Monoculture)	Only seaweed (*Alaria esculenta, Saccharina latissima*)	Not applicable
Scenario 2 (LTA)	Scenario 1 + bivalve molluscs (*Ostrea edulis*, *Pecten maximus)*	Synergies on infrastructure (same moorings and buoyancy elements)
Scenario 3 (LTA-improved)	Scenario 1 + bivalve molluscs (*Ostrea edulis*, *Pecten maximus)*	Same as scenario 2 + substitution by bio-based ropes

The Material Circular Indicator (MCI) was adapted to study low-trophic cultivation, to evaluate virgin feedstock, waste generation, linearity, and utility. To do so, the adaptation to the aquaculture sector of the methodology developed by the Ellen MacArthur foundation [1] was needed.

Table 2: Results from the MCI calculation

Scenario	Total Mass (M) in kg	Virgin feedstock (V) in kg	Unrecoverable Waste (W) in kg	Linear Flow Index (LFI)	Utility Factor (X)	Material Circularity Indicator (MCI)
Monoculture	9,501.607	8,575.435	6,471.310	0.833	1.000	0.250
LTA	12,175.876	10,290.504	6,657.088	0.787	1.089	0.349
LTA-improved	12,042.772	10,253.280	6,619.864	0.780	1.071	0.355

LFI decreased for LTA and LTA-improved scenarios by 5.54 and 6.35%, respectively when compared to monoculture (Table 2). The Utility Factor (X) increased by 8.90% for and 7.14% for LTA and LTA-improved, respectively. The MCI increased by 39.79% for the LTA scenario and 42.02% for the LTA-improved scenario. Even though MCI and X increased whilst LFI decreased, all results were positive since LFI showed better results as it is decreased, while utility and MCI showed an improvement when both indicators were increased.

Bibliography

1. Ellen MacArthur Foundation *Circularity Indicators: An Approach to Measuring Circularity. Methodology*; Ellen MacArthur Foundation, 2019

Acknowledgments

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EMERGING PROTEIN-RICH INGREDIENTS FOR AQUACULTURE - THE SEARCH FOR PROTEIN-RICH ALTERNATIVES TO EXPAND THE AQUAFEED INGREDIENT BASKET

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At 87.5 million tons in 2020, aquaculture growth rate has exceeded captured fisheries and is expected to reach 106 million tons by 2030. Global aquafeed production reached 52.9 million metric tonnes in 2023 and needs to accelerate in volume to meet growing aquaculture demand. This research aims to identify the most competitive emerging protein-rich ingredients with potential for market-wide inclusion in aquafeed that complements existing ingredients, expands the raw materials basket and bridges the feed protein gap. The report looks at the nutritional composition, digestibility, volume, production processes and challenges, cost, sale price and life cycle assessment of each ingredient to determine their scalability within the aquafeed market and summarize the challenges that need to be overcome before they can be adopted. The report provides a comparative analysis of these ingredients, an overview of the aquafeed market, lessons for aquafeed market entry and insights into neighboring markets that can provide additional revenue gains for ingredient producers.

Ingredient	Current Volume	Volume Potential	Cost/T	Capex/T	Price/T	Crude Protein	Digestibility
Definitions	1 - <1,000 MT/yr 2 - 1,000-10,000 MT/yr 3 - 10,000-100,000 MT/yr 4 - >100,000 MT/yr	1 - < 100,000 MT/yr 2 - 100k-1m MT/yr 3 - 1m - 5m MT/yr 4 - > 5m MT/yr	1 - $100-500 2 - $500-1,000 3 - $1,000-2,000 4 - >$2,000	1 - $100-500 2 - $500-1,000 3 - $1,000-2,000 4 - >$2,000	1 - $500-1,000 2 - $1,000-1,500 3 - $1,500-2,000 4 - >$2,000	1 - 50-55% 2 - 56-60% 3 - 61-65% 4 - 66-70%	1 - <85% 2 - 85-88% 3 - 89-92% 4 - >92%
Corn Fermented Protein	4	4	1	2	1	1	2
Fermented Soybean Meal	4	2	NA	NA	3	2	1
Barley Protein Concentrate	3	3	1	2	2	2	4
Insect Meal	3	4	4	4	4	3	1
Methanotrophic Bacteria	3	4	3	4	3	4	2
Mycelium	2	4	NA	NA	3	3	2
Grass Protein Concentrate	1	4	2	2	1	1	2
Canola Protein Concentrate	1	3	2	NA	2	4	3
Mixed Nut Meal	1	1	3	2	3	2	3

PROTEIN KINASE R (PKR) AN ANTIVIRAL PROTEIN THROUGH THE eIF2α IN THE GIANT GROUPER (*Epinephelus lanceolatus*) FOR VIRUS RESISTANCE

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The giant grouper (*Epinephelus lanceolatus*) is an important aquaculture species in Taiwan's aquaculture industry. However, its vulnerability to Nervous Necrosis Virus (NNV) during early stages hampers fry survival. Investigating the immune response, this study focused on Protein Kinase R (PKR), a vital antiviral protein pathway in fish and mammals. Cloning and sequencing the full-length PKR gene (1566 bp, encoding a 522-amino-acid peptide), followed by bioinformatics and phylogenetic analysis, elucidated its characteristics. Real-time PCR revealed highest expression of PKR in juvenile grouper fins, influenced by environmental temperature. Experiments with poly I:C and LPS injections showed elevated PKR expression in response to dsRNA. Fluctuations in PKR expression correlated with NNV infection severity in juvenile organs and behavior. Overexpression and siRNA inhibition experiments demonstrated role of PKR in suppressing viral replication via eIF2α phosphorylation. These findings unveil significance in grouper immunity, especially against NNV, shedding light on potential strategies for disease management.

BIOTECHNOLOGICAL INNOVATION AND BREEDING PROGRESS OF MARINE FISH IN CHINA

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Aquaculture is a vital industry in China, with significant advancements made in the basic research and breeding biotechnology of marine fish farming over recent decades. This paper systematically reviews these biotechnological innovations and breeding progress of marine fish in China, structured into four key areas: the current situation and problems in marine fish culture, progress in basic research of marine fish, progress in marine fish breeding biotechnology, and progress in improved marine fish varieties. China faces several challenges in marine fish culture, including slow growth rates, long breeding cycles, germplasm degradation, poor disease resistance, and significant sexual growth differences, which have impeded industry development. In basic research, since the first genome sequencing of the Chinese tongue sole in 2014, over 40 marine fish genomes have been sequenced, leading to key discoveries such as the identification of the dmrt1 gene as the male-determining factor in Chinese tongue sole, the dual regulation of metamorphosis in Japanese flounder by thyroid hormone and retinoic acid, and the revelation that bacterial disease resistance in marine fish is regulated by multiple minor-effect genes. In breeding biotechnology, significant strides have been made, including the development and application of molecular marker-assisted sex control technology, the establishment of a TALEN genome editing platform for Chinese tongue sole to address slow male growth, and the implementation of genome selection (GS) technology for breeding disease-resistant fish. These advancements have resulted in 19 new marine fish varieties, including “Pingyou No.2” and “Tayou No.1”, which demonstrate enhanced survival rates and growth performance. Overall, these innovations have significantly improved marine fish breeding in China, providing a robust foundation for future advancements in fish culture.

EMOPAMIL BINDING PROTEIN (*Cs-ebp*) INVOLVED IN THE GROWTH PERFORMANCE OF CHINESE TONGUE SOLE POSSIBLY THROUGH ITS REGULATION ON CHOLESTEROL BIOSYNTHESIS

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Sexual size dimorphism (SSD) with faster-growing females challenges sustainable aquaculture of Chinese tongue sole (*Cynoglossus semilaevis*), an economically important flatfish in northeastern Asia. Females grow faster than males, and eventually reached over twice in weight and length. We explored the steroid pathway's role in SSD using comparative transcriptomics. Notably, genes involved in this pathway, such as emopamil-binding protein (*ebp*) and its downstream genes, lathosterol 5-desaturase (*sc5d*), 7-dehydrocholesterol-Δ7 reductase (*dhcr7*) and 24-dehydrocholesterol Reductase (*dhcr24*), were highly expressed in female individuals of *C. semilaevis*. EBP is the key catalyzing enzymes in cholesterol biosynthesis. Its mutation caused abnormal bone development and growth retardation in humans; however, few investigation has focused on the role of ebp gene in the regulation of growth performance in teleost.

In this study, we characterized the Chinese tongue sole EBP gene (*Cs-ebp*) and its link to growth. *Cs-ebp* exhibited female-dominant expression in gonads and livers of *C. semilaevis*. Its abundance in ovaries were in accordance with the ovarian development and oocyte growth (Fig. 1). Promoter analysis identified JUNB and POU1F1α as potential transcriptional activators of *Cs-ebp* (Fig. 2A). Notably, *Cs-ebp* knockdown led to downregulation of downstream genes in cholesterol biosynthesis (*sc5d*, *dhcr7*, *dhcr24)* and pathways linked to estrogen synthesis (*cyp19a1a*) and body growth (*akt1* and *bmp2b*) (Fig.2B). These results suggested potential crosstalk between Cs-ebp, PI3K/Akt, TGF-β/Bmp, and estrogen signaling, potentially influencing female growth. The findings enhance our understanding of teleost SSD and pave the way for future investigations into its molecular mechanisms.

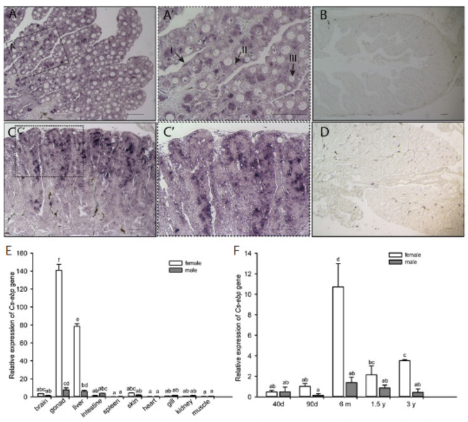

Figure 1. The spatial expression profiles of *Cs-ebp* in an ovary of 6 m female (A, A', and B) and a testis of 6 m male (C, C', and D). *Cs-ebp* expression patterns in different tissues (n=3) (E) and in different developmental stages (n=6) (F).

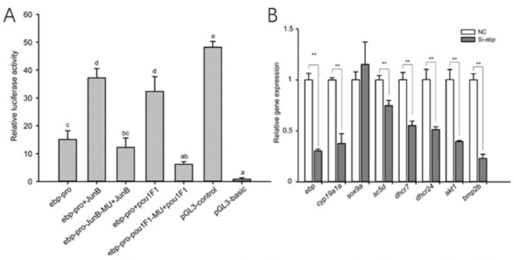

Figure 2. The transcription activity of *Cs-ebp* promoter (A) and the inhibitory effects of *Cs-ebp* siRNA (B).

ACUTE TOXICITY, BIOACCUMULATION AND ELIMINATION OF PROMETRYN IN TILAPIA *Oreochromis niloticus*

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Tilapia juvenile were aqueous exposed to different concentrations of the herbicide prometryn to investigate its acute toxicity, bioaccumulation and uptake and elimination rates. First, a 96-h acute toxicity test was carried out. The resulting 96 h LC_{50} was 5.49 mg/L, and the 96 h LC_{10} was 5.02 mg/L. Then, fish were exposed to 0.55 mg/L (1/10 96 h LC_{50}) and 0.055 mg/L (1/100 96 h LC_{50}) of prometryn solution for 28 days, followed by 14 days of elimination in clean groundwater.

The accumulated concentration levels in various tissues were always higher in the high concentration compared to the low concentration. The highest accumulated concentration of prometryn in various tissues in the 0.055 mg/L treatment were for muscle: 0.136 ± 0.0616 mg/kg (1 d), liver: 3.74 ± 2.95 mg/kg (7 d), gill: 0.971 ± 1.45 mg/kg (1 d) and blood: 0.0716 ± 0.0669 mg/kg (22 d). In the 0.55 mg/L treatment, the highest levels were for muscle: 1.27 ± 0.284 mg/kg (1 d), liver: 16.9 ± 12.7 mg/kg (7 d), gill: 8.11 ± 3.02 mg/kg (1 d) and blood: 0.751 ± 0.0775 mg/kg (22 d). The highest bioconcentration factor (BCF) of 93.1 was observed in the liver when exposed to the low concentration. Besides, for other tissues, the highest BCF were for muscle: 6.03, gill: 32.3 and blood: 2.91, all observed in the 0.55 mg/L treatment. Most of the accumulated prometryn was removed from all tissues within 24 h after the organisms were transferred to clean water. However, management of using prometryn in China aquaculture should be improved to prevent possible ecotoxicological effects and ensure food safety.

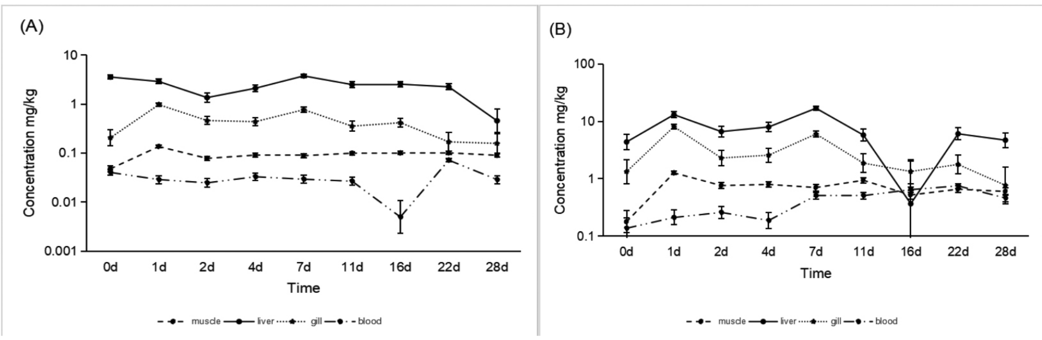

Figure 1. Tissue concentrations of prometryn in the 0.055 mg/L (A) and 0.55 mg/L (B) treatments during the exposure period.

AQUACULTURE OPPORTUNITY AREAS: A NEW APPROACH TO U.S. AQUACULTURE DEVELOPMENT

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Although the United States is a global leader in aquaculture science and technology, we lag in production. Executive Order 13921 "Promoting American Seafood Competitiveness and Economic Growth" aims to bridge this gap. With a new vision and strategic plan to support aquaculture development, the National Oceanic and Atmospheric Administration (NOAA) is leading a shift-change in U.S. aquaculture development through the identification of Aquaculture Opportunity Areas (AOAs). The search for AOAs in U.S. waters is a planning process, not a regulatory process, to identify defined geographic areas that are environmentally, socially, and economically appropriate for commercial aquaculture.

By pursuing AOAs, we increase the confidence of both entrepreneurs and regulators via proactive planning measures that inform the permitting and environmental review processes for projects proposed to be sited within an AOA. AOAs are intended to minimize interactions of aquaculture development with other ocean uses and important environmental resources, maintaining NOAA's commitment to ocean stewardship. The process to identify potential AOA siting options and complete a Programmatic Environmental Impact Statement (PEIS) for each AOA will rely on the best available scientific information and involve extensive public engagement.

In 2020, NOAA began the process of identifying potential AOAs in the Gulf of Mexico and Southern California federal waters. AOA Atlases, documenting the spatial modeling analyses that inform the AOA identification process, were released for both the Gulf of Mexico and Southern California in 2021. In 2023, NOAA announced a partnership with the State of Alaska to begin identifying AOAs in Alaska state waters. The AOA identification process in these three regions, the Gulf of Mexico, Southern California, and Alaska, mark an exciting new chapter for the development of sustainable aquaculture in the United States.

THE EFFECT OF STOCKING DENSITY ON THE GROWTH PERFORMANCE AND STRESS RESPONSE OF OLIVE FLOUNDER *Paralichthys olivaceus* IN RAS

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Olive flounder (*Paralichthys olivaceus*) sometimes referred to as Japanese flounder is a highly valued fish in East Asia due to its rapid growth, excellent aquaculture performance, and high market value. This species has been domesticated in Korea starting in the 1980's and has potential as a lucrative aquaculture candidate in other countries as well. Recirculating aquaculture systems (RAS) reduce the amount of water and space required to intensively produce seafood products. In landlocked states such as Kentucky where shrimp production in RAS systems has been growing, olive flounder can be reared in the same flat-bottom culture tanks used for shrimp. Rearing fingerlings in separate nursery tanks at high density ensures better utilization of space and animal inventory. Stocking density is one of the major factors affecting animal welfare and system productivity. The level of stress resulting from high stocking density may also affect energy and metabolism, potentially affecting growth rates and suppressing the immune response. This project focused on assessing the effect of stocking density in a RAS on production dynamics and stress response of olive flounder in nursery-level RAS.

In this study, fingerling (~58 gm) olive flounder were stocked at three densities: 2.7, 5.8 and 8.1 kg/m^2 (low-density: LD, medium-density: MD, and high-density: HD) in 1.2 m^2 fiberglass tanks. Each of these three treatments was replicated in three randomly assigned tanks connected to a common sump and shared filtration system. To maintain clear water, water from the tanks passed through a drum filter with 40 µm screen, foam fractionator, and a moving bed bioreactor (MBBR) aerobic bio filter. Ozone (O_3) gas was injected into the fractionator reaction chamber and water then passed through a UV radiation lamp to destroy ozone and further sterilize the water. In order to prevent the accumulation of nitrate, an anaerobic MBBR denitrification chamber was also used. To assess the health of olive flounder, stress indicators including blood glucose, cortisol, growth hormone and Insulin like growth factor (IGF-1) concentrations were analyzed using a Dynex DS2 System (Chantilly, Virginia, USA), an automated ELISA (Enzyme-linked immunosorbent assay) processor.

This research is currently ongoing; however, as of the most recent fish sampling event, fish were largest in the LD tanks followed by MD, and lastly the HD treatment. According to the assumed survival (very few mortalities have been detected), biomass is highest in the HD treatment, followed by MD, then LD. Upon harvesting fish from the experiment, data will be analyzed in detail comparing average weight, growth rate, total harvest, FCR, and condition factor between fish raised at the different stocking densities. Similarly, the collected blood samples will also be analyzed to compare stress hormone concentrations between the three treatment levels. Results of this study will be presented. At the end of this project we expect to better understand the tradeoffs of various stocking densities in olive flounder RAS. This trial will further develop inland production of marine species and provide growers with an alternative species for intensive, indoor production.

QUANTITATIVE ANALYSIS OF *Enterocytozoon epatopenaei* AMOUNT AND PATHOLOGIC CHANGE IN *Litopenaeus vannamei*

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Enterocytozoon hepatopenaei (EHP) has emerged as a significant protozoan disease affecting *Litopenaeus vannamei* in recent years. Caused by Microsporidia, it primarily infects the hepatopancreas, leading to damage, growth retardation, variable growth rates, and mortality. Reported cases have been documented in various countries including Thailand, Vietnam, China, Brunei, India, Indonesia, and Venezuela. Unlike acute infectious diseases such as monodon baculovirus (MBV), white spot syndrome virus (WSSV), yellow head virus (YHV), and Taura syndrome virus (TSV) which swiftly cause shrimp mortality, EHP typically manifests as a subacute or chronic infection, gradually impeding growth without immediate massive mortalities. Nonetheless, it continues to incur economic losses through growth retardation, decreased immunity, feed wastage, compromised production and quality, and heightened risks of concurrent diseases, comparable to acute infections.

Different types of diseases necessitate distinct diagnostic approaches. Rapid diagnostics are pivotal for acute infections to complement quarantine or eradication measures. In contrast, management strategies for subacute or chronic infections may involve environmental improvements, functional feeds, nutritional adjustments, and emergency harvests, besides eradication. The choice of strategy relies on factors like pathogen load, tissue damage extent, and environmental considerations. Focusing on EHP infection, this study compares pathological changes with molecular diagnostics for over 100 infections shrimp from different shrimp pounds, revealing a correlation between pathological severity and pathogen load. Based on this finding, it is envisaged that a diagnostic method associating with tissue damage severity could be developed, providing insights into subsequent management decisions through novel diagnostic systems assessing the severity of shrimp tissue pathology.

ANTIVIRAL PROTEINS AS POTENTIAL THERAPEUTICS AGAINST VIRAL INFECTIONS IN SALMONID AQUACULTURE

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Treatments to control infectious diseases in aquacultured fish commonly focus on the use of antibiotics (which are ineffective against viruses) and inorganic disinfectants. Currently, antiviral treatments are not available for use in aquaculture. The use of plasmid vectors to overexpress antiviral proteins in fish has been reported, with positive results against viral infections.

The aim of this work was to overexpress the antiviral proteins interferon-induced protein with tetratricopeptide repeats 5 (IFIT5) and natural killer enhancing factor (NKEF) using plasmid vectors (pIFIT5 and pNKEF) as novel therapeutic agents against viral infections. The antiviral activity of pIFIT5 against viral hemorrhagic septicemia virus (VHSV) and infectious pancreatic necrosis virus (IPNV) was evaluated *in vitro* in epithelioma papulosum cyprinid and Chinook salmon embryo cell lines. Moreover, pNKEF antiviral activity against IPNV was also evaluated. Results showed that pIFIT5 had antiviral activity against both viruses, but pNKEF did not protect against IPNV. In addition, we evaluated the use of pIFIT5 and pNKEF constructs as therapeutics in rainbow trout. Biodistribution assays demonstrated systemic distribution of the IFIT5 and NKEF proteins in rainbow trout injected with the plasmids, and pIFIT5 and pNKEF triggered an immune response in the injected fish. In a pilot study, we evaluated the level of protection conferred by pIFIT5 and pNKEF against VHSV in rainbow trout. Together, these results suggest the potential of pIFIT5 and pNKEF as therapeutic agents in aquaculture.

ENVIRONMENTAL IMPACTS OF BOTTOM COLD WATER MOVEMENT ON MARINE AQUACULTURE AREA AND ITS POSSIBLE CAUSE

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Introduction

The southwest coast of Korea is well known for one of the largest seaweed and abalone marine aquaculture farms. In recent years, many aquaculture farms in this region have suffered from low productivity of algae and abalone. Many factors have been suggested to be responsible for the decline in productivity, but the exact causes and solutions have yet to be found. In this study, we analyse the fluctuation characteristics of the bottom cold water, which is expected to affect the water temperature fluctuation and environmental conditions in aquaculture farms, and discuss the water temperature fluctuation in aquaculture farms and its impact on the environmental conditions.

Materials and methods

To understand the interannual variation of sea surface and bottom water temperature in the southwest coast of Korea, the daily observed long-time series data from the National Institute of Fisheries Science(NIFS) during the 40 years, 1981~2020, were analysed. The seasonal hydrographic surveys were carried out and time series datasets of hydrodynamic were collected to analyze the variability of the water movement, geochemical process and environmental characteristics around the abalone marine aquaculture area in the study area simultaneously.

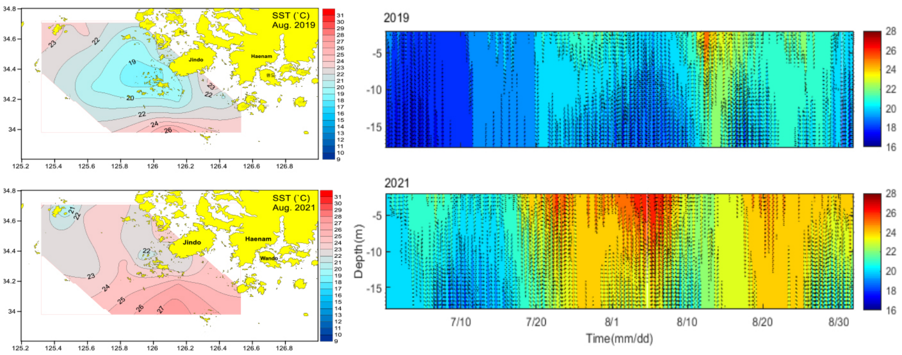

Fig. 1. The distribution of SST in the southwest coast of Korea and the vertical distribution of seawater temperature in the abalone aquaculture farm. This result shows that the vertical structure and fluctuation of seawater temperature in the aquaculture farm is strongly related to the distribution of cold water.

(Continued on next page)

Results

According to the results of linear regression of seawater temperature, the increasing trend of sea surface temperature(SST) in summer is more significant than that in winter with a rate of 0.01°C $year^{-1}$ over the whole period. This result suggests that the increased SST in summer is associated with the weakened cold water in the southwest coast (Jindo Cold Water, JCW). The JCW is known to be the upwelling of cold water by tidal mixing and pumping in the southwest coast of Korea, and the temperature of this water mass is strongly influenced by the low temperature of bottom water associated with the Yellow Sea Bottom Cold Water (YSBCW). It is well known that the YSBCW is formed by sinking to a deeper layer in the northern part of the Yellow Sea (YS) in winter, and occupies the central part and wide area below the YS thermocline in summer. The southern boundary (area) of the YSBCW in summer shows the interannual variability and is strongly correlated with the SST of the northern YS in winter. The more extended YSBCW to the south is bound to affect the bottom water temperature of the southwest coast of Korea. The hydrodynamic data shows that the seawater movement in the study area was characterised by tides and tidal currents with a semi-diurnal cycle. However, the distortion of the tidal current was observed in the vicinity of the abalone aquaculture cage. The minimum and maximum velocity of the tidal current were measured at the surface and in the middle layer, respectively. The change in the vertical structure of the current velocity appears to be related to the increase in abalone aquaculture facilities (cages). Finally, the increased current velocity in the middle layer would influence the increase in vertical velocity shear in the bottom layer. The periodic variation and high concentration of suspended particulate matter in the bottom layer and within the abalone aquaculture facilities can be explained by the increased shear velocity in the bottom layer. The reduced current velocity at the surface layer due to the dense and massive abalone aquaculture facilities can also be related to the main cause of environmental degradation in the study area due to the restriction of water mass exchange. Further research is needed to understand the mechanism of the south and westward movement and the variation of the southern boundary (area or volume) of the YSBCW.

Discussion and conclusion

From the preliminary results, two factors are expected to contribute to the decline in productivity of aquaculture farms: increased variability of SST and changes (distortions) of seawater movement. The increased variability of SST in summer is associated with the weakened cold water which is strongly influenced by the low temperature of bottom water associated with the YSBCW. The extent of the environmental impact on the abalone aquaculture area seems to be highly correlated with the change of seawater movement as well as geochemical processes such as the amount of organic matters and the concentration of nutrients. Further research for the interaction of the hydrodynamic and geochemical processes including the variation of the seawater temperature is essential to understand the environmental effects on the marine aquaculture area.

References

Butterworth, A., 2010. Integrated multi-trophic aquaculture system incorporating abalone and seaweeds. Nuffield Australia Project No. 0914. 1-38.

Korea Maritime Institute, 2012. Abalone Monthly Outlook Report. Fisheries Outlook Center, Feb. 2012-Jan. 2013.

Park, S., P.C. Chu and J.H. Lee. 2011. Interannual-to-interdecadal variability of the Yellow Sea Cold Water Mass in 1967-2008: characteristics and seasonal forcings. J. Mar. Syst., 87(3), 177-193.

EFFECT OF DIETARY PROTEIN LEVEL ON GROWTH AND FEED UTILIZATION IN HYBRID GROUPER (*Epinephelus akaara* ♀ × *E. lanceolatus* ♂)

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This study was conducted to evaluate the optimal dietary protein level for hybrid grouper (*Epinephelus akaara* ♀ × *E. lanceolatus* ♂). Four hundred fifty hybrid groupers (239.6±0.02 g) were randomly distributed into 18 tanks (6 treatments in triplicate groups) and fed six types of feed with varying protein levels (40, 45, 50, 55, 60, and 65%) for 11 weeks. Each diet was assigned to three tanks and the fish were hand-fed to satiation twice daily. At the end of feeding trial, weight gain and feed efficiency increased with increasing dietary protein up to 60%, and then decreased with further increase in dietary protein. The proximate composition of muscle and liver did not show significant differences based on dietary protein level. Hematological analysis results were also not influenced by dietary protein level. According to these results, the optimal dietary protein level for hybrid grouper (*Epinephelus akaara* ♀ × *E. lanceolatus* ♂) is 60%.

Experimental conditions

Twenty-five hybrid groupers [average body weight 239.6 ± 0.02 g (Mean ± SE)] were randomly distributed into 18 tanks (400 L) each. During the 11-week feeding trial, the average water temperature, dissolved oxygen, and salinity were maintained at 24.5 ± 2.02°C, 6.4 ± 1.3 mg/L, and 32.6 ± 1.3‰, respectively, while the fish were subjected to natural photoperiod conditions.

Results

The hybrid grouper accepted all experimental diets, with a consistent survival rate ranging from 96% to 100%, displaying no significant differences among the experimental diets. As dietary protein level increased from 40% to 60%, final weight (FW), weight gain (WG), and specific growth rate (SGR) also increased. However, further increasing the protein level to 65% led to a decrease in these growth parameters. The experimental group with a dietary protein level of 60% exhibited the highest growth performance in the study. The experimental findings indicated that feed intake (FI) normally decreased with increasing dietary protein level, except in the P60 group where this trend was not observed. Feed efficiency (FE) increased dose-dependently with dietary protein level from 40% to 60%, peaking at 114.4% in the P60 experimental group.

Table 1. Ingredients composition of the experimental diets (% dry matter)

	Experimental diets					
	P40	P45	P50	P55	P60	P65
Ingredient (%)						
Fish meal	26.5	34.5	42.5	50.5	58.5	66.5
Soybean meal	10.0	10.0	10.0	10.0	10.0	10.0
Wheat gluten	10.0	10.0	10.0	10.0	10.0	10.0
Wheat flour	43.6	36.3	29.0	21.7	14.4	7.1
Fish oil	5.9	5.0	4.1	3.2	2.3	1.4
Soybean oil	0.0	0.2	0.4	0.6	0.8	1.0
Others	4.0	4.0	4.0	4.0	4.0	4.0

INDUCED SPAWNING AND MESOCOSM-BASED STRATEGIES FOR ENHANCING LARVAL SURVIVAL OF THE PHILIPPINE NATIVE FISH, CLIMBING PERCH *Anabas testudineus*

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The climbing perch, *Anabas testudineus,* is a hardy air-breathing fish suitable for aquaculture due to its ability to withstand harsh environmental conditions. Successful breeding and early rearing practices are vital for sustainable captive production. This study aimed to determine the optimal dose of salmon gonadotropin-releasing hormone analogue (sGnRHa) for induced spawning and the impact of green water technology (GWT) on early rearing of *A. testudineus*. Mature male and female breeders (30–35 g) received intramuscular injections of varying sGnRHa doses (10, 20, and 30 µg/kg).

Results showed that the highest spawning fecundity, fertilization rate, and hatching rate were achieved at 30 µg/kg sGnRHa. Additionally, green water, produced from Nile tilapia culture, significantly influences the survival and growth of *A. testudineus* larvae to the fingerling stage (Table 1). Treatment comparisons revealed significant differences ($p<0.5$) with the highest survival rate and mean length recorded in Green water treatment (Table 2). These observations showed the potential of green water mesocosm-based approaches in enhancing larval rearing protocols of *A. testudineus* for sustainable aquaculture.

Table 1. Effect of different dosages of sGnRHa hormone on Latency (h) fertilization rate (%), incubation (h), and hatching rate (%) of climbing perch

Hormone dose µg/kg BW	Latency (h)	Fecundity/ gram (x100)	Fertilization rate (%)	Incubation Time (h)	Hatching Rate (%)
Control (saline)	0	0	0	0	0
10	7.79±1.52	511.31±1.22^{bc}	90.22±2.02	19.70±1.54	52.00±11.67^{a}
20	7.83±1.36	248.02±1.24^{ab}	90.84±9.25	19.57±1.53	66.00±3.05^{ab}
30	7.95±1.25	690.12±1.82^{c}	96.36±4.84	19.39±1.01	76.00±5.50^{b}

[1] Different subscript indicate significant differences ($p<0.05$).
[2] Duncan's new multiple-range tests were applied to compare differences at ($p<0.05$) level.

Table 2. Growth performance and survival rate of climbing perch for 30 days reared in tilapia water.

Parameters	Treatments		
	Control (tap water)	T1 (50%TGW:50%TW)	T2 (Tilapia Green water)
Initial length (mm)	4.533 ± 0.03^{a}	4.511 ± 0.01^{a}	4.544 ± 0.01^{a}
Final length (mm)	7.455 ± 0.22^{a}	14.444 ± 2.14^{b}	19.555 ± 0.67^{c}
Initial weight (mg)	6.711 ± 0.05^{a}	6.666 ± 0.08^{a}	6.7 ± 0.11^{a}
Final weight (mg)	493.33 ± 58.40^{a}	666.66 ± 16.67^{b}	876.66 ± 12.01^{c}
Survival rate (%)	4.466 ± 0.81^{a}	8.8 ± 1.97^{a}	23.133 ± 8.66^{b}
Length-weight relationship (*b*)	8.87	4.06	2.73
Condition factor (K)	0.63	0.42	0.54

1 Different subscript indicate significant differences ($p<0.05$).
2 Duncan's new multiple-range tests were applied to compare differences at ($p<0.05$) level.

MOLECULAR DETECTION AND CHARACTERIZATION OF *Ichthyophthirius multifiliis* INFECTING HATCHERY-REARED MUDFISH *Channa striata* FRY

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This study reports the detection and molecular characterization of *Ichthyophthirius multifiliis* or freshwater Ich, a parasitic ciliate infecting tank-reared mudfish *Channa striata* fry. The fry, at 18 days after hatching (DAH), exhibited signs of lethargy, increased mucus secretion, gasping, and eventual death. Prominent white spots, suggestive of *I. multifiliis* trophonts, were noted on the fry's body, primarily concentrated on the gills, dorsal, pelvic, and caudal fins.

Samples of the infected fry were collected and the trophonts were individually documented and isolated. Genomic DNA was extracted immediately from the isolated parasite, followed by polymerase chain reaction (PCR) amplification and sequencing of the small-subunit rRNA (SSU rRNA) and mitochondrial cytochrome c oxidase subunit I (MT-COI) regions. BLAST analyses of the sequences revealed a 100% similarity to the published sequences of *I. multifiliis* with accession numbers MN372056.1 (Philippines), OM302502.1 (China), KJ690572.1 (USA), and OM865867.1 (India). The phylogenetic analysis based on SSU rRNA revealed significant bootstrap probability with *I. multifiliis*, forming distinct clusters independently from other organisms within the order Hymenostomatida. Furthermore, the MT-COI primer failed to amplify the target gene in *I. multifiliis*. Between both primers it can be said that SSU rRNA gene can be used to detect the parasite in a random target environmental biodiversity screening. This study represents the first documentation and molecular detection of *I. multifiliis* infection in mudfish *C. striata* fry.

The protocols presented herein play a crucial role in developing a more rapid and sensitive method for detecting *I. multifiliis* infections in freshwater fishes in the Philippines.

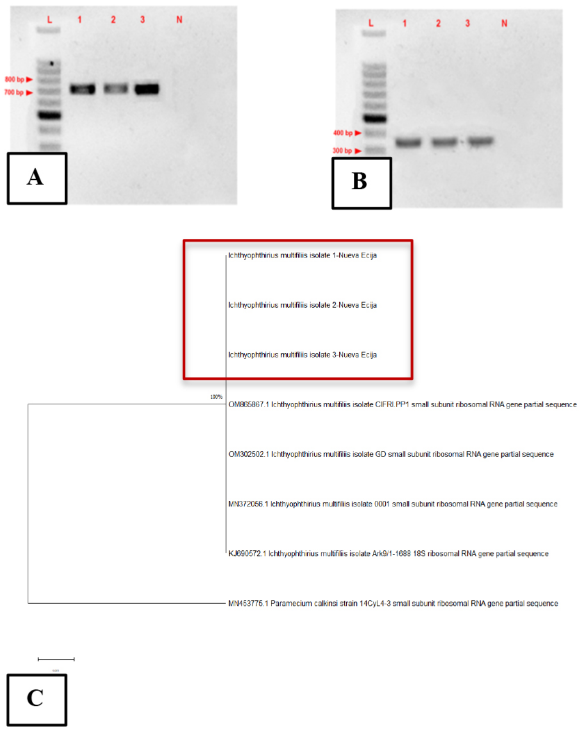

Figure 1. Gel electrophoresis results and phylogenetic investigation of samples: **(A)** Notable bands at approximately 600 bp were initially detected using the MT-COI primers originating from *C. striata*; **(B)** Clear bands of roughly 350 bp were observed using the SSU rRNA primers that matched with published *I. multifiliis* isolates in the NCBI GenBank; **(C)** Phylogenetic analysis using SSU rRNA demonstrated strong bootstrap probability with *I. multifiliis*

EFFECT OF FLORFENICOL ON CYP 1A GENE EXPRESSION AND ENZYMATIC ACTIVITY IN NILE TILAPIA

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To investigate the metabolism of florfenicol (FF) in Nile Tilapia (*Oreochromis niloticus*), the cytochrome P450 1A, one of the major drug-metabolizing enzymes in the terrestrial animals, was examined for its gene regulation in multiple organs and the hepatic enzyme activity in Tilapia at two regulated dosages and temperatures. The results showed that CYP 1A might not be the major CYP 450 superfamily that is responsible for FF metabolism in Nile Tilapia.

The tilapias were orally administered with either 5 or 15 mg/kg FF in 5 consecutive days when rearing at either 25 or 30°C water temperature. The CYP 1A gene expression levels (relative to the control group) in the liver, gill and kidney were measured by qPCR on day 1 and 5 after FF treatment. The results indicated that overall, the CYP 1A gene expression in the liver was not affected by FF at both temperatures, but was significantly up-regulated in the kidneys and gills, especially at the higher dosage (15 mg/kg). Statically significant increase of CYP 1A gene expression could be observed starting on day 1 (Fig.1). There were no significant temperature effects on CYP 1A expression at the genetic level.

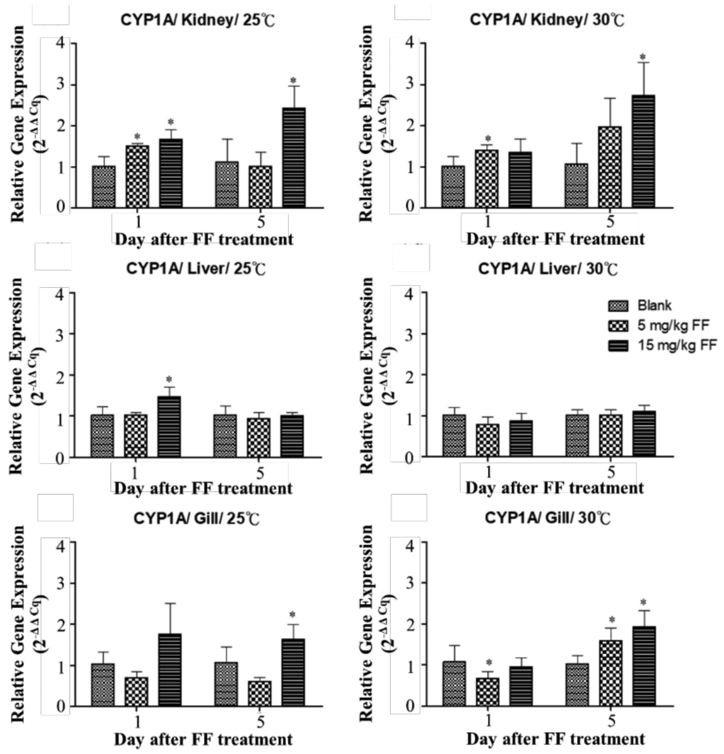

FIG 1. *CYP 1A* gene expression after administration of 5 or 15 mg/kg FF to Nile Tilapia rearing at 25 and 30°C.

To determine the effect of FF on hepatic CYP 1A enzyme activity, an enzyme activity assay was established using the CYP 1A-specific substrate α-naphthoflavone (α-NF) to define CYP 1A enzymatic activity. The liver S9 was prepared and tested with the same concentrations (0.02, 0.1, and 0.5 mM) of FF or α-NF at the presence of EROD. The results showed that FF has no apparent effect on CYP 1A activity at either dosage (Fig.2).

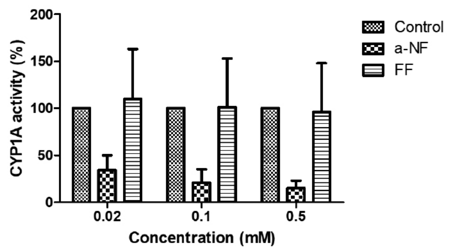

FIG 2. Comparative inhibition of CYP 1A activity by FF and α-NF.

Furthermore, after FF administration, the hepatic CPY 1A enzyme activities were not altered at both dosage and temperatures (Fig. 3).

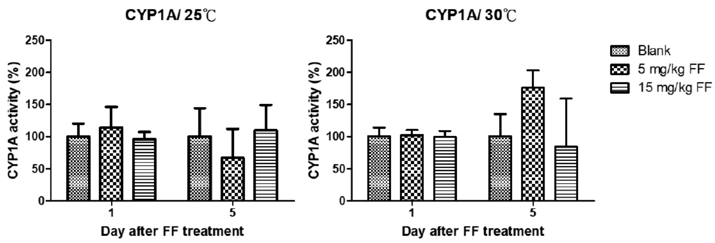

FIG 3. The inhibitory effect of FF on CYP 1A activity after administration of 5 or 15 mg/kg FF to Nile Tilapia rearing at 25 and 30°C.

In conclusion, FF at recommended dosage did not change the CYP 1A response at both genetic and enzyme levels. It is therefore suggested that CYP 1A might not be the major CYP 450 responsible for FF metabolism.

FLORFENICOL METABOLISM IS ASSOCIATED WITH CYTOCHROME P450 CYP 3A IN NILE TILAPIA

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Florfenicol (FF) is one of the widest used broad-spectrum antibiotics in aquaculture. While it is well known that cytochrome P450 superfamily CYP 3A metabolizes a myriad of drugs in the terrestrial animals, whether or not this is true for florfenicol metabolism in Nile Tilapia remains unknown. The study investigated multi-organ CYP 3A gene regulation and hepatic CYP 3A enzyme activity of Nile Tilapia at two dosages and two rearing temperatures. The results suggested that CYP 3A is involved in the FF metabolism in Nile Tilapia. FF at regulatory dosage could inhibit CYP 3A activity both at genetic and enzymatic levels.

The tilapias were orally administered with either 5 or 15 mg/kg FF in 5 consecutive days when rearing at either 25 or 30°C. The CYP 3A gene expression levels (relative to the control group) in the liver, gill and kidney were measured by qPCR on day 1 and 5. The results indicated that overall, FF at recommended dosage (15 mg/kg) significantly inhibited CYP 3A gene expression in all 3 organs; except for the group with lower dosage (5 mg/kg) at lower temperature (25°C), which exhibited an upregulation of the gene level. When inhibition occurs, it started after the first dose (day 1). (Fig. 1)

To elucidate whether hepatic CYP 3A enzyme activity was also inhibited by FF, the enzyme activity assay was first established using CYP 3A-specific substrate ketoconazole (KTZ) to define CYP 3A enzymatic activity. The liver S9 was prepared and tested with the same concentrations (0.125, 0.25, 0.5 mM) of FF or KTZ at the presence of BFCOD. The result suggested that FF showed approximately 60% of the inhibitory binding ability defined by KTZ (Fig. 2), proving the association of FF to CYP 3A enzyme.

Finally, after FF administration, the hepatic CPY 3A enzyme activities were in general decreased by 50% at both dosages and temperatures, suggesting the inhibitory effect of FF on CYP 3A at protein level (Fig. 3).

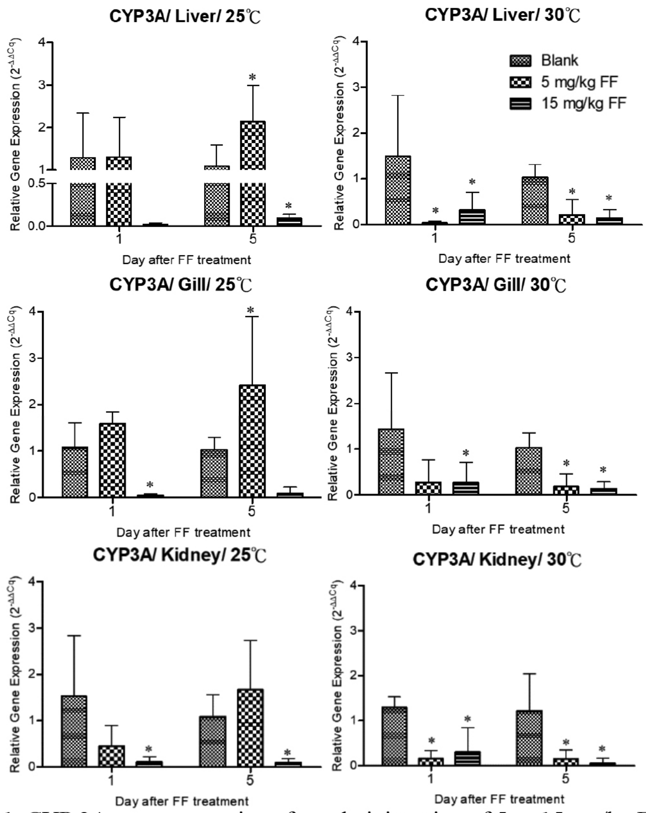

FIG 1. CYP 3A gene expression after administration of 5 or 15 mg/kg FF to Nile Tilapia rearing at 25 and 30°C.

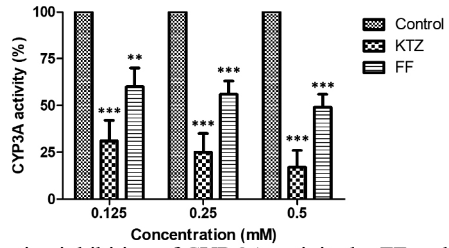

FIG 2. Comparative inhibition of CYP 3A activity by FF and KTZ.

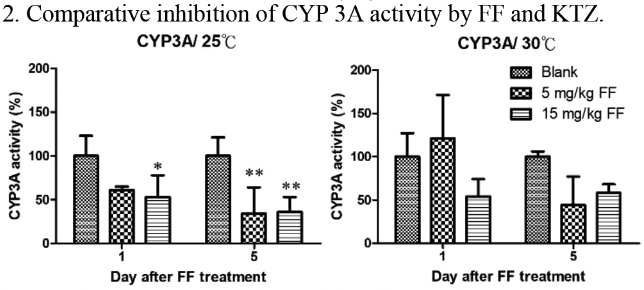

FIG 3. The inhibitory effect of FF on CYP 3A activity after administration of 5 or 15 mg/kg FF to Nile Tilapia rearing at 25 and 30°C.

In conclusion, the current study demonstrated the association of FF metabolism through CYP 3A enzyme and revealed the mainly inhibitory effects of FF on CYP 3A activity both at the genetic and the enzymatic levels. The inhibition could be both dose and temperature dependent.

TEMPERATURE AND DOSE-DEPENDENT METABOLISM OF FLORFENICOL IN TILAPIA: PATHWAYS TO FLORFENICOL AMINE

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Florfenicol (FF) is a broad-spectrum antibiotic widely used in aquaculture to treat and prevent bacterial infections. It has two reported primary metabolic pathways in terrestrial animals: an oxidative route through florfenicol alcohol (FFOH) and a reductive path potentially involving florfenicol monochloride (FFCL) and has the final product florfenicol amine (FFA).

FF was administered at a recommended dose of 10 and 15 mg/kg body weight for 5 days to evaluate its chronological and demographical metabolites formation in Nile Tilapia; in addition, the effects of temperatures (25°C and 30°C) on metabolites production was also evaluated.

Homogenized Tilapia liver and skin/muscle weighing 0.5 g was extracted by acetonitrile (ACN) containing 1% acetic acid, followed by injection to LC-MS/MS for identification of FF metabolites.

Based on the proposed metabolic pathways in land animals, the absence of FFCl across all conditions underscored a preferential metabolic route via FFOH to FFA in tilapia (Tables 1-3). Interestingly, skin/muscle concentrations of FF is consistently higher than that in the liver, suggesting quick hepatic conversion of FF and deposit to the tissue. In addition, elevated water temperatures expedite the metabolic process, as indicated by the higher conversion rates of FF to FFA at 30°C compared to 25°C (Tables 1 & 2).

Results comparing FF and metabolites production between Day 1 and Day 5 (15 mg/kg/day, 30°C) agreed with the assumption that FFOH being the most prominent intermediate, maintaining a FFOH/FFA ratio of 2 throughout the process.

The study demonstrates that FF metabolism in tilapia primarily occurs through the FFOH pathway, with higher temperatures potentially enhancing this process. The absence of detectable FFCL suggests a minor role for the reductive pathway. These findings offer insights for potential FF metabolic pathways in fish.

TABLE 1: Concentrations (µg/g) of FF and its metabolites following FF administration for 5 days at 25°C (n=5).

DAY 5	**10 mg/kg/day at 25°C**	
Analytes	**skin/muscle**	**Liver**
FF	5.8 ± 0.4	0.4 ± 0.5
FFOH	2.7 ± 0.3	1.4 ± 0.2
FFCl	nd.	nd.
FFA	1.2 ± 0.3	0.7 ± 0.1

TABLE 2: Concentration (µg/g) of FF and its metabolites following FF administration for 5 days at 30°C (n=5).

DAY 5	**10 mg/kg/day at 30°C**	
Analytes	**skin/muscle**	**Liver**
FF	3.9 ± 0.2	0.6 ± 0.4
FFOH	3.0 ± 0.5	0.8 ± 0.1
FFCl	nd.	nd.
FFA	1.3 ± 0.3	0.5 ± 0.2

TABLE 3: Concentrations (µg/g) of FF and its metabolites following 15 mg/kg/day administration for 5 days at 30°C (n=5).

DAY 1	**15 mg/kg/day at 30°C**	
Analytes	**skin/muscle**	**Liver**
FF	3.6 ± 0.1	0.2 ± 0.2
FFOH	1.5 ± 0.1	1.6 ± 1.2
FFCl	nd.	nd.
FFA	0.8 ± 0.03	0.7 ± 0.2
DAY 5	**15 mg/kg/day at 30°C**	
Analytes	**skin/muscle**	**Liver**
FF	4.6 ± 1.9	0.2 ± 0.2
FFOH	6.0 ± 0.5	1.6 ± 1.2
FFCl	nd.	nd.
FFA	2.9 ± 0.4	0.7 ± 0.2

IMPROVING SURVIVAL AND PERFORMANCE – FUNCTIONAL LIPIDS AS ANTIBACTERIAL AND ANTIVIRAL TREATMENT IN AQUACULTURE

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Aquaculture production from shrimp to support global population growth has increased the production of farmed shrimp globally. The practice of aquaculture intensification is impeded by health and nutrition – affecting growth performance. To untangle these consequences, functional feed additives have been used to stimulate shrimp immune and improve shrimp performance specially to control viral and bacterial pathogens in recent treat shrimp diseases such as V. parahaemolyticus that caused Acute Hepatopancreatic Necrosis Disease (AHPND), or more commonly known as Early Mortality Syndrome, EMS). One of the traditional and widely used shrimp farming practices being the use of antibiotics to reduce the likelihood of the disease breakout, or the use of short chain fatty acids (SCFAs) to directly reduce the GI tract pathogen load.

One of the recent innovations – LipoVital Protect (a functional lipid) includes the use of monoglycerides of short to medium chain fatty acids that combines the benefit of natural bioactive compound, with enhanced performance improvement. Further extending the benefits of organic acids, the composition of the organic acids is optimized by the monoglyceride technology to deliver a more specific approach towards different bacterial pathogens, reducing stress factors and thus increasing performance and yield.

In vitro trial results showed that the LipoVital Protect could inhibit the targeted *Vibrios spp*. and some other bacterial species. Tank trial done by CENAIM in Ecuador has shown an improvement in shrimp immune response to the disease – with ~3.3% improvement in survival rate and ~9% improvement in weight gain compared to control in AHPND challenge study (Table 1). Similar trial done by Kasetsart University in Thailand showed the results point to improved growth performance and tolerance to Vibrio indicating LipoVital Protect helped to promote a robust immune response in shrimps (Figure 2)

LipoVital Protect's ability to activate shrimps' innate immune system could be transformative for the shrimp industry. As a key additive in feeds, it can form the backbone of a comprehensive disease prevention strategy for shrimp farmers, all while improving animal health and increasing yield.

Table 1. Tank trial in shrimps conducted under normal and vibrio challenge that caused AHPND in Ecuador

Day 56, Pre-challenge

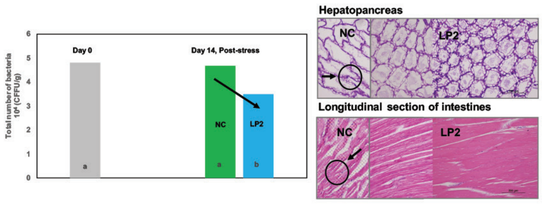
Treatment	Survival Rate (%)	FCR	Biomass (g)
PC	88.9 ± 2.57[a]	2.6 ± 0.14[a]	340.1 ± 9.06[a]
LVPA1	92.2 ± 2.05[a]	2.2 ± 0.11[a]	371.1 ± 16.74[a]

Post-challenge

Treatment	Survival (%)	Approximate survival time (Hr)
NC	37.77 ± 10.03	26.51 ± 1.90
LVPA2	48.89 ± 16.41	30.20 ± 1.94

PC: Positive control, basal diet, no challenge, NC: Negative control, basal diet, challenge, LVPA1: LipoVital Protect group, LipoVital Protect supplemented at 3kg/MT, no challenge, LVPA2: LipoVital Protect group, LipoVital Protect supplemented at 3kg/MT, challenge

Figure 1. Tank trial in shrimps conducted under normal and hypoxia condition in Thailand



PC: Positive control, basal diet, normal condition, NC: Negative control, basal diet, stress condition, LP1: LipoVital Protect group, Lipovital Protect supplemented at 3kg/MT, normal condition, LP2: LipoVital Protect group, Lipovital Protect supplemented at 3kg/MT, stress condition

INTEGRATING A VERTICAL SEAWEED CULTIVATION SYSTEM INTO THE RAS INDUSTRY

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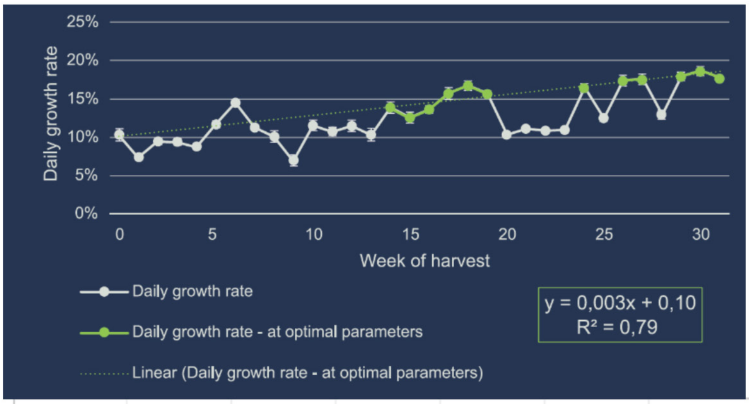
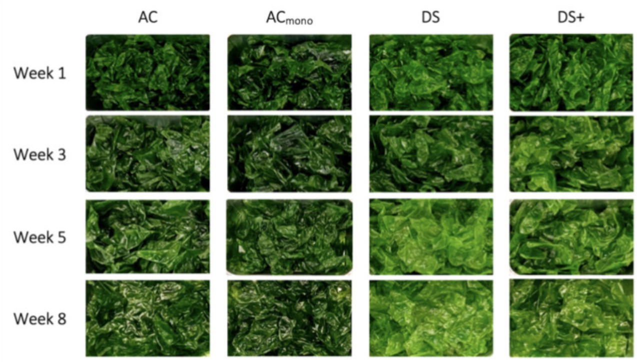
Scarcity of resources increase while linear nutrient flows from land to sea contribute to marine eutrophication, and consequently loss of biodiversity. It is imperative to change the linear nutrient flows from land to sea into circular nutrient flows in order to conserve bioavailable nitrogen and phosphorus in the technosphere. Land-based fish production in recirculated aquaculture systems (RAS) provide opportunities to capture and utilise emissions of nutrients and CO2 for cultivation of macroalgae in Integrated Multitrophic RAS (IMRAS). Hereby emissions are turned into revenue streams generating a double crop production, while alleviating the biosphere from negative impacts of nutrient and CO2 emissions.

Ulva – opportunistic green macroalgae, grows explosively under favourable conditions, generates high biomass production, and are attractive for European and global food and feed markets. Pure Algae has found a unique method and developed a technology for increasing the yield and bioremediation capacity of Ulva (figure 1) and keep this process stable over a long period of time (figure 2), while monitoring the quality of the seaweed as a result herof (figure 3).

Results prove that Ulva biomass can be produced in high output and high quality when integrating a vertical seaweed cultivation system into the RAS industry. This will be further developed and scaled up in the ValueFarm project.





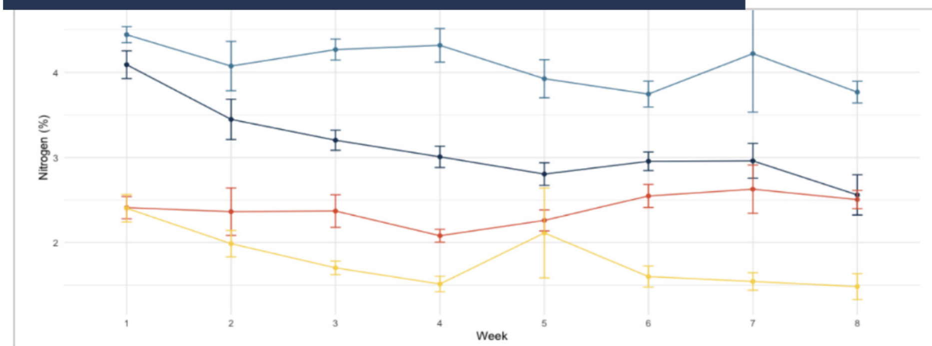


Figure 1: Top left

Figure 2: Buttom left

Figure 3: Top right

USING MITOCHONDRIA GENE AS A BIOMARKER FOR SPECIES IDENTIFICATION OF COMMON FISH AND FISHERY PRODUCTS IN TAIWAN

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High seafood demand in the supply chain often leads to seafood mislabelling, where the supplier substitutes higher commercial value fish species with less valuable ones. Thus, having an effective method for species identification is crucial to reduce mislabelling of common fish and fishery products. Besides morphological feature identification, many DNA-based species identification methods using the biomarker have been developed. As compared to nuclear DNA, mitochondria DNA (mtDNA) is more favourable as it is a circular genome that exhibits maternal inheritance, has higher copy numbers per cell for easy isolation and evolves faster which allows identification of closely related species easier. In this study, a mitochondria gene, Cytochrome c Oxidase Subunit I (*COI*) was chosen as a DNA biomarker for species identification. Universal biomarkers were developed to identify fish species and fish commercial products that are commonly sold in the Taiwan market. The genomic DNA of fish species was extracted and amplified to ensure accurate PCR amplification of the target region. Based on the results, the universal biomarker can amplify the *COI* targeted region of more than 10 fish species. Besides, to mimic the manufacturing process of the fish commercial products, samples were treated under various heat and pressure conditions to observe the DNA stability. The results showed that the denatured DNA can still be amplified after high-temperature and pressure treatment. Moreover, species identification was studied by using species-specific *COI* primer to carry out multiplex PCR. This PCR amplification technique is a rapid and lower-cost identification method for species identification based on the size variation of the target region. To conclude, this study showed that both universal and specific biomarker works well for species identification, which provides insight for future research in species identification to reduce seafood mislabelling.

FORMULATION OF SUSTAINABLE FEEDS FOR EUROPEAN SEA CUCUMBERS: A SHORT-TERM FEEDING TRIAL WITH *Holothuria forskali*

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European sea cucumbers have recently been targeted for commercial exploitation, resulting in increased attention to aquaculture development. Since information on effective diets for European species is still scant, a short-term feeding trial was conducted to evaluate sustainable feed performances on adult specimens of *Holothuria forskali*.

Three feed formulations were used to enrich natural sediment and fed sea cucumbers for 5 weeks (Figure 1). Two control treatments, with only natural sediment (Control^{+}) or without both sediment and feeds (Control^{-}), were also included in the experimental design for a total of 5 treatments. After 1 week of starvation, 60 individuals were weighed and randomly distributed into 15 tanks (3 tanks/treatment) of a flow-through system. During the experiment (after 2 weeks, T1, and after 4 weeks, T2) feeding rates (FR) were evaluated through defecation, and at the end of the end of the experiment, survival rate and weight changes were assessed.

A 100% survival rate was observed. FR values at T1 were comparable among feeding treatments, while at T2 values increased significantly for individuals fed with enriched sediment (Figure 2). Total weight did not show significant differences between feeds and times, although a positive trend was observed in individuals fed with the experimental feeds against a negative trend observed in the controls. Similarly, positive values of specific growth rate and weight change were observed for Feed-1, Feed-2 and Feed-3, while Control^{+} and Control^{-} showed negative values. This study provides initial evidence for the potential use of sustainable feeds based on food industry discards for sea cucumber aquaculture, although longer trials are needed to have a more comprehensive picture.

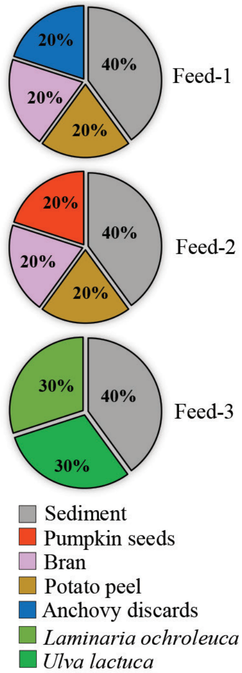
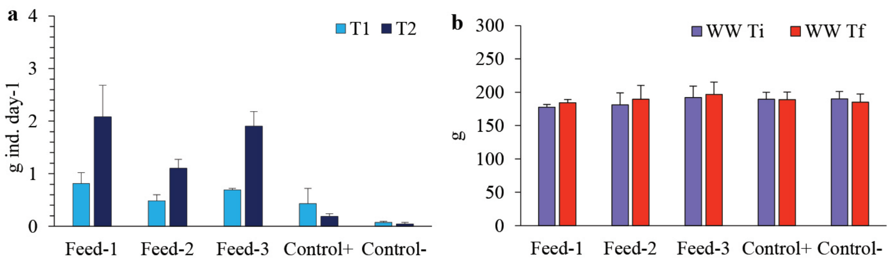


Figure 1. Ingredients (%) of experimental



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	SGR	WC
Feed-1	0.12±0.04	3.77±1.53
Feed-2	0.15±0.16	4.70±5.32
Feed-3	0.07±0.14	2.43±4.50
Control+	-0.10±0.01	-0.32±0.54
Control-	-0.08±0.07	-2.55±2.18

Figure 2. Feeding rates (g individual/day) after 15 (T1) and 30 (T2) days of controlled feeding, b) total weight of sea cucumbers (g) and c) specific growth rate (SGR, % day^{-1}) and weight change (WC, g) measured before (Ti) and after (Tf) the feeding experiment.

CAMERA TECHNOLOGY FOR EARLY STAGE DETECTION AND PREDICTION OF RED MARK SYNDROME IN RAINBOW TROUT, *Oncorhynchus mykiss*

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Red mark syndrome (RMS) is a skin condition characterized by deep, chronic dermatitis associated with panniculitis and myositis, affecting mainly rainbow trout (*Oncorhynchus mykiss*) significantly impacting the aquaculture industry. The aquatic environment where fish live presents challenges for diagnosing and treating water-borne diseases. Traditional diagnostic methods are often time-consuming and used when the disease has progressed significantly. Therefore, early and accurate diagnosis is crucial to minimize the impact of the disease on fish populations. In this study, we tested the possibility of early detection of disease symptoms by camera technology under real conditions. RMS was used since the pathology mainly manifests itself externally and in addition is fairly distinct from pathology with other causes.

In total, 160 rainbow trouts (*Oncorhynchus mykiss*) were used in our study. The size of the fish at the cohabitation with the seeders was 22.5 ± 1.4 cm 150 ± 30 g. Two 1m^3 tanks were used for infected fish (60 fish in each tank), and one tank (60 fish) was used as negative control. The fish were followed for 12 weeks. All tanks were monitored by the colour digital camera in underwater housing during the cultivation. The video clips, which lasted 30 minutes, were recorded for all three tanks. These video records were used to simulate the detection of disease symptoms under real conditions. The dataset of the still images was created from five fish photographing sessions (every two weeks starting from week six). An image of the right and left side of the fish was captured for all fish. The expert scored the levels for oedema, scales, color, and RMS severity for each fish.

For the automatic detection of the RMS symptoms, six classes were defined to cover different skin deviations that belong to the RMS and other diseases. Three classes (early symptoms, late symptoms, and healing) were defined for RMS. Three classes were defined for RMS on fins, snout/lower jaw and other symptoms. All images were manually annotated to prepare the data for CNN-based RMS symptom detection. The CNN with YOLOv8 architecture was used for the detection and classification of annotated symptoms without the detection of individual fish.

The accuracy (mAP50) of RMS symptoms detection (still images) using the CNN approach was 87% (84% for early symptoms and 89% for late symptoms). The early symptoms were detected by the system on the same day as the expert detected them. The study proved that the early symptoms of RMS disease can be automatically detected by deep learning methods from the still images of the fish. The next step is the RMS detection in video records to demonstrate the ability to detect the symptoms earlier than the expert.

Figure 1. Detected early RMS symptom

CELLULAR REGENERATION AND GENE EXPRESSION DURING MOULTING IN THE PACIFIC WHITELEG SHRIMP (*Litopenaeus vannamei*)

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Moulting is the primary process by which crustaceans grow. It involves a complex series of hormonally controlled metabolic and physiological changes that result in the construction of a new cuticular layer beneath the existing shell. This culminates in the removal of the old cuticle layer, a process called ecdysis, and an intake of water leading to expansion of the animal's size before hardening and calcification of the new exoskeleton. The exoskeleton development is crucial for metamorphosis and growth throughout the life of shrimp with failure of moulting resulting in production losses from either mortality or stunted growth. The histological changes associated with the moult stage in prawns has been well described, particularly methods by which to visually assess moult stage in Penaeid prawns (Figure 1). Modern bulk transcriptomic and proteomic approaches have also discovered unique hormonal regulatory mechanisms. However, this has not yet been resolved at the cellular level, with the underlying cell types and molecular mechanisms driving these phenotypic changes poorly understood. In the present study, we used a single-cell nuclei RNA-seq (snRNAseq) approach (Parse Biosciences) to identify the cell types and changes across key stages of the *L. vannamei* moult cycle. We identify the subcellular expression patterns unique to each epithelial cell type and how they change during moulting, expanding on and integrating bulk transcriptomic datasets to enhance our knowledge of exoskeleton development. This study defines the dynamic patterns of cell specific expression and cellular regeneration that occurs during this fundamental biological process. Implications for utilising this information to improve growth performance will be discussed.

INVESTIGATING THE RESPONSE OF NORWEGIAN BLUE MUSSELS (*Mytilus spp.*) TO THERMAL STRESS

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As the sea surface temperatures in the North Atlantic Ocean continue to rise, it will be imperative to understand how increased temperature will affect ectothermic organisms such as *Mytilus spp*. Innerpollen, Norway is a shallow, pond-like inlet with limited water exchange with the open ocean during summer months, exposing wild mussels to high water temperatures (19.9 ± 1.8°C, 13.1-26.7°C July-August). Approximately 11 km southwest of Innerpollen lies the IMR research station on the island of Austevoll, where wild mussels are exposed to comparatively cooler water temperatures (15.0 ± 0.9°C, 11.0-20.0°C July-August). Mussels were collected from these sites with the objective of comparing the thermal biology of animals originating from distinct thermal environments. Mussels were held under four temperatures (15, 20, 25, and 30°C) and their thermal biology was characterized using: (1) median lethal time (LT_{50}), (2) valve gaping behaviours, (3) respiration, and (4) feeding rates, where physiological rates were measured after a 7-day exposure to the target temperature. Differential physiological and behavioural responses between mussel sources suggest that individuals from Innerpollen outperform those from Austevoll at 25°C and above, as exhibited by increased survival. This study highlights the potential for mussels from distinct thermal environments within a close geographic range to have diverse responses to future climatic scenarios, which could provide insight to how the distribution or abundance of mussels may be affected by increasing temperatures due to differences in survival between sources. Results could have important ecological implications for Norwegian waters and can assist in identifying traits associated with thermal tolerance, which could have applications to selective breeding programs for mussel aquaculture within Europe.

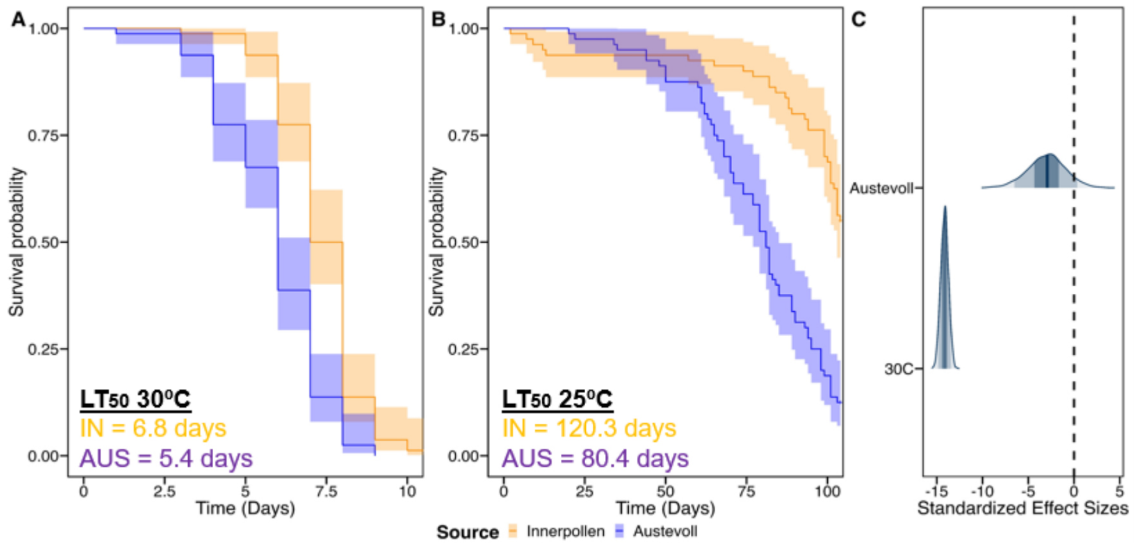

Figure 1. Effect of source and temperature on survival in *Mytilus spp*. (A/B) Kaplan Meier survival probability curves for 25/30°C with LT_{50} values, and (C) posterior predictive distributions from Bayesian analysis of source and temperature on survival probability. The reference category for Austevoll (AUS) and 30°C are Innerpollen (IN) and 25°C.

CREATING BETTER EDUCATIONAL VIDEOS FOR EXTENSION AND OUTREACH

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Welcome to the video age. The spread of high speed internet access and simple video editing software makes the creation and delivery of online educational videos easier than ever. YouTube videos provide an excellent mechanism for Extension specialists to share information and reach a large potential audience. Putting a Power Point presentation in video format does not make the most of this medium. The creation of good video content requires careful planning and practice. The average attention span of most adults is 15 minutes and students approximately 7-10 minutes. Most lecture presentations, however, last 30 minutes to an hour. How can you get your point across as quickly as possible? We must learn to provide entertainment in addition to education. YouTube suggests that the first 15 seconds of a video are critical for engagement and retention of the audience.

A central figure or "hero" and a story with a beginning, middle and end will go a long way towards retaining the attention of the audience. Join us for a discussion these and other simple tips and techniques that can improve your ability to create watchable or perhaps even enjoyable educational Extension videos.

BACTERIAL SELECTION FOR ENHANCED BIOFILTRATION AND CONTROL OF FISH PATHOGENS IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction: Recirculating aquaculture system (RAS) are nowadays considered to be particularly resource-efficient and sustainable. The biofilter of a RAS is a key element for the water treatment, since it removes byproducts originated during the fish production. A proper balance of the microbial community maintains safe levels of these residual compounds. An imbalance in the microbial community of the system results in poor water quality, which causes stress to the fish physiological system. In the worst cases this situation results in a disease outbreak. New strategies for disease prevention contributes to the development of a safe and sustainable aquaculture production.

Aim: The objective of the present work is the selection of microorganisms to enhance the biofiltration system in a RAS, and investigate its capacity to reduce the abundance of pathogens in the system.

Methods: Samples were obtained from the biofilter of a freshwater RAS with reared *Clarias gariepinus*. Different bacterial colonies were isolated and biochemically characterized. Isolates with interesting metabolic profiles were selected and tested for biofilm formation ability, quorum sensing activity and antagonistic effect against seven potentially pathogenic bacteria. Two bacterial candidates were tested for its capacity of inhibiting *Escherichia Coli* and enhancing water quality inside small scale in vitro biofilters, which consisted on bottles containing bioballs and water that simulated a RAS effluent. During a four-day trial, the abundance of R5, R28 and E. Coli were calculated and water samples analyzed for physicochemical parameters.

Results: Two strains, namely R28 and R5, showed a strong inhibition of the growth from *Staphylococcus aureus* and *Pseudomonas veronii*. R28 was able to catalyze phosphate, glucose, casein, gelatin, cellulose and reduce nitrate. R5 had an antagonistic effect on the growth of *Vibrio anguilarum*, *Aeromonas hydrophila* and *E. Coli,* and could reduce nitrate and catalyze another five compounds. Results of the four-day trial showed how R5 colonizes the bioballs and water while preventing *E. Coli* to increase in abundance. R28 did not perform as efficiently as R5 in controlling the growth of E. Coli.

Conclusions: The two candidates had essential functional traits with the potential to control the overgrowth of some potentially pathogenic bacteria. They also had advantageous biotechnological and functional characteristics for the future development of probiotics which could be used at the production sector.

ALTERNATIVE MICRODIETS SUSTAIN GOOD PERFORMANCE OF ATLANTIC COD (*Gadus morhua*) LARVAE

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Atlantic cod larviculture protocols need to be optimized, as a high variability in growth performance, survival rates and incidence of skeletal deformities is commonly observed. Still, considerable progress has been obtained in recent years, with introduction of alternative live prey in feeding protocols. In addition to enriched rotifers and Artemia, several cod hatcheries already use cryopreserved barnacle nauplii and/or copepod nauplii. Significant progress has also been achieved with microdiet technology and tailored formulations for different fish species, which may be applied to cod microdiets. The present study evaluated the impact of two innovative microdiets, and compared to a commonly used microdiet for cod and other marine fish larvae, all used in a protocol with enriched rotifers, plankton eggs and barnacle nauplii, on growth performance and skeletal anomalies in Atlantic cod (Gadus morhua) larvae.

Larvae from Ode AS (Norway) were randomly distributed in triplicate 400 L tanks at 2 days post-hatch (dph) and kept until 67 dph (trial end). The light regime was 24h and temperature ranged from 8.0 to 10.0°C. All tanks were fed with the same live feed protocol: a sequence of enriched rotifers, plankton eggs (Cryo-μ), small barnacle nauplii (Cryo-S), and large barnacle nauplii (Cryo-L). The 3 microdiets were introduced at 22 dph in co-feeding with live feed, and alone from 45 dph onwards. Diet CTRL is a commercially available diet, and diets D3 and D4 are experimental prototypes. At the end of the trial (67 DAH), fish fed all 3 microdiets had an identical final dry weight (1W-ANOVA, p=0.319) and standard length (p=0.882). Evaluation of skeletal deformities is still underway. Mortality rate between 15 and 67 dph was identical among treatments and around 19%.

The three microdiets tested brought high growth in Atlantic cod larvae. The evaluation of skeletal deformities will be an important add-on to these results, as a previous study on Atlantic cod has shown that even at comparable growth performances, feeding regime can have a strong impact on skeletal deformities incidence, and hence juvenile quality.

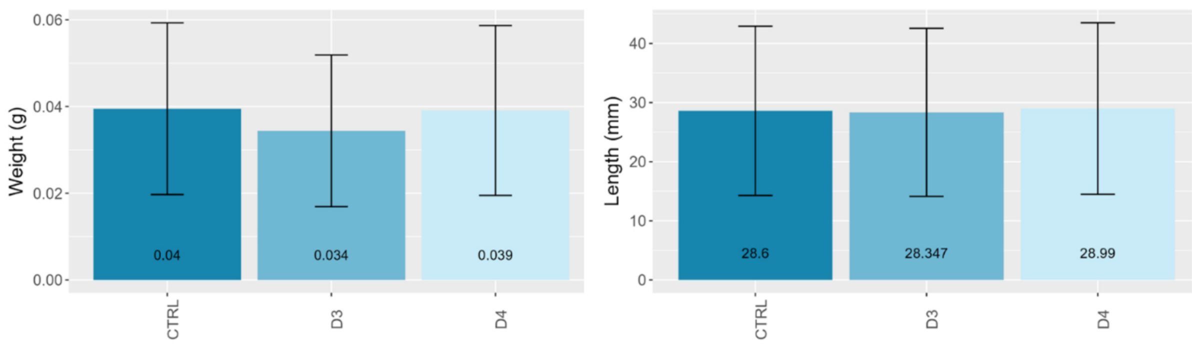

Figure 1. Dry Weight (Left) and standard length (Right) in Atlantic cod at 67 dph, after being fed a commercial microdiet (CTRL), and two experimental microdiet prototypes diets (D3 and D4). Results are means ± SD (n=3).

DAILY RHYTHMS OF THERMAL PREFERENCE IN COMMERCIAL INTEREST SPECIES: OPTIMIZING REARING AND WELFARE IN DIURNAL AND NOCTURNAL FISH

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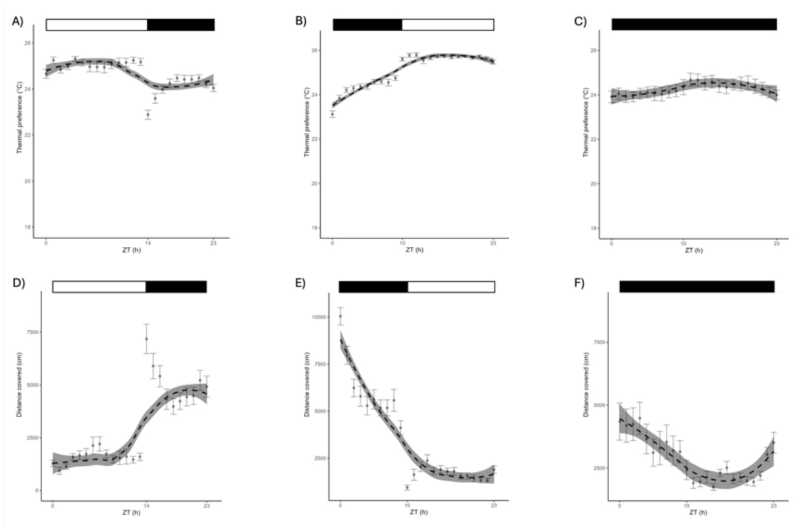
Introduction: Daily cycles of light and temperature are powerful interrelated stimuli in the wild that entrain animals' physiological functions and behaviour [1,2]. Being ectothermic, fish are strongly affected by water temperature and they have evolved behavioural strategy to actively choose a thermal environment that favours their physiological performance [3,4]. The aim of the present study sought to characterise the daily rhythms of thermal preference of nocturnal and diurnal fish of commercial interest using an automated system that controls and maintains a horizontal temperature gradient. The two species investigated were the nocturnal black bullhead catfish *Ameiurus melas* and the diurnal largemouth bass *Micropterus salmoides*.

Methods: We developed two separate automatic systems to control water temperature and record fish behaviour for long period. The experimental apparatus consisted in a multi-chamber tank (180×30×20cm) in which fish are freely to move across five equal compartments (30×30×20cm). A microcontroller compatible with Arduino recorded the temperature in each compartment via a probe and switch ON/OFF a single heater in order to maintain a horizontal thermal gradient (from 18 ºC to 26 ºC). We built three replicates of the system. The two species (n=8 catfish/system; n=12 bass/system) were subjected to 14:10 h light-dark cycle (LD lasted 10 days) which was subsequently reversed to a 10:14 h dark-light cycle (DL lasted 10 days), concluding with the constant darkness condition (DD lasted 7 days) to ascertain whether rhythms are driven by an endogenous circadian clock [5]. Fish were randomly fed once a day during the night (catfish) and light (bass) phase, and the food was evenly distributed in all chambers. During the DD period, the fish were fasted to avoid the food could become a synchronizer of their behavioural activity. To investigate difference of daily thermal preference between species, we continuously recorded fish behaviour across the experiment by using a Raspberry Pi, remotely controlled for avoiding disturbance to fish during the experiment. Then, we performed video analysis of animal behaviour using Ethovision XT software (Noldus, the Netherlands).



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Results: *A. melas* selected higher temperatures during the light phase and cooler temperatures during the night phase (Fig. A), as it would experience in the wild. Conversely, the analysis of locomotor activity confirmed that *A. melas* is a nocturnal species, i.e. higher activity during the night phase (Fig. D). No differences emerged in their thermal preference and locomotor activity when the light-cycle changed (Fig. B, E). In DD condition, catfish showed a similar pattern those observed in the DL (Fig. C, F) suggesting that daily thermal preference and locomotor activity are endogenous. Regarding *M. salmoides*, data will be analysed by the end of the month. In agreement with previous works on diurnal species [6,7], we expect to find a daily rhythmicity in thermal preference displaying the same pattern as catfish. However, we expect that *M. salmoides,* being a diurnal species, might show an increased activity during the light phase.

Conclusion: Our results show that the thermal preference may be primarily related to endogenous systems rather than a metabolic needs for fish. Providing a thermoregulatory environment would be resemble the natural conditions in which fish live and increased their welfare [8,9], thus daily variation of thermal preference is an important cue to be considered when designing husbandry protocols.

1. Bertolucci, C., & Foà, A. (2004). Extraocular photoreception and circadian entrainment in nonmammalian vertebrates. *Chronobiology international*, *21*, 501-519.
2. Bell-Pedersen, D., Cassone, V. M., Earnest, D. J., Golden, S. S., Hardin, P. E., Thomas, T. L., & Zoran, M. J. (2005). Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nature Reviews Genetics*, *6*, 544-556.
3. Angilletta Jr, M. J., Niewiarowski, P. H., & Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. *Journal of thermal Biology*, *27*, 249-268.
4. Christensen, E. A., Norin, T., Tabak, I., van Deurs, M., & Behrens, J. W. (2021). Effects of temperature on physiological performance and behavioral thermoregulation in an invasive fish, the round goby. *Journal of Experimental Biology*, *224*, jeb237669.
5. Herrero, M. J., Madrid, J. A., & Sánchez□Vázquez, F. J. (2003). Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiology international*, *20*, 1001-1017.
6. Vera, L. M., de Alba, G., Santos, S., Szewczyk, T. M., Mackenzie, S. A., Sánchez-Vázquez, F. J., & Planellas, S. R. (2023). Circadian rhythm of preferred temperature in fish: Behavioural thermoregulation linked to daily photocycles in zebrafish and Nile tilapia. *Journal of Thermal Biology*, *113*, 103544.
7. de Alba, G., Conti, F., Sánchez, J., Godoy, L. M., Sánchez-Vázquez, F. J., López-Olmeda, J. F., & Vera, L. M. (2024). Effect of light and feeding regimes on the daily rhythm of thermal preference in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *578*, 740122.
8. Sanhueza, N., Donoso, A., Aguilar, A., Farlora, R., Carnicero, B., Míguez, J. M., ... & Boltana, S. (2018). Thermal modulation of monoamine levels influence fish stress and welfare. *Frontiers in Endocrinology*, *9*, 717.
9. Sanhueza, N., Fuentes, R., Aguilar, A., Carnicero, B., Mattos, H., Rubalcaba, Y., ... & Boltana, S. (2023). Behavioral Thermoregulation in Captive Fish: Molecular, Physiological, and Welfare Implications. bioRxiv, 2023-10.

SUPPORTING SUSTAINABLE AQUACULTURE THROUGH INDEPENDENT SCIENTIFIC ADVICE

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Aquatic foods play a vital role in healthy and sustainable food systems around the world, but their dietary, economic, and ecological values are often undervalued and overlooked by society. This is especially evident when comparing society's treatment of aquatic foods to terrestrial foods, and this disparity is even greater for aquatic foods produced from aquaculture.

In the North Atlantic region, wild capture fisheries are long-held, traditional activities with special rights and privileges. Governed by regulations guided by global agreements, the industry benefits from relatively straightforward, single-source government policies. These regulations are complemented by well-organized stakeholder networks, coordinated data streams, and scientific cooperation, facilitating an established science-policy interface essential for sustaining wild capture fisheries.

Yet, in recent decades, landings from wild capture fisheries have declined while aquaculture production has increased. As demand for aquatic foods continues to rise, the North Atlantic region faces increasing pressure to meet this demand from aquaculture using sustainable practices at a faster pace. Unlike capture fisheries, aquaculture is typically a national competency where sharing best practices is not the norm. To confront the mounting pressure to sustainably meet escalating demands, governments must navigate intricate environmental, economic, and social factors inherent to this multifaceted sector. Collaboration and collective intelligence are imperative.

This presentation will argue that international scientific cooperation and independent, consensus-driven scientific advice is needed to support the demand for high-quality aquatic foods that are produced according to globally agreed best practices. The International Council for the Exploration of the Sea (ICES) is an international, inter-governmental organization that has long provided consensus-based, independent scientific advice to national governments, RFMOs and the European Union on the sustainable exploitation and management of marine commercial fisheries. Today, ICES' work on aquaculture is part of a wider portfolio of work under the Ecosystem Approach that seeks to advance and share scientific understanding of marine ecosystems and the services they provide, and to use this knowledge to generate state-of-the-art advice for meeting conservation, management, and sustainability goals. This presentation will highlight successful case studies where scientific cooperation and independent advice has facilitated the development of sustainable marine resource policies and practices, and it will encourage discussion on how stakeholders, industry, science, and policy makers can work together to support sustainable aquaculture in the region.

INSECT MIXTURES IN PRACTICAL DIETS FOR EUROPEAN SEABASS: WORKING TOWARDS FLEXIBILITY IN DIET FORMULATION

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Black soldier fly (BSF, *Hermetia illucens*) and yellow mealworm (YM, *Tenebrio molitor*) larvae meals have been tested individually as fishmeal (FM) substitutes in fish diets, due to their potential for sustainable production, high nutritional value, and composition in bioactive compounds with immunomodulatory or antimicrobial properties. However, combining both insect meals in a mixture (IM) could provide feed companies with increased flexibility for formulation, ensuring a well-balanced dietary amino acid profile while allowing for a strategic use of FM. This study assessed the potential of combining BSF and YM larvae meals as a protein source and functional ingredient in practical diets for European seabass (*Dicentrarchus labrax*) juveniles.

Four isoproteic and isolipidic diets were formulated. The control (CTRL) followed commercial trends for seabass diets, incorporating plant proteins (55.3%), processed animal proteins (9%), and moderate levels of FM (15%). The remaining diets included a 50:50 mixture of defatted BSF and YM larvae meals, replacing 3% (IM3), 25% (IM25), or 50% (IM50) of FM in CTRL. Amino acid supplementation was not necessary, and equivalent levels of EPA and DHA were maintained across diets by adding extra 0.4 and 1.1% salmon oil to IM25 and IM50, respectively, at the expense of rapeseed oil. Diets were tested in triplicate in a 75-day feeding trial conducted in a RAS with water at 22 ºC and 35 ppt salinity, under a 12 h light/12 h dark photoperiod.

The diets containing IM promoted similar growth, feed efficiency, and nutrient digestibility to the CTRL diet, while also reducing phosphorus fecal losses. Moreover, intestinal integrity was maintained in all fish, but those fed IM50 displayed the longest villi. All diets ensured a high muscle nutritional value, with 360-400 mg of EPA+DHA per 100 g of fillet. The muscle fatty acid profile of fish fed IM closely resembled that of those fed CTRL. However, the IM25 and IM50 diets promoted a higher deposition of lauric acid (2.4 and 3.5 mg per 100 g, respectively) compared to both the CTRL and IM3 diets (0.9 mg per 100 g). Given that lauric acid has been suggested to possess antioxidant properties, its potential impact on muscle lipid peroxidation and antioxidant capacity is presently being explored. IM50 also promoted a higher hepatic response of the glutathione antioxidant system after a salinity stress challenge. Overall, our findings demonstrate the potential of utilizing a combination of BSF and YM to partially substitute FM in practical diets for European seabass. Future work will evaluate fish health and resilience upon stress-inducing conditions.

Acknowledgments
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THE IMPACT OF WATER SALINITY ON THE DIGESTIVE CAPACITY OF EUROPEAN SEABASS FED DIETS CONTAINING AN INSECT MEAL MIXTURE

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Black soldier fly (BSF, *Hermetia illucens*) and yellow mealworm (YM, *Tenebrio molitor*) defatted larvae meals have been tested as novel protein sources in aquafeeds. Thus far, studies have shown adequate nutrient digestibility of these ingredients for European seabass (*Dicentrarchus labrax*) reared in saltwater conditions (35-36 ppt). However, seabass are euryhaline fish, tolerating a wide range of water salinity (5-50 ppt). Since salinity can impact intestinal morphology and nutrient digestion, this study explored the impact of low water salinity (5 ppt) on the digestibility of practical diets for seabass containing a BSF and YM mixture, in comparison to the Atlantic Ocean's salinity (35 ppt).

Four isoproteic and isolipidic diets containing 1% Cr_2O_3 were tested in a digestibility trial with juveniles: a practical control diet (CTRL) with moderate fishmeal (FM) levels (15%) and three experimental diets with a 50:50 mixture of BSF and YM larvae meals (IM), both defatted, included at 0.5% (IM0.5), 4.3% (IM4) and 8.4% (IM8) to replace FM. Similar EPA and DHA levels were maintained across the diets by adding extra salmon oil to IM4 and IM8 (0.4% and 1.1%, respectively), at the expense of rapeseed oil. The two salinity conditions were tested in parallel, in two RAS equipped with tanks with a settling column connected to the outlet for faeces collection. Triplicate fish groups were fed three times daily (2% of body weight) and faeces were collected daily and stored at -20 ºC. After freeze-drying, faecal chromium, crude protein, total lipids, crude energy, and phosphorus levels were determined, and their apparent digestibility coefficients were calculated.

The lower water salinity (5 ppt) promoted a higher digestibility of crude protein, lipids, and phosphorus than the higher water salinity (35 ppt), independently of the diet. Still, gross energy digestibility was not significantly affected by rearing salinity. Additionally, the IM8 diet presented higher lipid digestibility than CTRL, but protein and energy digestibility remained similar. Lastly, the IM4 diet presented higher phosphorus digestibility than CTRL.

Our results suggest that lower water salinity increases the digestibility of diets for European seabass. Thus, these conditions could be strategically used for its inland culture. Moreover, the diets containing the IM performed comparably to the control diet, supporting their utilization for feeding European seabass independently of rearing salinity. Future work will focus on the impact of water salinity on fish intestinal morphology and the enzymatic machinery involved in nutrient digestion.

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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. These compounds will be obtained after developing an optimal bioactive compounds extraction strategy from residual halophytes, and thereafter testing their pro-and pre-biotic activities in shrimp and fish diets. By bolstering stress and disease resistance, the project also 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.

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UNRAVELLING WHITE SPOT SYNDROME VIRUS (WSSV) TRANSMISSION DYNAMICS IN *Litopenaeus vannamei* SHRIMP

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Crucial knowledge gaps remain in the understanding of white spot disease (WSD) transmission dynamics. To accurately characterize these dynamics, it is necessary to first understand the time course of WSD in an individual host (Jewell et al. 2016). Then, the epidemic pattern of spread can be characterized. Studying this pattern can potentially reveal the primary transmission dynamics, aid in the determination of the point at which host-to-host transmission occurs (Giesecke et al. 2017), and which risk factors are at play (Jewell et al. 2016). The aim of this study was to analyse the horizontal transmission dynamics of WSD in *L. vannamei*, and to evaluate the role of some environmental components that might be involved.

First, we performed a peroral inoculation with WSSV-infected tissue inoculum (Thai-1 strain) (Thuong et al., 2016) in individually housed shrimp to characterize WSD progression. Second, we developed a peroral group infection model. Third, this model was used to identify the characteristics of an WSSV epidemic. Finally, we investigated the role of molts, feces, and water from infected populations in WSSV transmission by exposing naïve shrimp.

The WSSV Thai-1 strain had an incubation period of 24–54 hpi, and an irreversible disease progression leading to death within 78 hpi. Infected shrimp were shedding viral DNA, and this shedding reached a peak within 12 h of the time of death. The threshold density for the occurrence of a WSD epidemic in a group infection model was 10 shrimp per 10 L. At this density, the first cases of host-to-host transmission occurred between 30 and 48 hpi in parallel with the occurrence of the first mortalities. Ingestion of WSSV-infected tissues did not significantly increase the number of index cases during an epidemic compared to immersion into water in which cannibalism had occurred. Moreover, the investigation of the role of water, feces, molts, showed that exposing sentinels to rearing water collected from WSSV-infected tanks resulted in a significantly higher probability of infection than exposure to feces or molts. Therefore, we postulate that the occurrence of cannibalism of infected shrimp contributes to indirect water-borne WSSV transmission by the spread of free infectious viral particles.

This research received funding from Flanders Innovation and Entrepreneurship (Belgium).

References:
Giesecke, J., (2017), *Modern Infectious Disease Epidemiology: Third Edition, 1-233.*
Jewell, N., (2016), *Clin. Pharm. Therap., 100 (4), 353.*
Thuong, K. et al., (2016). Vet. Research, 47(1).

KEY ASPECTS OF *IN VIVO* WHITE SPOT SYNDROME VIRUS (WSSV) INFECTION MODEL DEVELOPMENT

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In vivo WSSV infection models occupy a crucial first step in the research flow that tries to elucidate the infectious disease process to develop new anti-viral treatments. Thus, the development and use of standardized *in vivo* infection models is a necessity. This review critically examines key aspects of *in vivo* WSSV infection model development that are often neglected and that can greatly affect the experimental outcomes.

A review was conducted by retrieving literature from searches of computerized databases, hand searches, and authoritative texts with the overarching purpose to synthesize the existing literature in the field (Sargeant & O'Connor, 2020).

First, standardizing and reporting factors pertaining to the components of the disease triad is crucial, because each component may influence the results of the experiments that are performed (Arbon et al., 2023). Second, the characteristics of the experimental shrimp can potentially influence the dynamics of disease expression. A characteristic that is very rarely addressed specifically in relation to WSSV infection studies is the 'shrimp seed quality'. This can vary significantly between shrimp batches, regardless of genetic or geographic origin, which could in turn cause variation between study results. Third, the choice of viral inoculum is important. The inoculum should be specific pathogen free to prevent co-infections that might influence the outcome of the study. Liquid viral stocks can be purified which minimizes potential confounding factors (Dantas-Lima, et al. 2013). Tissue inoculum is cruder and made from unprocessed or minced WSSV-infected tissues. Including a mixing or blending step in the processing procedure is advised. Fourth, the most effective inoculation method is intramuscular injection, but it artificially by-passes the host's natural defense barriers. Oral intubation and intra-bladder inoculation mimic natural transmission routes, but regurgitation and/or perforation are a risks. Inoculations through immersion, cohabitation, or feeding are less invasive, and also represent natural WSSV transmission routes, but they risk being less controllable. Fifth, the housing conditions determine for a large part which information will obtained and possibly also its validity. Individually housing allows for collection of data in a more controlled scientific setting and clinical outcomes can be evaluated individually. Group housing simulates more closely the on-farm reality of a WSSV outbreak, which might be beneficial for extrapolation to the field.

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References:
Arbon, P., et al., (2023). *Rev. Aquacult., 1-19*;
Dantas-Lima, J.J., et al., (2013), *J. Fish. Dis.*, 36, 841-851;
Sargeant, J. M., et al., (2020). *Front. vet. sci.*, *7*, 502756.

GENETIC BASIS OF THERMAL ADAPTATION IN WILD POPULATIONS OF EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Understanding the impact of warming surface waters on fish growth is crucial for both fisheries and aquaculture. We investigated the growth of three genetically distinct populations of European seabass: Atlantic (AT), West-Mediterranean (WM) and Est-Mediterranean (EM) in three seasonal thermal regimes mimicking temperatures encountered in the Atlantic (*rAT*), the western Mediterranean (*rWM*) and the eastern Mediterranean (*rEM*).

At 20 days post-hatch, the three AT, WM, EM populations were reared in common garden in three separate seasonal temperature regimes, representing *rAT*, *rWM* or *rEM*. A total of 5148 fish were genotyped with the ThermoFisher AxiomTM Seabass 57k SNP array DlabChip and PIT-tagged (± 10 g average weight) to study their growth over 1.5 years, as their daily growth coefficient (DGC). We used GBLUP models to investigate the genetic bases of local adaptation to temperature by estimating heritability and genetic correlations, to study genotype by environment interaction (GxE) for AT and EM populations (the WM population did not have enough individuals to compute robust estimations).

During the first year after tagging, growth of the AT population was higher than those of EM and WM populations in all regimes, with greater differences in colder regimes (*rAT* and *rWM*). As of the 2nd year, the growth of AT remained higher only in *rAT*. Modelling of growth rate as a function of temperature and fish size revealed that the thermal optimum of the AT population was between 24 and 26 °C whereas the optimum was above 27 °C for the WM and EM populations. At 2 years old, the AT population was on average bigger in all thermal regimes, followed by the EM and WM populations. Heritability of growth rate during the winter (0.55) was higher than in summer (0.33) in both AT and EM populations. During the summer, there were negative genetic correlations between the extreme regimes (*rAT* and *rEM*; Table 1).

These results reveal countergradient variation for growth of the AT population during the first year and, then, a specific adaptation of AT to *rAT* and EM to *rEM*. The strong GxE interaction, particularly in summer, indicates an inversion of genotype rankings for summer growth between the warmest and coldest regime. Further analyses are in progress to further explore and explain these observations.

This study is part of FishNess project (ANR-21-CE20-0043).

TABLE 1 – Genetic parameters for growth rate in summer in the AT (a) and the EM (b) populations in the three thermal regimes. Heritabilities are in bold on the diagonal. Genetic correlations are below the diagonal.

a) AT

	rAT	*rWM*	*rEM*
rAT	**0.37 (± 0.06)**		
rWM	0.75 (± 0.09)	**0.35 (± 0.06)**	
rEM	-0.41 (± 0.19)	0.29 (± 0.20)	**0.23 (± 0.08)**

b) EM

	rAT	*rWM*	*rEM*
rAT	**0.30 (± 0.09)**		
rWM	0.37 (± 0.21)	**0.34 (± 0.07)**	
rEM	-0.27 (± 0.27)	0.61 (± 0.23)	**0.37 (± 0.11)**

TREATMENT TECHNOLOGIES FOR THE DECONTAMINATION OF PHARAMCEUTICALS AFTER SEA LICE BATH TREATMENTS OF ATLANTIC SALMON

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Introduction
During salmon production, the bath product Alpha Flux (active pharmaceutical ingredient (API): hexaflumuron (HEX)) is used to treat infestations by the parasitic sea louse *Caligus rogercresseyi*. To reduce possible environmental impacts from these treatments, it is desirable to limit the discharge of pharmaceuticals after use. This can be achieved by water treatment technologies such as ozonation, chlorination, and/or ultraviolet (UV) radiation. Ozonation and chlorination in seawater leads to the formation of oxidants, commonly expressed as total residual oxidants (TRO), which can react with a variety of compounds and result in their degradation[1]. UV radiation can initiate photodegradation of chemicals due to its high photon energy[2]. Both ozonation and UV equipment is already in use on modern well boats.

Several lab-scale studies have been conducted in collaboration with NIVA through the MARTERA project WeBoat to investigate the degradation of the API HEX by ozonation, UV radiation, and chlorination.

Materials and methods
Three experiments were conducted in seawater collected from a wellboat after fish transport. In each experiment, Alpha Flux was administered at 2 mg L^{-1} HEX into 10 L wellboat water and continuously stirred for 1 h to simulate a delousing treatment. Afterwards, ozonation, UV radiation, and chlorination were applied. The ozonation and chlorination process was adjusted according to the TRO concentration (conc.).

The following treatments were tested in separate experiments:
(1) One single and two consecutive ozonations to TRO concentration (conc.) 5 mg L^{1} at pH 10, with an intermediate incubation to TRO conc. 4 mg L^{1}.
(2) Ozonation to TRO conc. 5 mg L^{1} at pH 10, followed by UV treatment at 900 mJ cm^{2} after different intermediate incubation periods.
(3) Administration of hypochlorite to TRO concentrations 10, 100, and 1000 mg L^{-1} at unadjusted pH and pH 10.
After each treatment, the water was incubated at room temperature for 24 h. Prior to sample collection, excess oxidants were neutralized by adding sodium thiosulfate.

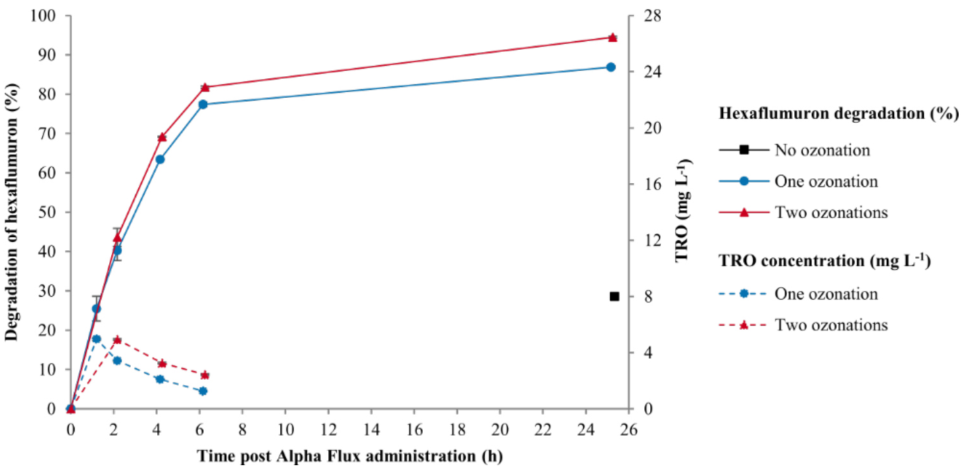

Figure 1 Degradation of hexaflumuron (%; n=2) and total residual oxidant (TRO) concentration (mg L^{-1}; n=3) after a single ozonation (blue) and two consecutive ozonations (red).

(Continued on next page)

Results

Two consecutive ozonations to TRO conc. 5 mg L^{-1} at pH 10 resulted in 44 ±2.3% HEX degradation (Fig. 1). During the subsequent 23-hour incubation period, the degradation increased to 94 ±0.2%. A single ozonation led to 4-7% less HEX degradation.

UV radiation after ozonation resulted in a lower degradation of HEX than incubation without UV. After UV treatment, the TRO conc. dropped immediately by 85%. UV radiation as a final treatment after both ozonation and 24 h incubation increased the degradation of HEX by 2.6%.

Chlorination at pH 10 resulted in a higher removal rate of HEX than chlorination at unadjusted pH. The degradation of HEX continued during the subsequent 24 h incubation period. The highest HEX degradation, 99.98%, was achieved after chlorination to TRO conc. 100 mg L^{-1} at pH 10 and 24 h incubation. At TRO conc. 10 mg L^{-1} at pH 10, 47% degradation was reached after 24 h incubation.

Discussion and conclusion

In seawater, both ozone (O_3) and free chlorine ($HClO/OCl^-$) quickly react with bromide ion (Br^-) and lead to the formation of reactive oxidants such as bromine (BrO/HOBr) and bromate/chlorate (BrO_3/ClO_3^-)[1]. These oxidants may react with HEX and result in its degradation. They can persist in the water after ozonation or chlorination, leading to the degradation of HEX during the incubation period.

In the present studies, the highest HEX degradation, 99.98%, was achieved by chlorination to 100 mg L^{-1} TRO. An increased TRO conc. is, however, accelerating corrosion[3] of wellboat equipment. In ballast water treatment, chlorination is therefore usually conducted below 10 mg L^{-1} TRO.

UV treatment after ozonation reduced the degradation of HEX. UV light can break the bond of oxidative molecules, leading to the formation of less reactive species[2]. Accordingly, UV radiation reduced the TRO concentration in the water, which may have diminished degradation reactions with HEX.

Taken together, the highest realistically feasible degradation of HEX was achieved by two consecutive ozonations to 5 mg L^{-1} TRO at pH 10.

References

[1]Kim H., H. Lee, C.-M. Kim, and A. Jang. 2020. Enhancement of ozonation of seawater-based wastewater containing pharmaceutical compounds by total residual oxidants: Salinity, ammonia, and organic matter. Chemosphere. 259: 127513.

[2]Guo K., S. Zheng, X. Zhang, L. Zhao, S. Ji, C. Chen, Z. Wu, D. Wang, and J. Fang. 2020. Roles of bromine radicals and hydroxyl radicals in the degradation of micropollutants by the UV/bromine process. Environmental Science & Technology. 54 (10): 6415-6426.

[3]Song Y., K. Dang, H. Chi, and D. Guan. 2009. Corrosion of marine carbon steel by electrochemically treated ballast water. Journal of Marine Engineering & Technology. 8(1): 49-55.

EVALUATING THE IMPACT OF DIETARY *Tetraselmis chui* EXTRACTS ON IMMUNITY AND DISEASE RESISTANCE IN EUROPEAN SEABASS *Dicentrarchus labrax* JUVENILES

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Improved nutrition through fortified diets may bring additional benefits for the industry such as growth and health enhancement. The present study aimed to evaluate how dietary supplementation of *Tetraselmis chui.* extracts would act on European seabass (*Dicentrarchus labrax*) immune system after a short-term feeding period and its outcome on resistance to a bacterial pathogen.

European seabass juveniles (14.16 ± 1.01 g) were randomly sorted into triplicate tanks according to dietary treatments. Four experimental diets were designed: (i) a control diet based on a commercial formulation for the species, and three others (with the control as basis) supplemented with (ii) low dose of *Tetraselmis chui.* extract, (iii) high dose of *Tetraselmis chui.* extract, and finally (iv) a diet supplemented with β-glucans from algae origin. A feeding period of 5 and 10 days was applied for all dietary treatments and after which fish were sampled. At the end of each feeding period, fish were intraperitoneal injected with 2.75×10^5 cfu/mL of *Photobacterium damselae piscicida* strain MM415 and mortalities were tracked for 14-days, to evaluate disease resistance. Samplings of fish gut, head-kidney, plasma and liver were performed before and 24 hours post infection.

Evaluation of immune related genes in the gut showed an upregulation of pro-inflammatory transcripts in response to the infection. In agreement, it was observed a significant decrease of peroxidase activity in the plasma, total glutathione content and lipid peroxidation of the liver in response to the bacteria. Feeding times and experimental diets, were not able to significantly modulate the immune status and immune response in the presence of the pathogen. Nevertheless, other feeding times and supplementation levels should be considered to better understand the potential of *Tetraselmis chui.* extracts to improve fish health.

TREATMENT OF SLUDGE PRODUCED IN A RECIRCULATING AQUACULTURE SYSTEM: TEST OF DIFFERENT FLOCCULANTS TO IMPROVE THE SEDIMENTATION OF SUSPENDED SOLIDS

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Sludge is a semi-solid by-product generated by aquaculture activities that contains faeces, uneaten feed, as well as a variety of dissolved compounds such as nitrogen, phosphorus, and other minerals. The management of the sludge produced is a challenge as the release of sludge into the environment represents an environmental concern. If this product is not managed properly, it can lead to eutrophication of natural waters and generation of greenhouse gases. One of the main issues related to sludge management is the removal of the liquid fraction and the concentration of the solid part. Flocculation is a common method to increase the size of the suspended solid and promote their precipitation. Potentially, the supernatant could be freed from most solids and re-used in the system avoiding its discharge.

In the present study, sludge produced by Atlantic salmon (*Salmo salar*) reared in freshwater and European seabass (*Dicentrarchus labrax*), both farmed in Recirculating Aquaculture System (RAS), was treated in different ways in order to identify the most efficient methodology. Flocculation using ozone and acrylamide-based flocculants was tested. The flocculants were applied before the sludge entered the drum-filter, in order to increase the size of the solids by forming flocs and therefore to make easier their removal with the back-wash function of the drum-filter. The highest concentration of ozone added to the sludge was 680µg/L in 30 minutes. This concentration was not sufficient to induce flocculation. However, higher concentrations could not be tested as the saturation point of the gas in the water was reached at this concentration, causing ozone release in the air, and constituting a health hazard.

When the acrylamide-based flocculants were applied after the drum-filter, the collected sludge from treated wastewater did not reach the desirable levels of solids content (>20%). However, 87.4% turbidity removal was achieved for wastewater of *D. labrax* and 90.64% for *S. salar*. Moreover, flocculants resulted in a 3.4% Total Dissolved Solids removal in the supernatant of sea bass wastewater and 32.8% less solid content in the supernatant of the flocculated wastewater of Atlantic salmon.

Flocculation provided the desired results in less than 5 minutes. If the wastewater is not treated, the sedimentation takes more than 30 minutes, resulting in supernatant water containing more solids and with higher turbidity. Trials using different flocculants are currently ongoing.

The present study is part of the SEA2LAND Project funded by EU, under GA number 101000402.

ALTERNATIVE PROTEIN SOURCES TO BOOST FISH HEALTH AND GROWTH TRHOUGH AN EUROPEAN FUNDED PRIMA PROJECT "CIPROMED"

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The aquaculture sector is a major contributor to global fish and seafood production, heavily relying on marine resources to create balanced and nutritious diets. However, the rising cost of such raw materials and the environmental footprint caused by their overexploitation pose a significant challenge to the aquaculture production. To address these challenges, a growing interest has been showed to the utilisation of unconventional feed ingredients, including plant by-products, macro and microalgae, single cell protein, insect meal and others.

In this context, the CIPROMED project, funded by the European Prima program, focuses on the valorisation of agri-industrial side-streams for insect and microalgae production. The resulting high-value proteins will be tested in marine and freshwater fish of commercial interest through feeding trials and challenge tests to evaluate growth performance, digestibility, product quality and health parameters.

Novel ingredients such as insect meal (*Hermetia illucens*) and a blend of insect and microalgae (*Chlorella vulgaris* or *Spirulina platensis*) will be tested in rainbow trout (*Oncorhynchus* mykiss), European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). At the AquaBioTech testing facility in Malta, the digestibility of each ingredient will be assessed and subsequently used to formulate nutritious and balanced diets to evaluate growth performance, health parameters and histology or microbiota in rainbow trout and European seabass. Additionally, ABT will perform challenge tests using commercially relevant pathogen in juveniles of both species to assess the efficacy of bioactive compounds contained in the ingredients tested. General health, necropsy, histology, bacteriology, and PCR analysis will be undertaken to determine the feed's efficacy against the selected pathogens.

Acknowledgement
The CIPROMED project is supported and funded by the PRIMA programme under Horizon 2020. Grant agreement No 2231.

EVALUATION OF BLUEFIN TUNA, *Thunnus thynnus* BY-PRODUCT MEAL AS FISHMEAL REPLACEMENT IN DIET FOR EUROPEAN SEABASS, *Dicentrarchus labrax*

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The aquafeed industry is showing a growing interest in alternative raw materials such as fish by-products (heads, bones, viscera, gills, spines, fins and skin), to be used as feed for aquatic organisms due to the overexploitation of conventional marine resources.

The aim of this study was to evaluate the use of bluefin tuna side-streams from a Maltese fish farm as a fishmeal replacement in formulated diets for European seabass (*Dicentrarchus labrax*).

The trial was conducted in a 12 x 650L RAS system. Three experimental diets with 10%, 20% and 50% fishmeal replacement (10TBM, 20TBM and 50TBM) were formulated to be isonitrogenous, isolipidic and isoenergetic, and were tested in triplicate. European seabass, *Dicentrarchus labrax* (100 fish/tank; initial weight of 17.49 ± 0.04g; stocking density of 2.7kg/m^3). Fish were fed four times a day until satiation for 10 weeks, after which a bulk weight of each replicate was performed. 5 fish from each tank were randomly selected to record total weight and length as well as liver, spleen and total viscera weight. Growth performance (FCR, SGR, SFR%, feed intake) as well as K, VSI, HSI, SSI, were evaluated and compared among diets.

Overall, there were no significant differences in growth and biometric indexes among the treatments. Fish fed 10TBM showed comparable growth to the control diet (69.36±3.33g and 69.42±2.64g), while fish fed TBM20 and TBM50 had slightly lower growth values (67.36±3.83g and 65.82±1.84g). FCR, SGR, SFR%, and feed intake also showed no significant differences among treatments. The K factor was highest in fish fed 10TBM (1.34±0.03) and the lowest in fish fed the CTRL diet (1.31±0.03). VSI showed the lowest value in fish fed 10TBM (12.38±1.41) and the highest value in fish fed 20TBM (13.03±0.68). The HSI and SSI indexes followed a similar pattern, with the highest values recorded in fish fed CTRL and 10TBM diets and the lowest in the remaining treatments.

The tuna-based fish meal proved to be an efficient protein source.

Acknowledgements
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PRELIMINARY INSIGHT INTO THE INCORPORATION OF COMMON HOP *Humulus lupulus* L. INTO THE FISH FEED: EFFECT ON GROWTH PERFORMANCE, BLOOD BIOCHEMICAL AND ANTIOXDANT PROPERTIES OF CARP *Cyprinus carpio* L.

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Common hop (*Humulus lupulus* L.), as a medicinal plant, has health-promoting properties due to phenolic compounds. In the present study, common carp (*Cyprinus carpio* L.) juveniles (Initial weight of 32.22±0.65 g) were fed with experimental diets, without supplements (Control), with hop extract at levels 0.75 g/kg ($H_{0.75}$) and 1.5 g/kg ($H_{1.5}$), manually for eight weeks. The experiment comprised three groups, three replications, and nine tanks, and each tank housed 6 fish and was fed twice daily based on 3% biomass. At the end of the feeding trial, growth performance, whole-body proximate composition, blood biochemistry, antioxidant activity, and fatty acids profile were evaluated.

No significant differences in growth performance were observed among groups (p>0.05). No significant changes in lipid and ash contents in the whole-body proximate composition among all groups were found, but the $H_{1.5}$ group showed higher crude protein content compared to the control group (p<0.05). The activity of LDH in group $H_{1.5}$ was significantly higher among the groups (p<0.05). The activities of ALT and ALP were significantly lower in both hop groups compared to the control (p<0.05). LPO and GSH were significantly higher in 0.75 g/kg common hop compared with the control group (p<0.05). The content of n-3 polyunsaturated fatty acid, n-6 polyunsaturated fatty acid, monounsaturated fatty acid, and saturated fatty acid significantly differed in the $H_{0.75}$ and $H_{1.5}$ groups (p<0.05). $H_{0.75}$ significantly increased the proportion of heptadecenoic acid, α-linolenic acid, heneicosanoic acid, docosapentaenoic acid, and docosahexaenoic acid compared with the control treatment (p<0.05). In addition, both doses of hop raised considerably the value of oleic acid and eicosatrienoic acid (p<0.05), and octadecenoic acid and eicosapentaenoic acid were changed significantly by $H_{1.5}$ (p<0.05).

ADAPTIVE EVOLUTION FOR OPTIMIZATION OF AN INDUSTRIALLY-RELEVANT MICROALGA

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Microalgae are the primary producers of omega-3 fatty acids in the marine food chain and could therefore serve as a direct resource of these valuable nutrients, however microalgal production needs to be optimized. This would help to mitigate the alarming decrease in wild fish stocks that are currently the main resource of these fatty acids. Thraustochytrids accumulate lipids in the cell as a defence mechanism against various stress factors, and can produce biomass with more than 50% of their cell dry weight as lipids, in which omega-3 fatty acids account for more than 40% of total lipids. Lack of genetic resources for these non-model strains makes rational genetic engineering challenging. Therefore, this project aims to enhance the phenotype of a thraustochytrid strain by using the Adaptive Evolution approach, focusing on increasing the oxidative stress resistance and production of biotechnologically relevant metabolites. Both long-read and short-read sequencing data were used to generate a high-quality genome assembly that is used as a reference for the subsequent mutant analysis. The final genome assembly showed improved quality and completeness compared to other publicly available references, and consists of 33 contigs, >95% sequence identity and 91.09% of complete single-copy orthologous genes. Generation of a completely annotated genome and transcriptome combined with multi-omics analysis is used to elucidate the connection between genotype and phenotype in the evolved strains. We will discuss the key-genes involved and molecular mechanisms underlying such an adaptation, and provide novel biomarkers for targeted genetic engineering for metabolic products and pathways of interest.

MINIMIZING PHOSPHORUS DISCHARGE FROM AQUACULTURE

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While there are effective methods for removing nitrogen and organic matter from the effluent of land-based fish farms, there is a lack of cost-effective strategies for removing phosphorus.
Phosphorous is an important potential pollutant to natural water bodies. Any excess dietary phosphorus that fish don't absorb and incorporate is excreted in their feces and urine, and fish farming might lead to phosphorus eutrophication. The waste production from aquaculture, especially the fraction in dissolved form (orthophosphate), is difficult to remove and withhold in treatment devices. Therefore, minimizing excretion from the fish is a promising way to reduce farm discharge.

To minimize the environmental impact of fish farming in terms of phosphorus, the total dietary phosphorus content must be minimized while ensuring that the available dietary phosphorus matches the fish's needs. This study tested how much total dietary phosphorus can be lowered without compromising fish performance. This was achieved by carefully selecting raw materials with knowingly high phosphorus availability and adding phytase to improve the uptake of plant-based, phytin-bound phosphorus. Several low-phosphorus diets, tailored to match fish requirements, were produced and fed to rainbow trout (*Oncorhynchus mykiss*) in a mass-balance setup as well as to rainbow trout in a commercial recirculating aquaculture system (RAS).

Overall, the study showed that it is possible to significantly reduce the excretion of total phosphorus including reducing the excretion of dissolved phosphorus to a minimum without affecting fish performance, thereby reducing the discharge and/or the need for subsequent effluent phosphorus treatment.

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IN VIVO INFECTION MODELS TO TEST THE EFFICACY OF ANTIVIRAL THERAPIES AND IMMUNOMODULATORS AGAINST WHITE SPOT SYNDROME VIRUS (WSSV) IN *Litopenaeus vannamei* SHRIMP

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In vivo white spot syndrome virus (WSSV) infection models are indispensable tools to support the development of antiviral therapies and immunomodulators against white spot disease (WSD). Still, the selection of the most appropriate models for anti-WSSV product development is typically not straightforward. The objective of this review was to further elaborate on this subject to provide investigators with solid arguments for or against the selection of certain types of WSSV infection models in specific research contexts.

A review was conducted by retrieving literature from searches of computerized databases, hand searches, and authoritative texts with the overarching purpose to synthesize the existing literature in the field (Sargeant & O'Connor, 2020).

To determine if anti-WSSV product candidates have virucidal properties, it is advisable to carry out *in vivo* antiviral activity tests. Multiple product candidates can be screened in parallel, which reduces the costs and the time spent in development. Since immunomodulators target the host rather than the virus, antiviral activity testing is not applicable. Both for antiviral and immunomodulators product candidates, it is recommended to test *in vivo* if the chosen delivery method manages to deliver a sufficient concentration of the active substance to intended site(s) of action. Next, the efficacy of the product candidate can be tested during *in vivo* infection experiments. An inoculation procedure that resembles natural infection (immersion, feeding of WSSV-infected tissues) is expected to help translate study results to the field. Housing shrimp individually instead of in groups when the delivery methods and the efficacy of the therapy still need to be established, is advised, because individuals can easily be monitored and sampled. The evaluation of the product candidate's protective properties against WSSV can be based on clinical observations (survival rates and pathogen counts), or additional health parameters that can be evaluated through the model and/or additional laboratory tests. At a later development stage, it is more beneficial for the extrapolation of the results to house shrimp in groups instead of individually. This facilitates natural transmission between hosts, which mimics the situation in the field, but in a laboratory setting the biotic and abiotic parameters can still be controlled. It is therefore recommended to test the product candidates in these controlled conditions, before proceeding to assess their effectiveness under 'real-world' conditions in field studies on farms.

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Reference:

Sargeant, J. M., et al., (2020). *Front. vet. sci.*, *7*, 502756.

EFFICACY OF VARIOUS CONCENTRATIONS OF SYNTHETIC HORMONES ON THE INDUCED BREEDING OF *Channa marulius* (Sole)

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A study was conducted to determine the artificial breeding response, embryonic and larval development of giant snakehead, Channa marulius, with the application of various dosages of stimulatory hormones: Suprefact® (LHRH) agonist and Ovaprim® (GnRH + dopamine inhibitor). First and second hormonal dosages of Suprefact® (diluted) and Ovaprim® (0.3, 0.4, and 0.5 ml for male and 0.8, 0.9, and 1.0 ml for females per kilogram body weight) were used. There were three treatments T1, T2, and T3 and each treatment had three replicates. Male fish were treated with T1 (0.3), T2 (0.4), T3 (0.5) ml kg^{-1} of body weight while female fish with T1 (0.8), T2 (0.9), T3 (1.0) ml kg^{-1} of body weight. The results showed that fish stimulated with T3 (1.0) obtained better fecundity rate (2951.7) followed by T2 (0.4) (2678.3), while the lowest fecundity (466.7) was recorded in T1. The numerically higher values of gonado-somatic index (males and females) and fertilization rate appeared in T2. The highest survival rate (97.1%) was found in T3 followed by T2 (97.0%), while the lowest (32.2%) in T1. In conclusion, the application of Suprefact® and Ovaprim® at 0.4 and 0.5 ml kg^{-1} body weight for male and 0.9 and 1.0 ml kg^{-1} for female fish successfully stimulated snakehead fish induced breeding.

INTESTINAL FLUID TRANSPORT CAPACITY IN ATLANTIC SALMON SMOLTS AND EFFECTS OF SEAWATER TRANSFER TIME

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Smoltification, is a transition stage where Atlantic salmon parr prepare for a life in seawater (SW) while still in freshwater (FW). In SW, fish experience osmotic water loss, therefore the intestine is responsible for net water absorption through ion-driven fluid uptake to prevent dehydration. During smoltification, there is a gradual increase of fluid and nutrient transport capacity driven by increased Na^+/K^+-ATPase (NKA) activity, stimulated by cortisol. This capacity is further enhanced when smolts are transferred to SW. Further, in the Atlantic salmon aquaculture, SW transfer remains a significant challenge that may result in poor growth, and high mortalities. At present, optimal time for SW transfer is commonly determined based on the NKA gene transcript in gill and a seawater challenge test. However, the readiness of the intestine is often overlooked. In addition, fish are fasted for 3-4 days before SW transfer to maintain the water quality during well-boat transportation. Yet, the consequences on intestinal osmoregulatory capacity have not been studied. The aim of the study was to investigate how fasting and seawater transfer time affect intestinal fluid transport capacity and thereby SW acclimation of Atlantic salmon smolts.

Atlantic salmon smolts were produced at the Lerang Research station, Norway (Skretting Aquaculture Innovation) by use of a winter light regime (12 hours light and 12 hours darkness, 12:12) for 6 weeks, before the onset of continuous light (24:0) at 12 °C in FW. Smolts were transferred to SW at three timepoints as defined by the degree days (dd); (Early 168 dd, Normal 348 dd, and Late 516 dd). On each day of transfer, fish had been fed or fasted for 4 days. Sampling occurred; (1) in FW (right before SW transfer) and (2) in SW (6 weeks after SW transfer) where fish body weight (g) and intestinal mucosal wet weight (g) were measured. Gut sac method and Ussing chamber technique were used to measure the intestinal fluid transport and nutrient transport capacity respectively. NKA activity was also determined.

Mucosal weight and fluid transport capacity significantly increased with transfer time. Smolts transferred at 516 dd had higher mucosal weight and fluid transport capacity after 6 weeks in SW compared to 348 dd (Fig1). Interestingly, four days of fasting significantly reduced mucosal weight and fluid transport capacity in FW smolts even though NKA activity increased by fasting. The reduced transport tended to be sustained even 6 weeks after SW transfer and normal feeding (Fig1). Our results show that smolts transferred later (516 dd) than the normal (348 dd) have increased fluid transport capacity due to higher intestinal mass and perform better the first month after SW transfer. Further, fasting reduce the mucosal tissue and the intestinal fluid transport capacity regardless of the transfer time.

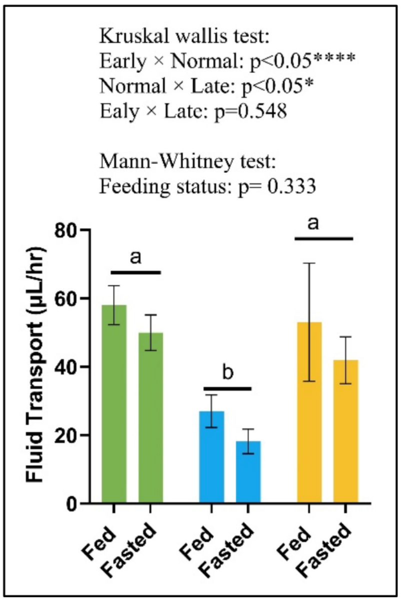

Figure1: After 6 weeks in SW (**Early 168 dd**, **Normal 348 dd**, **Late 516 dd**)

BYSSUS ALTERATIONS IN MUSSELS EXPOSED TO A PROLONGED THERMAL STRESS

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Mussels are farmed in suspended net socks, to which they are attached by byssus. This is a natural fiber with a particular composition and structure, affected by the changing of chemical/physical sea water parameters, like the temperature. In the present study, byssus production and resistance to traction were evaluated in mussels (*M. galloprovincialis*) after a prolonged exposure to high temperature.

For 30 days, farmed mussels were divided in two groups: the control one at temperature of 20 ± 0,5°C and the high temperature exposed group at 28° ± 0,5°C. They were placed on square of polypropylene (PP) film in order to simulate the attach to PP socks used in farming. A portion of the basal foot tissues was collected at 3, 5, 10 and 30 days after high temperature exposure to evaluate the expression of the three precursors genes of the byssus structural collagen, *preCOL-D, preCOL-P* and *preCOL-NG*. At the final time point, all the mussels attached on PP squares were removed from the supports, cutting the byssus, for the traction resistance test.

A collapse was found for the expression of *preCOL-P*, *preCOL-D* and *preCOL-NG* at 30 days of exposure (Fig.1). About the mechanical resistance to the traction, the exposed threads are less resistant and deformable, and support half of the maximum force of control group. For the plaque adhesion resistance, no difference emerged between the groups.

The prolongation of the thermal stress affected the capacity of the basal gland of the foot to express *preCOLs* genes. The cost for byssus production depends on the available animal energy budget, high temperatures reduce it, consequently mussels do not have enough energy to spend for byssal collagen synthesis, which is then reflected in the reduced byssogenesis and in the lower tensile strength. Moreover, the heat stress creates an interference in collagen polymerization leading to a decrease in byssal thread resistance. Lower tensile strength represents a serious risk for the production quantity and sector economy: the more lasting the warming of sea water, the more mussels might break away from the farming socks.

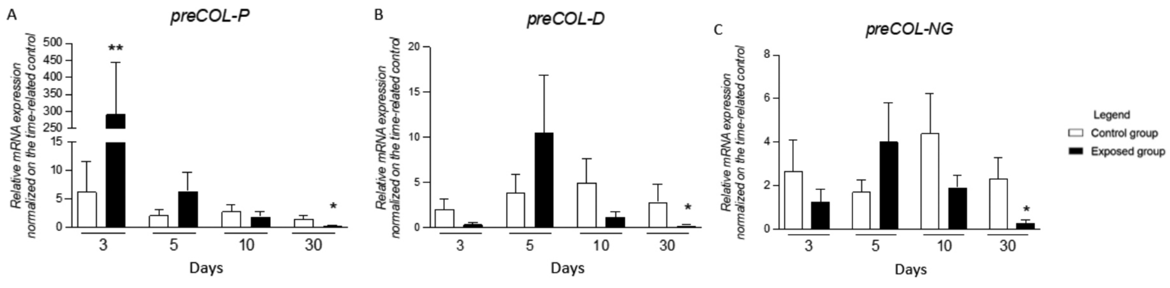

Fig. 1: Effect of temperature on *preCOL-P* (A), *preCOL-D* (B) and *preCOL-NG* (C). mRNA expression in foot tissue of the exposed group was normalized on the time-related control for each time point. Asterisks represent the differences between the high temperature-exposed and non-exposed group at the same time point (* $p < 0.05$; ** $p < 0.01$).

ASSESS THE IMPACT OF UNSTABLE ENVIRONMENTAL CONDITIONS ON WELFARE OF MANILA CLAM *Ruditapes philippinarum*

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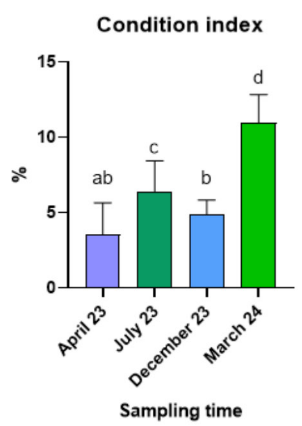
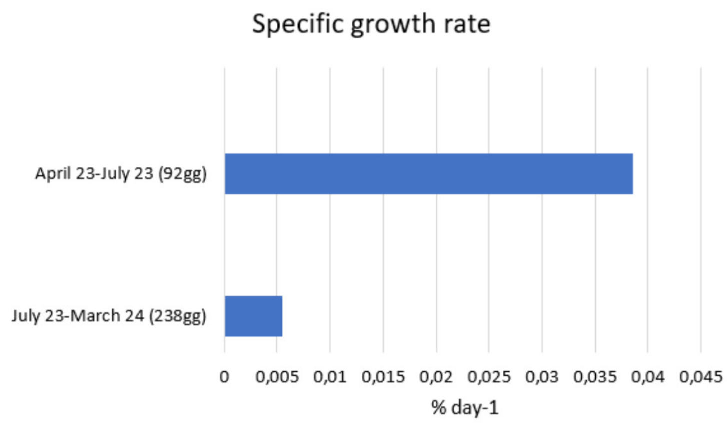
Shellfish farming productivity is greatly affected by climate change. Through the activities of this study it was possible to highlight how the welfare of Manila clam changes currently during the seasons and against unpredictable and unstable weather/environment. The data collected were cross-referenced with each other in order to assess the repercussions of climate change on the Manila clam production chain from spat to commercial size.

Every four months from April 2023 to March 2024, a batch of Manila clam fattened in the Goro lagoon (northern Adriatic Sea), was collected and analyzed in university facility in order to evaluate the biometric parameters of performance, welfare and growth during the seasons and climate changes. The parameters were: the length, the weight of the soft body, the clearance rate, the condition index (CI). In addition, physic-chemical water parameters were monitored, like temperature, salinity, pH and concentration of nitrites, phosphates and chlorophyll.

From the results obtained so far, it is evident that the Manila condition index increased in July, considered the hottest season in 2023, but the values return to initial conditions in the winter season. With low temperatures, the metabolic rate of clams is reduced and this has repercussions on the pulp yield. At the beginning of spring 2024, the values reach a peak, probably due to a rise in temperatures that stimulates the start of gonadal maturation that inevitably also defines an increase in CI.

It is impressive how the specific growth rate in 4 months between spring and summer 2023 was so high, compared to the growth had until the following spring, proof of the rapidity with which the spat grows especially in the early and warmer seasons.
This research was undertaken under the BIVALVI (ERA-NET BlueBio: Advancing European bivalve production systems) project, which has received funding from the European Union's Horizon2020 Research and Innovation programme under grant agreement no. 817992 (https://bluebioeconomy.eu/advancing-european-bivalve-production-systems/).





TACKLING THE SHRIMP HATCHERY BOTTLENECKS FOR EUROPEAN SHRIMP AQUACULTURE AND ASSESSING THE DEVELOPMENT OF A MULTISPECIES HATCHERY IN EUROPE (BLEUMARINE[3].COM)

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There is a growing interest in the development of local aquaculture production throughout Europe. Many initiatives exist, such as indoor penaeid shrimp farming, bivalve and seaweed culture, or restauration of the flat oyster (*Ostrea edulis*) reefs in the North Sea. However, one of the common problems across many initiatives is the struggle to source high quality and continuously available starting material, pathogen free and with a suitable genetic background. During the BlueMarine[3].com project, the expertise of Ghent University and six Flemish companies was joined to tackle the challenges related to sourcing material. For this, hatchery protocols for shrimp, bivalves and seaweeds were adapted to the local conditions, keeping in mind economic feasibility and sustainability for the environment. The aim of the project was not only to develop a compact, closed recirculation system for larval production on land considering the high costs for land, labor and energy in Europe; but also to study and to value the synergy between hatchery techniques for the different species in terms of infrastructure, resources, breeding and management (i.e. a multispecies hatchery concept).

For shrimp (*Litopenaeus vannamei*) larval rearing, IMAQUA joined forces with the Ghent University and Proviron to tackle the current bottlenecks. During the project, broodstock diet composition and feeding schedules were optimized. The project revealed that lowering the water temperature by 3°C during the maturation of the broodstock animals, had no impact on the breeding success of the animals (up till PL1), increasing the economic feasibility of the shrimp hatchery in the European temperate climate. At the level of the larval rearing, several larval diet formulations were finetuned, ensuring high-quality postlarvae through the use of (live) microalgae. Within the project, also several seawater sources were compared, indicating that both natural and artificial seawater can be used to perform high-quality shrimp breeding and production, although the sea salt mixture source must be carefully chosen. The successful cultivation of *L. vannamei* under conditions more suitable for European realities laid the foundation for the successful development of the European shrimp farming industry. Additionally, this increases the possibility of incorporating *L. vannamei* into multispecies recirculating systems with colder water species.

"LIKE MOTHER, LIKE LARVAE": MATERNAL CONTRIBUTION TO OFFSPRING PERFORMANCE AND MOLECULAR PROFILE

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There is a number of evidences, that maternally-derived molecules are crucial for proper embryo development. However, developmental consequences purely maternal origin are poorly understood at both zootechnical and molecular levels. The current research involves comparative analysis of two extreme maternal phenotypes (wild vs. domesticated) as an original approach to delve into maternal factors, mediated by maternal effect genes, on larvae phenotype. Additionally, this research not only sheds light on the importance of maternal impacts in shaping larval traits but also provides insights into the broader implications of domestication on offspring development.

We established 12 experimental groups by crossing Eurasian perch (*Perca fluviatilis*) females from both domesticated (D) and wild (W) origins with cryopreserved semen from the same wild males each time, resulting in two types of crosses: DW and WW (denoting the maternal lineage as the first letter). After hatching, the larvae were reared for 30 days, during which zootechnical data (i.e., length, weight, swim bladder inflation rate, cannibalism rate, mortality) were recorded. Additionally, larvae at the mouth-opening stage were sampled for transcriptomic analysis. Obtained RNA-sequencing data were mapped to the reference genome *P. fluviatilis* and analyzed to detect differentially expressed genes between groups.

The zootechnical analysis revealed no significant differences ($p > 0.05$) in fertilization and deformity rates. Mortality rate was notably higher ($p < 0.05$) in WW larval group. Conversely, larvae from DW group showed increased cannibalism rate and more efficient swim bladder inflation rate ($p < 0.05$). At mouth opening stage, DW larvae were larger and heavier than WW larvae, but the latter exhibited compensatory growth, surpassing domesticated ones in size and length as development progressed. Transcriptome analysis between groups highlighted genes involved mostly in metabolic- related processes and muscle development and suggested *perm1*, *pgr* and *egln3* as potential maternal-origin markers of domestication.

The study found significant differences in larval performance based on maternal origin. Despite standardized rearing conditions, significant differences emerged between offspring from wild and domesticated females. Higher mortality rates in offspring from wild females indicate potential maternal effects on larval survival, while domesticated larvae displayed superior swim bladder inflation and increased cannibalism tendencies suggesting a maternal effect on behavior. However, compensatory growth observed in wild larvae suggests adaptive mechanisms to overcome initial disadvantages. Transcriptomic analysis revealed metabolic processes predominating among differentially expressed genes, indicating a maternal role in influencing offspring metabolism. These findings contribute valuable insights into the interplay between maternal origin, larval traits, and gene expression patterns, crucial for understanding the complex dynamics of fish domestication and its implications for aquaculture practices.

DIETARY POTASSIUM REQUIREMENT OF *Litopenaeus vannamei* UNDER DIFFERENT SALINITY CONDITIONS

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White-leg shrimp (*Litopenaeus vannamei*) can tolerate a wide salinity range (0.5 – 40 ppt), which makes it possible to cultivate this species under different water salinities. Shrimp farmers however face problems, among others with reduced shrimp performances at low salinity. This might be caused by changes in water ion profiles, including potassium (K^+) concentrations. K^+ is the primary intracellular cation directly involved in maintaining ionic regulation in shrimp. Insufficient availability of K^+ can potentially have negative effects on the osmoregulation ability of shrimp and thereby affect shrimp performances. To improve ionic profiles of the rearing water, shrimp farmers add mineral fertilizers, which can be costly, particularly for large shrimp farms. One possible alternative could be dietary K^+ supplementation. To date, studies on dietary K^+ requirement of *L. vannamei* under iso-osmotic and challenging conditions (e.g., low salinity) are limited. This study aims to determine dietary K^+ requirement of *L. vannamei* by investigating growth, feed utilization, and body mineral compositions under iso-osmotic (25 ppt) and challenging (5 ppt) salinity conditions.

Five semi-purified diets were formulated with different levels of K^+ supplementation: 4, 8, 12, 16, and 20 g/kg diet. The diets were tested in 4 replicates at two different salinity levels using the same shrimp in two periods; 8 weeks under 25 ppt salinity (Period I, iso-osmotic condition) and 2 weeks under 5 ppt salinity (Period II, challenging condition). In period I, 30 shrimp (mean initial weight of 5.51 g) were stocked per tank, and in period II, the number of shrimp was reduced to 15 shrimp/tank (mean weight of 15.44 g). At the end of period I, salinity was gradually decreased from 25 ppt to 5 ppt (over 8 hours). Shrimp were fed to apparent satiation. Whole shrimp and hemolymph samples were collected at the end of periods I and II to determine mineral compositions. These samples are being analysed in the lab at this moment.

Repeated measure ANOVA showed that dropping salinity from 25 to 5 ppt affected shrimp survival ($p < 0.05$). In both water salinity conditions, no effect of dietary K^+ levels was observed on shrimp survival (Fig. 1-A), growth (Fig. 1-B), feed intake, and FCR ($p > 0.05$). These findings indicate that the different dietary K^+ levels used in this study did not influence survival, growth, and feed intake of white-leg shrimp both in iso-osmotic and challenging conditions. Hemolymph and body mineral composition, and body mineral retention will be presented during the presentation, but are now being analysed.

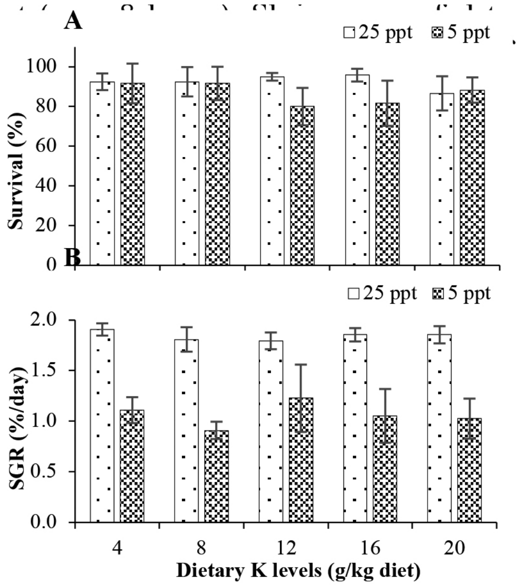

Figure 1: A. Effects of dietary K^+ levels on survival (%) and B. SGR (%/day) of *Litopenaeus vannamei* under two different salinity levels (25 & 5 ppt).

GROWTH, LIPID PEROXIDATION, ANTIOXIDANT ENZYME ACTIVITY AND BIOCHEMICAL STATUS OF SIBERIAN STURGEON *Acipenser baeri* FED DIETARY FERMENTED PRODUCT WITH KOMBUCHA AND MILK KEFIR GRAINS

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The primary objective of this study was to assess how incorporating a fermented product with kombucha and milk kefir into feed impacts the growth, oxidative stess levels, and overall health of Siberian sturgeon. Kombucha is known to have several beneficial effects on health due to the higher source of citric acid, butyric acid, lactic acid, acetic acid, and isovaleric acid and may be used as an alternate source of probiotics. Milk kefir granules also contain a wide variety of microbiota, which includes lactic acid bacteria, acetic bacteria and yeast. Fermented product, designed to have increased bioactive properties, was obtained using a blend of artisanal cultures comprising kombucha and milk kefir granules cultivated in black tea, sucrose and bovine colostrum. For the present experiment has been used lyophilized product contains postbiotics with functional proprieties, such as organic acids (citric - 18.38 mg / mL, butyric - 104.14 mg / mL and isovaleric - 6.03 g / mL) and phenolic compounds (gallic and caffeic acids -170.36 and 21.56 µg /mL, epicatechin - 9946.63 µg /mL, isorhamnetin- 4,20 µg/mL, hesperidin - 456,05 µg/mL, quercetin 3-β-D-glucoside - 18,42 µg/mL). The compounds detected in the obtained product had a positive impact on the functional properties of the product in terms of acidification potential, and antimicrobial and antioxidant properties. For the trial , the fish with an initial weight of 263.80±81.43 g were randomly distributed into four feeding treatment groups, in triplicates (17 fish/tank): V_1- control group, where fish were fed normal diet containing 54% crude proteins and 15% lipids, and experimental groups were feremented product was supplemented in the fish diet at 1 g/kg feed (V_2), 2 g/kg feed (V_3) and respectively 3 g/kg feed (V_4). The feed was administered for 40 days. The results showed that the administration of fermeneted product in the feed had no significant effect on the growth performance of the fish (p>0.05). Parameters such as feed conversion factor (FCR), specific growth rate (SGR), and protein efficiency ratio (PER) remained comparable across all experimental variations (FCR- V_1- 1,1±0.06 g; V_2-1.01±0.08 g/g; V_3-1.02±0.18 g/g and V_4-1±0.03 g/g; SGR- V_1- 1.17±0.08 g day^{-1}; V_2-1.16±0.07 g day^{-1}; V_3-1.17±0.03 g day^{-1} and V_4-1.17±0.03 g day^{-1}; PER- V_1- 1.84±0.05 g/g; V_2-1.84±0.14 g day^{-1} g/g; V_3-1.84±0.32 g/g and V_4-1.86±0.05 g/g). However, the condition factor showed slightly improved values in V_3 variants where fermented product was added at a dosage of 2g/kg feed. Furthermore, the best antioxidant enzyme activity (GSH, SOD) outcomes were observed in the V4 variant, which also exhibited the lowest MDA concentration. Notably, the introducing the product into the fish feed, particularly at the 2g/Kg level, enhanced the overall health profile of the sturgeons.

INDOLE SIGNALING, A PROMISING TARGET TO CONTROL VIBRIOSIS IN AQUACULTURE

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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. Therefore, novel methods to control vibriosis are needed. 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.

We found that indole controls several virulence-related phenotypes in vibrios, most notably biofilm formation and motility. Further, we found that 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), acute hepatopancreatic necrosis disease (AHPND)-causing *V. parahaemolyticus* (in brine shrimp) and *V. tasmaniensis* (in blue mussel). At the tested 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. Most notably is the auxin plant hormone indole-3-acetic acid. We found that indole-3-acetic acid decreases the biofilm formation and motility of *V. campbellii*, *V. harveyi* and *V. parahaemolyticus* strains and protects brine shrimp larvae from these pathogens. Moreover, addition of indole-3-acetic acid to the feed completely protected whiteleg shrimp from AHPND. Interestingly, auxins are not only produced by terrestrial plants, but also by algae and seaweeds. 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. A second interesting group of synthetic indole analogues are halogenated indoles. We investigated the impact of 31 halogenated indoles on *V. campbellii*. Five of these compounds increased the survival of brine shrimp challenged with *V. campbellii* to over 80% when added at 10 μM.

ADDRESSING THE NEED FOR HIGH-CONTAINMENT AQUACULTURE PATHOGEN FACILITIES THROUGH THE UNIVERSITY OF MAINE'S AQUATIC ANIMAL HEALTH LABORATORY

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The need for robust biocontainment facilities for studying pathogens in aquaculture research has escalated parallel to the intensifying risk of aquatic disease outbreaks in large-scale commercial fish farming. This necessity is met by the University of Maine's Aquaculture Research Institute which provides an integrated, advanced infrastructure designed to address the complex challenges of aquatic animal health and disease. The University of Maine's Aquaculture Research Institute employs cutting-edge facilities and protocols, highlighting a comprehensive approach to biocontainment in aquaculture research.

The Diagnostic Research Laboratory (DRL) features over 1,000 square feet of dry lab space, equipped for a broad spectrum of *in vitro* research, including virology, cell culture, bacteriology and molecular biology. This facility supports high-level research and fosters an environment for groundbreaking discoveries in aquatic pathogen containment, diagnostics, and novel therapeutic treatments.

The adjoining Aquatic Animal Health Lab consists of over 2,150 square feet of flexible wet lab space and adheres to Biosafety Level 3 standards to enable research in exotic species and/or high risk pathogens. This facility is equipped with sophisticated systems for disease trial environments, wastewater disinfection, and air handling to mitigate biohazard risk.

The integration of these high-containment facilities showcases a forward-thinking approach to aquaculture disease management and research. Through its sophisticated design and equipment, the Aquaculture Research Institute is not only advancing aquaculture health research but also playing a pivotal role in ensuring the sustainability and biosecurity of aquaculture practices.

ESTABLISHING AN ATLANTIC SALMON (*Salmo salar L.*) PRIMARY GILL CELL LINE FOR ADVANCING RESEARCH ON INFECTIOUS SALMON ANEMIA VIRUS (ISAV) HPR0

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Infectious Salmon Anemia Virus (ISAV), belonging to the *Orthomyxoviridae* family, consists of eight negative single-stranded RNA segments and is the causative agent of infectious salmon anemia (ISA), a serious disease of Atlantic salmon (*Salmo salar L.*). ISA can cause significant mortalities of up to 90% in infected aquacultured Atlantic salmon . ISAV can be classified into two subtype groups, a highly virulent HPR-delete variant and avirulent non-delete variant (ISAV-HPR0). Whether or not avirulent HPR0 leads to virulent HPR-delete remains in question. Understanding the dynamics between these two variants could play a vital role in helping to control disease outbreaks caused by ISAV-delete and thus lead to a more economically sustainable Atlantic salmon aquaculture industry. One major hurdle to better understanding the role that ISAV-HPR0 plays in virulence and disease is due to the inability to propagate and amplify ISAV-HPR0 in cell lines. Because ISAV-HPR0 appears to mainly target Atlantic salmon epithelial gill cells, a method for culturing primary gill cells from Atlantic salmon was developed. In repeated trials, 100% confluency was obtained in 25cm^2 cell culture flasks but subculture of these cells was not achieved. Establishing Atlantic primary gill cell lines could lead to amplification of ISAV-HPR0 for further investigation of the relationship between virulent HPR-delete and avirulent HPR0. With the ability to culture primary gill cells, continued research aims at preparing primary gill cells from Atlantic salmon that have tested positive for ISAV HPR0 to determine if amplification of the viral agent can be achieved in these cells.

ENHANCING GENOMIC SELECTION EFFICIENCY FOR VIRAL NERVOUS NECROSIS RESISTANCE IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Viral Nervous Necrosis (VNN) is a major threat to sea bass populations, with mortality rates of up to 90% and an expected increased incidence with global warming. Genetic selection is a promising strategy to reduce the frequency and severity of epidemics that French hatcheries have been developing for several years, and which has recently benefited from the identification of a genomic region on chromosome 12, significantly involved in resistance to infection. The heritability of resistance to nervous necrosis viruses (NNV) was estimated to be 0.21, and in the studied population, the presence of the identified marker resulted in up to a 40% increase in survival rates. In this study, we investigate the impact on progeny survival to NNV infection when incorporating or omitting this marker in the genetic evaluation model.

To get the genomic estimated breeding values (GEBV) of 98 EMG-Ichtus males, we used genotypes obtained from a comprehensive dataset. Specifically, genotypes were available from 5251 siblings, all of which were genotyped on ThermoFisher's 57,000-marker DlabCHIP SNP chip. Among these siblings, 1051 sibs of the candidate males had been assessed for VNN survival, allowing for a thorough investigation of their genetic resistance to this disease.

A total of 98 EMG-Ichtus males were then crossed with a group of 8 females. This mating strategy was designed to obtain precise progeny-based estimates of the breeding value of the 98 males. Subsequently, to evaluate the susceptibility of the 4210 offspring to NNV, an experimental challenge was conducted at Fortior Genetics. Mortalities were recorded and all offspring were genotyped with the AgriSeq™ targeted genotyping-by-sequencing (tGBS) technology comprising 563 SNP markers among which 199 were used for parentage assignment and 282 were chosen for their association to NNV resistance. A total of 3663 offspring were assigned to their parents, and each sire had from 17 to 70 offspring, except one sire with 3 offspring which was removed from the analysis.

Based on all this information, we observed a correlation $r = 0.38$ between the GEBV of the 98 males obtained by ssGBLUP (Single-Step Genomic Best Linear Unbiased Prediction, combining the use of pedigree and genotypes) and the survival of their offspring challenged for NNV resistance. When the genetic effect of the SNP marker most significantly associated with NNV resistance was integrated as a fixed effect in the evaluation model, the correlation between GEBV and offspring survival increased significantly ($r = 0.52$).

These results confirm that the genetic selection of parents increases the resistance of their offspring to NNV. They also show that the additional use of the information provided by the QTL marker explaining a large part of the resistance (marker-assisted selection) improves the efficiency of "simple" genomic selection using the 57,000 markers available. The synergy between marker-assisted selection and genomic evaluation underscores its potential for improving natural disease resistance in aquaculture populations.

This work was financially supported by the FEAMP MedMax project (no. FEA470020FA1000002) co-funded by the French government and the European Union.

MICROPLASTIC POLLUTION IN *Genypterus* GENUS: DIFFERENCES BETWEEN TISSUES, SPECIES, AND GEOGRAPHICAL LOCATION

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The Chilean teleost fish includes several economically important relevant species for Chilean marine aquaculture diversification and fisheries. A relevant group of these species is included in the *Genypterus* genus, with red cusk-eel (*Genypterus chilensis*), pink cusk-eel (*Genypterus blacodes*), and black cusk-eel (*Genypterus maculatus*) as the main native species of this genus in Chile. These species are relevant for artisanal fisheries, as well as for aquaculture diversification. A broad geographical distribution is observed for these species, with populations in the north, center, and south of the country. These fish could be exposed to different pollutants, including plastic pollution. Microplastics are small plastics that are abundant in marine environments. However, little is known about the microplastic pollution in the population of these species, including the differences between species, distribution in tissues, and geographical effect in the pollution level of microplastic in the *Genypterus* genus. This study aimed to evaluate the prevalence of microplastic in different tissues of *Genypterus* 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. Additionally, we determined differences in pollution levels between the digestive tract, gills, and muscles. Additionally, we observed higher prevalence compared to other fish species, with some differences according to species and location under study. This information is evidence that microplastic pollution level is a relevant issue for species of the *Genypterus* genus and that the species and location are factors that influence the microplastic pollution, requiring more studies to determine its effect at the physiological level on these species.

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HIGH TEMPERATURE INDUCE OXIDATIVE DAMAGE, IMMUNE MODULATION AND ATROPHY IN BLACK CUSK-EEL (*Genypterus maculatus*)

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The Genypterus genus contains native species of economic relevance with high potential for aquaculture diversification, including the black cusk-eel (*Genypterus maculatus*), which is distributed on the south Pacific coast of Chile and Peru. Environmental factors such as heat waves can generate an increase in sea temperature, which could induce stress on fish. In this study, we determine the effect of increased temperature on stress response and oxidative status of black cusk-eel juveniles and the effect on immune genes in gills and atrophy-related genes on skeletal muscle.

Control and stress groups were subjected to different temperatures (13°C and 18,5°C), sampling plasma for cortisol determination, gills and muscle for DNA, protein, and lipids oxidative damage evaluation, and for relative gene expression evaluation through RT-qPCR. The increase in temperature induces a stress status in the fish (1A), with oxidative damage in both tissues (1B). Additionally, an up-regulation of heat shock protein was observed in both tissues (1C and D), with oxidative stress-related genes up-regulated (1E, F, and G). In muscle, an up-regulation of atrophy-related genes was observed (1H), while in gills, up-regulation of some immune genes was observed. This study shows how temperature related to heat waves could induce negative effects on marine fish.

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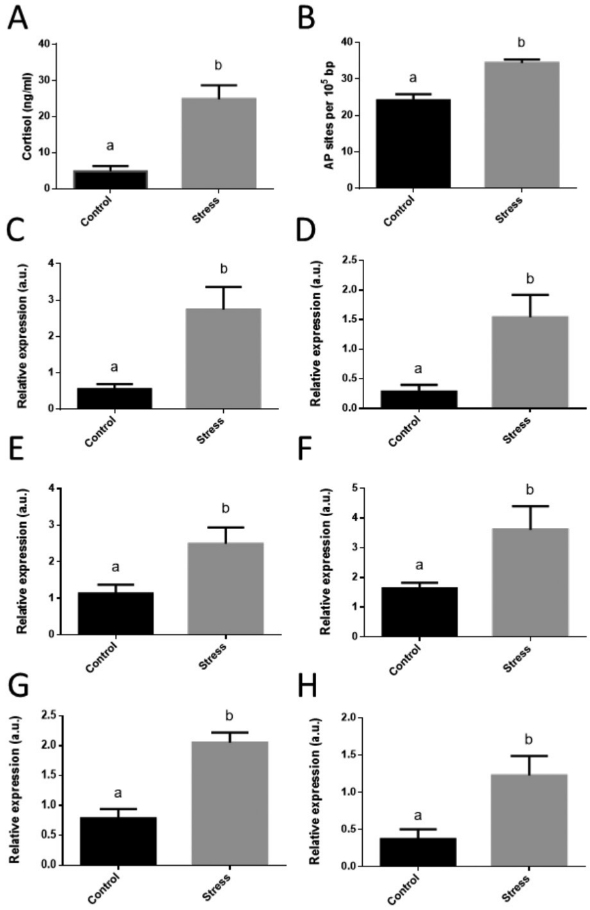

Figure 1. Comparison of cortisol level, AP sites and genes expression between Control and stressed groups. A = Cortisol levels in plasma, B = AP sites in DNA on gills, C= hsp70 expression in gills, D= hsp70 expression in muscle, E= sod1 expression in gills, F= ndua4 expression in gills, G= gpx1 expression in muscle, H= atg16l expression in muscle. Different letters indicate significant differences between the groups (p value < 0,05).

CORN AND BARLEY PROTEIN CONCENTRATES: A BIOENGINEERING TAKE ON PLANT PROTEINS FOR THE AQUACULTURE INDUSTRY

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ClonBio Group operates the largest single biorefinery in Europe to process 300 000 tons of Barley and 1.3 million tons of corn each year. With protein extraction at its core operation, the engineering company additionally produces dietary fibers, food/pharma grade ethanol and biogas for its internal use and domestic supply through grid injection.

Combining enzyme technology and mechanical separation, ClonBio has developed a sustainable protein concentrates portfolio from barley and corn grains with an emphasis on nutritional quality and minimal damage to the protein fraction for use in aquaculture feeds.

In order to support and validate this strategic engagement for the company to supply feed ingredients of the highest quality to the industry, an ingredient performance trial was performed to assess the effect of various levels of two novel plant protein sources, corn protein concentrate (TSR) and barley protein concentrate (BPC) on the growth performance, nutrient utilization and digestibility in rainbow trout *(Oncorhynchus mykiss*).

The trial comprised six dietary treatments: a control diet (CTRL) mimicking a practical formulation for Trout, with moderate levels of fishmeal (10%), fish protein hydrolysate, soy protein concentrate (SPC, 18%), corn gluten meal (CGM, 8%), wheat gluten, and pea protein concentrate, as main protein sources; and five other diets, in which the digestible protein supply of SPC and CGM was replaced at 50 and 100% by TSR (TSR50, TSR100), BPC (BPC50, BPC100) or its combination (TSR+BPC100).

Quadruplicate groups of 40 rainbow trout, with a mean initial body weight of 30.6 ± 2.2 g were fed one of the six experimental diets for 97 days. At the end of the trial, the final body weight (FBW) ranged between 122.8 and 132.2 grams and the specific growth rate (SGR) varied between 1.43 and 1.51 %/day.

Fish fed diet TSR50 showed a significantly higher FBW and SGR than those fed all other diets ($P<0.05$). The feed conversion ratio (FCR) varied between 0.94 and 1.07. Fish fed the TSR50 and BPC50 diets showed a significantly lower feed intake and FCR, and a higher protein efficiency ratio (PER) than those fed the CTRL, TSR100, BPC100 and TSR+BPC100 diets ($P<0.05$). Somatic indexes (HSI, VSI and fillet yield), whole-body composition and apparent digestibility for protein, fat, phosphorus and energy were not affected by dietary treatments ($P>0.05$).

Overall data shows that the replacement at 50% of the digestible protein supply of SPC and CGM by TSR, resulted in a significant enhancement of overall growth performance criteria. A similar replacement by BPC, although without a significant effect on weight gain (FBW and SGR), resulted in a significant improvement of feed utilization criteria (FCR and PER).

ANALYSIS OF *Edwardsiella piscicida* DERIVED EXTRACELLULAR VESICLES PROTEOME AND THEIR IMMUNOMODULATION

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Extracellular vehicles (EVs) are nanospheres emitted by most prokaryotic and eukaryotic organisms into their surrounding environment. Extensive research has underscored the ability of bacterial EVs to function as molecular transporters and to modulate immune responses, thereby playing a critical role in host immune system regulation. This study aims to analyze the proteomic profile of EVs isolated from *Edwardsiella piscicida* (*Ep*EVs) and investigate their immune responses.

The isolation of *Ep*EVs was done by ultracentrifugation with transmission electron microscopy confirming their spherical morphology. The isolated *Ep*EVs had an average size of 85.3 ± 1.8 nm and a zeta potential of -8.28 ± 0.41 mV. Proteomic analysis identified 1,487 proteins, with subcellular localization indicating that "cell" and "cell part" constituted 61.31% of the structural proteins. Key proteins associated with virulence, such as Type III secretion system components, flagellin, outer membrane proteins, and several chaperones involved in protein folding and stability, were also identified. Protein-protein interaction (PPI) predictions with *E. tarda* proteins revealed 673 interactions among the 1,487 identified *Ep*EV proteins.

Exposure of fathead minnow (FHM) cells to *Ep*EVs up to 100 µg/mL resulted in cell proliferation without toxicity, while Raw 264.7 cells exhibited mild toxicity at 12.5 µg/mL. Fluorescent-labeled *Ep*EVs demonstrated cellular internalization in FHM cells after 24 hours. In vitro gene expression analysis showed upregulation of interleukin (Il)6, IL1β, interferon (Ifn)β, and pro-inflammatory cytokines, along with simultaneous upregulation of the anti-inflammatory cytokine Il10. In vivo gene expression, except for heat shock protein (hsp)70, indicated upregulation of other genes, with a timely response suggesting *Ep*EV-induced immune gene expression. Western blot analysis revealed elevated levels of tumor necrosis factor (Tnf)α at 6 hours post-treatment (hpt) and increased Il10 expression at 24 hpt in spleens treated with 10 µg/fish *Ep*EVs.

These findings confirm that *Ep*EVs can be effectively isolated via ultracentrifugation and highlight the necessity of further investigation into their immunomodulatory mechanisms to develop novel therapeutic applications in fish medicine.

CHARACTERIZATION OF PROTEIN CARGO OF *Aeromonas hydrophila* EXTRACELLULAR VESICLES AND INTERACTIONS WITH HOST

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Bacterial extracellular vesicles (BEVs) are recognized for containing diverse protein cargos including virulence factors and signaling molecules that promote cell communication and host-pathogen interactions. This study aims to characterize protein cargos of *Aeromonas hydrophila* BEVs (*Ah*EVs) and investigate their potential interactions with host through in silico analysis.

BEVs were isolated through ultracentrifugation and characterized. Proteomic analysis was conducted using an Extractive HF-X Hybrid Quadrupole-Orbitrap Mass Spectrometer along with the *A. hydrophila*-specific database. Identified proteins were subjected to subcellular localization and Gene Ontology (GO) annotation, followed by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. To elucidate potential biological functions between *Ah*EV proteins and the host, protein-protein interactions (PPIs) were predicted using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database.

TEM imaging confirmed that the ultrastructural morphology of the particles was spherical, and nanoparticle tracking analysis (NTA) determined their average size to be 105.5 ± 2.0 nm. Proteomic analysis identified 49,015 total spectra, 5,597 peptides (including 4,825 unique peptides), and 1,284 proteins. The most prevalent protein mass range was 20-30 kDa, comprising 228 proteins. Subcellular localization indicated that "cell" and "cell part" were the primary protein localization areas, together accounting for 46.32% of the structural proteins. GO analysis showed a higher number of proteins related to "catalytic activity" (566) compared to other functions. Biological processes were categorized into 19 functional annotations, with cellular process-related proteins being the most abundant (458). Protein-protein interactions (PPIs) with *A. hydrophila* proteins revealed 532 predicted interactions among the 1,284 identified *Ah*EV proteins. Additionally, KEGG pathway analysis identified 21 enriched pathways, related to various biological functions such as protein synthesis and transportation, RNA degradation as well as amino acid metabolism. Further PPIs with host (human) revealed 102 interactions where 88 pathway enrichments were observed from the KEGG analysis. Key pathways enriched were MAPK signaling, NF-ϰB signaling, Il17 signaling, toll-like receptor signaling, RIG-I-like receptor signaling, as well as NOD-like receptor signaling which are some of the crucial interconnected pathways regulating immunomodulatory effects. Further studies could unravel the functional role of specific protein cargo in *Ah*EVs.

INVASIVE FISHES AS AN UNDERUTILIZED PROTEIN RESOURCE: THE U.S. CARP CHALLENGE

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Successful marketing and sustainability efforts targeting invasive bluestriped snapper (ta'ape, *Lutjanus kasmira*) in Hawaii and lionfish (*Pterois* spp.) in the Caribbean as excellent cuisine support conservation efforts to control populations of these non-native species, while contributing to local food security and economies. Awareness campaigns have resulted in increased commercial harvest and fisher prices, as well as recognition and growth of complimentary industries including the animal feed and leather markets. Similar harvest and utilization efforts for U.S. invasives including northern snakehead (*Channa argus*), blue and flathead catfish (*Ictalurus furcatus* and *Pylodictis olivaris*, respectively) along the eastern coastal water, as well as 4 carp species present throughout the Mississippi River drainage, need to be implemented as control measures, but also for optimization of sustainable protein sources.

Non-native carp species remain an established and nuisance group in US Midwest river systems, negatively impacting native aquatic ecosystems and water quality, recreation, and threatening ecology of not only the river systems but also the Great Lakes. Concentrated efforts and budget have been expended to limit spread; technical eradication approaches have had limited success, and market potential has not resulted in significant population declines. Although carp remain the highest volume aquaculture species globally (over $105 billion; growth >6%), a perception of low palatability and quality impedes food market growth in some locations, and optimal processing remains a challenge. Nutritionally, carp represent a low-fat, high protein nutrient resource, with an excellent amino acid profile and can achieve higher omega-3 fatty acid concentrations compared with some marine species, thus adding health benefits to consumers.

Efforts need to be directed towards supporting the supply chain, developing better carp products for human consumption from these invasives, and capitalizing on carp as an alternative protein for various animal feeds through improved harvesting and processing technologies that incorporate by- and co-products to build increased demand that also represents sustainability principles of responsible sourcing and circularity. Such actions will create employment opportunities while simultaneously offsetting some of the negative impacts of feed ingredients in the livestock, aquaculture, and pet feed industries, leading to ecosystem improvements and environmental benefits for widespread messaging.

PHENOTYPIC AND GENOMIC VALIDATION OF CONTINOUS GROWTH MEASURES OF ATLANTIC SALMON AT SEA WITH DIODE FRAMES AND PIT TAG RESPONDERS

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Most Atlantic salmon production occurs in large sea cages, limiting individual growth recording opportunities except at stocking or harvest. This hampers management for monitoring mean weight, biomass, feed usage, and genetic selection. Integrating passive integrated transponder (PIT) identification with non-invasive diode frames allows continuous, minimally disruptive growth monitoring. However, diode frame accuracy for growth traits needs evaluation as the phenotypes can have some level or measurement error and missing phenotypes as not all fish are guaranteed to be recorded each day. Random regression genetic models enable predicting breeding values despite missing data. We stocked over 5000 individually PIT tagged Atlantic salmon post-smolts in a sea cage, monitoring growth using diode frames. Our study aims to assess diode frame accuracy, estimate genetic parameters for growth, and validate this approach through genetic correlations with harvest phenotypes.

The details of animal husbandry and phenotyping is published in detail elsewhere and briefly mentioned herein [1]. We stocked 5500 Atlantic salmon individually tagged with passive integrated transponders (PIT-tags) from the MOWI strain and transferred them to a sea cage. A diode frame (Biomass Daily, Vaki AS) and PIT tag antenna was installed. At the end of the trial all remaining 5133 Atlantic salmon were then manually recorded for weight and length for comparisons with diode frame data. Phenotypic validation was conducted between the latest measurements at sea and the manually recordings at harvest. Random regression models with second order polynomials were run using DMU v6 [2]. The last 5 measurements obtained within two weeks of the final harvest were averaged to obtain a single "corrected phenotype" and then run in bivariate with the harvest phenotypes to assess the genetic correlations and the effect of averaging diode frame phenotypes.

In total more than 35 000 reliable measurements were obtained on 4980 fish over 125 days [1]. A subset of 510 individuals with more than 10 measurements each were used for random regression models. At the population level diode frames were highly accurate with a mean difference of 0.002% for length and repeatable 0.35 with moderate concordance (CCC) of 0.52 for length. The random regression model for length was best fit by a second order Legendre polynomial on the additive genetic and permanent environmental effects. Heritability for length was low for diode frame measurements starting at 0.13 and increasing to 0.22 over the 125 day period and length at harvest was 0.38 (Figure1). The residual variance of diode frame measurements were proportionally higher, thus lowering heritability. The genetic correlation between harvest length and corrected length was 0.90 ± 0.01. Diode frame measurements hold promise for continuous growth measurements at sea, however they have higher residual and lower heritability, but are highly correlated to harvest length.

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1. Difford et al https://doi.org/10.1016/j.compag.2020.105411, 2. Jensen J, Madsen P. Calculation of Standard Errors of estimates of genetic and phenotypic parameters in DMU. 2005;

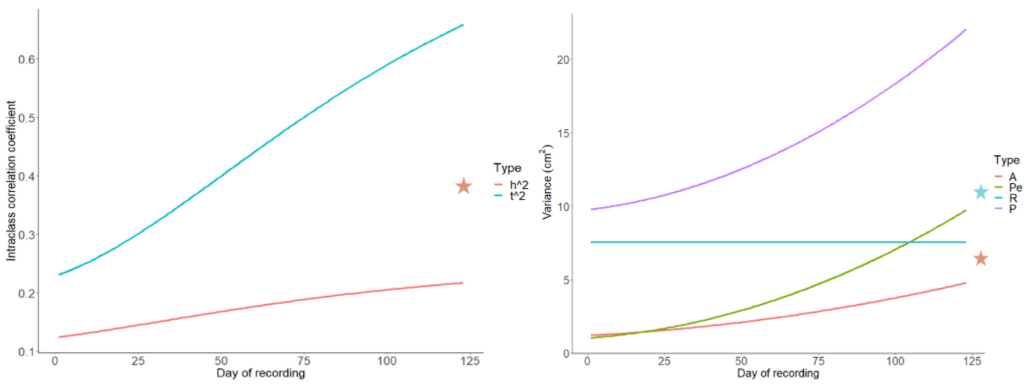

Figure 1. Intraclass correlation coefficients (heritability h^2 & repeatability t^2) for diode frame measurements of length (left insert) & Variance components (right insert) with stars for manual harvest length.

CIRCULAR SOLUTIONS FOR PLASTIC EQUIPMENT FROM THE AQUACULTURE INDUSTRY

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The UN's Global Resource Outlook 2024[1] highlights that excessive resource exploitation stands as the primary driver behind the world's environmental crises. Without more efficient and sustainable resource utilization, global natural resource extraction could increase by 60 percent by the year 2060. The Norwegian government has taken steps to promote a circular economy by proposing a new law for sustainable products and value chains[2]. The new legislation sets requirements regarding circular value chains including reuse, life extension, repair and recycling in addition to reducing waste.

To increase the circular economy for the Norwegian plastic system, a report from Systemiq, Handelens Miljøfond and Mepex (2023)[3] are pointing at better waste collection, product design, new delivery models, and recycling to achieve 77% circularity and reduce GHG emissions by 90% by 2040. Furthermore, they show that it is possible to reduce the need for virgin plastic material in the fisheries and aquaculture industry by 48%.

Scale Aquaculture, a technological supplier for the aquaculture industry, acknowledge the need to increase the circular economy for discarded plastic equipment in the fish farming industry. In the aquaculture industry there are about 192,000 tonnes of plastic in use in sea-based aquaculture in Norway. About 16,000-29,000 tonnes of this amount is plastic waste annually[4]. In 2023 Scale Aquaculture together with seven partners, started the project "Circular solutions for the aquaculture industry" (SirkAQ). The aim of the SirkAQ project is to drive the transition from a linear to a circular economy in aquaculture industry by establishing and implementing sustainable circular value chains for plastics from discarded equipment. Through reuse, repair, life extension and the use of recycled material in new products, we seek to optimize the use of resources and reduce the environmental and climate footprint of aquaculture.

We are implementing new circular value chains with a special focus on the reuse and recycling of equipment from the pens. We will present the work that Scale Aquaculture is doing regards implementing circular value chains of discarded plastic equipment. We will give examples on possible technological solutions. Results from the ongoing project "SirkAQ" will be presented.

Acknowledgement
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Hallingplast AS, SinkabergHansen AS, OsloMet, Norner Research AS, Sintef Ocean AS and Future Materials.

GRO24_Summary_for_Policymakers.pdf (unep.org)
Prop. 69 LS (2023–2024) (regjeringen.no)
Achieving Circularity | Systemiq
SINTEF Open: Avfallshåndtering fra sjøbasert havbruk (unit.no)

AQUACULTURE IMPACT ASSESSMENT USING BENTHIC ECOLOGICAL STATUS IN CYPRUS FISH FARMS OVER A DECADE: A MULTI-DEPTH MONITORING APPROACH

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The impact of aquaculture on the marine environment is known for causing organic enrichment and eutrophication. Aquaculture monitoring in Cyprus (Eastern Mediterranean) has been consistently conducted since 2010, using annual sampling. The locations of the monitored fish farms have remained largely unchanged. As a result, the time series dataset created is optimal for long-term monitoring studies of aquaculture effects and the aim of the study is to assess these effects in the coastal zone of Cyprus and evaluate the efficacy of the current monitoring plan.

During 2010-2021, sediment samples were collected from 8 fish farms in accordance to the Cypriot National Environmental Monitoring Protocol. Two different monitoring companies were responsible for the monitoring process with different fish farms assigned to each company each year. Sampling was done at increasing distances from fish cages (0m, 50m, 200m, 500m, and a reference station), with three replicates per station, totaling 1244 samples. The macrofaunal community identified at the species level was used for Ecological Quality Status (EcoQs) assessment based on different benthic biotic indices (i.e. BENTIX, AMBI and others). Environmental data measured included depth, sediment type, organic matter content, sediment phosphorus levels, and water column nutrients, and Chlorophyll-a.

The results revealed that the factor related to the company in charge of the analysis of samples (codenamed "Prepared by"), was the most influential factor in explaining the variation of the macrofaunal community. Other significant factors included sediment type, depth and distance from the fish farm.

Despite this company-related variation in macrofaunal community attributed to differences in sample analysis, the ecological status assessment remained consistent between the two companies, as indicated by the levels of agreement (Kappa values) in the EcoQs observed between each benthic index. Notably, the BQI-Family index aligns closely with BENTIX, the current index utilized by WFD (Water Frame Directive) in Cyprus. The majority of EcoQs of the fish farms was Good, and did not deteriorate over the years, despite variability in benthic species composition. The national aquaculture legislative framework in relation to the operation of open sea aquaculture farms and the environmental monitoring programme of the Republic of Cyprus that is being implemented, seems to be an effective tool in preventing ecosystem degradation

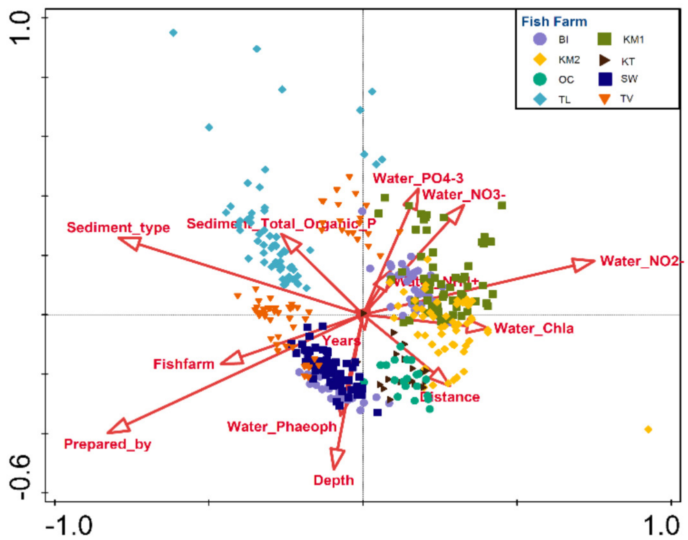

Figure 1. Canonical Correspondence Analysis (CCA) of Benthic Samples with Environmental Variables and Categorical Factors

EFFECTS OF DIETARY YEAST HYDROLYSATE IN JUVENILE GILTHEAD SEABREAM (*Sparus aurata*) NUTRITIONAL PERFORMANCE

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Yeast hydrolysate (YH) have gained increasing interest in animal nutrition as non-conventional raw material presenting a high proportion of highly digestible, quickly absorbed proteins (free AA, small peptides) in addition to fermentable carbohydrates. Thus, feed formulation with YH may allow to optimise the feed performance and functional benefits while reducing reliance on marine or land-based ingredients such as soybean meal due to high digestible protein content.

A 10-week trial used juveniles gilthead seabream (25g; 20 fish/tank) under optimum rearing conditions within 12 * 150 L tanks connected to a fully controlled RAS supplied with natural seawater (29 ppt; 22°C). The trial tested 3 diets in quadruplicate tanks. The control diet was formulated to the species life-stage requirements according to standard industry practices (45% plant-ingredients; 20% land-animal meal, 20% fish-meal (FM), 9.6 % fish-oil; 48% crude protein; 14.5 % crude lipids). The second experimental diet (YH-Iso) was formulated by reducing FM by 10%, adding 2% YH and balancing the other ingredients to achieve an iso-protein, -lipidic and -energy levels. The third experimental diet (YH-LowP) had the same FM and YH inclusion as the second diet but with 1% reduced total crude protein content (47% protein) to evaluate the performance of high digestible protein of YH. Small inclusion of DL-methionine in all experimental diets was necessary to balance the low methionine content of YH. Extruded feeds were hand-fed at a fixed ration. Statistical differences were assessed by 1-way or 1-way nested ANOVA and Tukey's post-hoc test, significance was accepted at $P < 0.05$.

Over the trial's duration, growth was significantly higher in the YH-iso compared to the Control which was associated with a numerical improvement in FCR (-5.1%) and a slight increase in feed intake. In comparison, there was no significant difference in growth and feed performance between the control and YH-LowP diets. Liver transcriptomic, gut histology and microbiota data will shed light on how YH may bring the production benefits measured.

In conclusion, incorporating 2% YH within balanced formulation has the potential to improve the nutritional performance and sustainability of gilthead seabream production. This may represent a valuable option in the context of increasing pressure on conventional raw material.

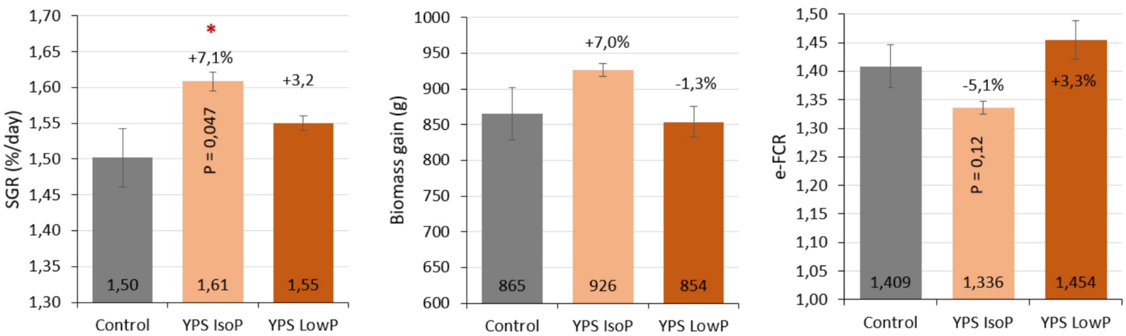

Fig 1: Growth and feed performance over the trial's duration. Mean ± SEM, n = 4. * denote a significant differences between groups.

MICROALGAE AS A POTENTIAL FUNCTIONAL INGREDIENT FOR AQUAFEEDS: ENHANCING ANTI-INFLAMMATORY ACTIVITIES THROUGH CULTIVATION FACTORS

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Aquaculture is the fastest growing food production sector and its expansion is being attained by the use of intensive production systems and aquafeeds formulated with ingredients that fish do not consume in their natural environment. Despite the fact that these diets promote an adequate fish growth and survival, the use of pro-inflammatory ingredients (e.g. soy) to substitute fisheries derived ingredients (e.g. fish meal) can promote foodborne inflammation, intestine injury and enteritis in fish. Inflammation is the first defence of the organism against pathogens and excessive intestinal inflammation can cause inflammatory bowel disease in fish. The inclusion of bioactive ingredients in aquafeeds to alleviate intestinal inflammation is a highly valuable tool to improve fish welfare and biological performance. Microalgae are promising sources of bioactive compounds with antioxidant, anti-inflammatory and cancer-preventive properties. The H2020-funded project Algae4IBD explores this potential to develop commercial products for Inflammatory Bowel Disease (IBD) prevention and treatment using aquatic natural biological resources. The project offers a nature-to-bedside approach, using the entire value chain to discover new biotherapeutic approaches by developing algae-based compounds. Necton S.A. aims to optimize the cultivation of several microalgae species to promote target-bioactive compound induction. Different cultivation modes regarding nutrients supply, such as batch (adding nutrients once in inoculation), fed batch (daily nutrients adjustment) and replenish mode (adding nutrients in the day prior to harvesting), are known to modulate microalgae bioactivities. Therefore, metabolites induction through nutritional modulation was investigated in *Tetraselmis chui* and *Phaeodactylum tricornutum*. *T. chui* was cultured at 22ºC and *P. tricornutum* at 19ºC in a volume of 100 mL (*n*=3), using NutriBloom® Plus as culture media during 7 days. In the nitrates trial, *T. chui* was cultured in batch, fed batch (2 mM NO_3) and in replenish mode and in the phosphates trial the same cultivation modes were tested (0.25 mM PO_4). The same conditions were applied for the trial with *P. tricornutum*. Overall, specific productivity (μ) of *T. chui* was higher than *P. tricornutum*. *T. chui* μ was negatively affected by the nutrient's limitation under batch growth but similar between fed-batch and replenishment cultivation modes. *P. tricornutum* μ did not reveal significant differences between conditions however, its photosynthetic efficiency was lower than expected under all conditions, particularly compared to *T. chui*. This work will contribute to the species and cultivation mode selection that allows the enhancement of microalgae bioactivities relevant for inflammation bowel disease alleviation. This initial process will contribute to developing novel and sustainable algal-based and high-quality bioactive compounds for IBD prevention and treatment. The application of microalgae with enhanced bioactivity that allow the prevention and alleviation IBD adds value to the biomass and can be leveraged through its application in aquafeeds, as a mitigation strategy of the pro-inflammatory ingredients commonly present in fish diets, to improve animals' biological performance.

Acknowledgements
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BEYOND CONVENTIONAL SOLUTIONS: VITABLOOM® NEW PRODUCTS RANGE COMBINES MICROALGAE AND PROBIOTICS FOR GREEN WATER TECHNIQUE, ROTIFERS NUTRITION, AND LIVE FEED ENRICHMENT

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The development of new microalgae products to solve current marine fish larvae production challenges in aquaculture is essential to enhance this fast-growing food production sector. The combination of different microalgae and probiotics is a powerful tool to improve animals' nutrition and can be used as a prophylaxis to enhance biological performance, resilience to pathogens and fish stress coping abilities. Microalgae are used in green water technique, rotifer cultivation and live feed enrichment (rotifers and artemia). VitaBloom® Green Water was applied to meagre (*Argyrosomus regius*) larviculture until weaning in comparison to live *Nannochloropsis* sp. (*n*=3 tanks). Larvae at 19 days post fertilization (dpf) showed a significant reduction of skeletal malformations affecting phenotype (24%) compared to the control (50%), displaying similar survival (20dpf) and reduction of *Vibrionaceae* bacteria (TCBS) in the water (1.93Log10CFU/ml, 1.23Log10CFU/ml respectively). A stress challenge of transport was performed post-weaning, where larvae cultured with VitaBloom® Green Water revealed higher survival (93.0±1.3%) compared with control (68.2±18.3%). VitaBloom® Rotifers+ was used in rotifers cultivation in batch (3 days) compared to a commercial product. VitaBloom® Rotifers+ showed a significant reduction of product decantation (0.160±0.03cm), microalgae agglomerates abundance (0.05±0.04%) and dimensions (16.8±7.7μm) compared to control (0.280±0.07cm, 0.5±0.4% and 21.0±8.3μm respectively). VitaBloom® Rotifers+ significantly improved rotifers growth rate (266±16.7%) and amictic females (28.8±7.5%) compared to rotifers produced with a commercial product (160±13.2% and 23.3±9.8% respectively). VitaBloom® Enrich was used in gilthead seabream (*Sparus aurata*) larviculture until weaning, with an initial density of 76larvae/L, in comparison with a commercial enrichment product (*n*=4 tanks). In this experiment, green water was performed with live *Nannochloropsis* sp. and rotifers cultured with a standard product (PhytoBloom®Green Formula) in both treatments. Although there were no significant differences, larvae fed with enriched live feed with VitaBloom® Enrich revealed 18.7±7.3% of larvae survival at weaning and larvae fed with live feed enriched with a commercial product resulted in a 15.5±7.7% survival at weaning. The synergetic effect of the 3 VitaBloom® products was tested in *S. aurata* larviculture until weaning compared to larvae cultured with 3 commercial products (*n*=3 tanks). Larvae fed with VitaBloom® showed higher survival at weaning (11.0±2.3%) compared to control (6.6±5.0%). The development of VitaBloom® innovative, sustainable and effective microalgae solutions in powder is highly advantageous for the aquaculture industry due to the logistics simplification, displaying a balanced nutrition for fish and supporting their health. It is noteworthy, that the synergistic effect of VitaBloom® range can enhance by up to 13.4% (6064€) the nursery *S. aurata* sales per million larvae, boosting production in this relevant food sector.

Acknowledgements
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AQUALOOP – TO DEVELOP, PROMOTE AND SUPPORT THE CONCEPT OF CIRCULAR AQUACULTURE

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The objective of the AquaLoop project – Aquaculture expert floor for circular economy practice, a three year collaboration between seven partners from the South Baltic (SB) region, is to develop, promote and support the concept of circular aquaculture in the South Baltic region for the green transition, with a focus on nutrient loop containment with by-products production towards responsible consumption.

The reason for the project idea is a recent stagnation of aquaculture production in the SB region, due to environmental, legal, economic and social restrictions, indicating a need for the adaptation of new production methods. This is where AquaLoop steps in. The collaboration of universities, municipalities, associations and SMEs within the project reflects the strong interest and link between research, education, awareness raising and application in industry. The foreseen actions, supporting circular economy and blue biotechnology, are designed to develop and showcase original, innovative solutions, and prepare present and future employees, business sector and customers for the circular aquaculture.

The actions are based on three pillars: PilotLoops, TrainingLoop and SupportLoop. PilotLoops stand for Circular aquaculture South Baltic pilots, with 3 cross-border pilots being developed, focused on testing algae applications in recirculating aquaculture systems (RAS) to improve aquaculture circularity potential in the SB region, increasing the nutrient efficiency of commercial aquaculture through increased application of circular economy concepts, and development of the fish-shrimp-vegetables integrated system of aquaponics to showcase the potential of circular economy. The second pillar – TrainingLoop, standing for Circular aquaculture training pool, focuses on training activities for school youth, students and professionals in innovative aquaculture methods, and exchanging knowledge and experience in the sector. And finally, SupportLoop – Circular aquaculture stakeholder support, focuses on investigating best practices, experiencing the cross-sector cooperation possibilities in an international setting and forming international networks with organizations pursuing the same mission.

EFFECT OF GREEN SEAWEED EXTRACT OBTAINED BY SUPERCRITICAL CO_2 ON GROWTH PERFORMANCE AND HEALTH STATUS OF *Cyprinus carpio* REARED UNDER DIFFERENT WATER QUALITY REGIME

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In recirculating aquaculture systems (RAS), water exchange has a direct impact on the removal of fish metabolic wastes and unconsumed feed. For this reason, practicing an inadequate exchange rate can lead to the accumulation of toxic compounds that can act as stress factors for cultivated organisms. The inclusion of seaweed in the fish feed is of interest both from the perspective of farmers, who want functional feed that strengthens fish immunity and counteracts the negative effects of stressors, and from the perspective of feed producers, who are in direct competition and for whom, the development of innovative feed, represents the premise of the competitive advantage. Green algae, such as *Ulva lactuca*, are of increasing interest from this point of view, due to their composition rich in bioactive compounds, such as antioxidants, antimicrobials and anti-inflammatories, which can help fight diseases and promote the general health of fish. However, their inclusion in fish feed is limited by antinutritional factors that they also contain. In this context, algae extract can have advantages to the biomass, both in terms of fish growth and in combating stress or enhancing the immune response.

The primary objective of this study was to assess the effect of seaweed extracted by supercritical CO2 on the *Cyprinus carpio* growth and general health profile, under different exchange rate regime. Reducing the water exchange rate in a RAS could help save energy, but requires careful monitoring of water quality to maintain optimal conditions for fish. Thus, in a 50 day study, in addition to monitoring the main parameters of water quality (nitrates, nitrites, ammonia, ph and temperature), we also investigated the potential effects the extract on fish health (serum biochemical parameters, haemoleucogram, antioxidant enzyme activity, lysozyme activity). For the experiment, there were 3 groups each with different exchange rates: ER1: 7.5 l/kg/d, ER2: 3.75 l/kg/d and ER3:2,5 l/kg/d. For each group three diets were tested: C-control diet, E5 – experimental diet supplemented with 5% extract and E10 - experimental diet supplemented with 10% extract. Extractions were carried out using the SC CO_2 pilot-plant (Natex, Prozesstechnologie GesmbH, Austria, Fabr. no. 10-023/2011) designed with a cylinder extraction vessel and two separators. Before extraction, the lyophilized Ulva macroalga was grinded (manual grinder model Tristar KM 227, power 150 W). For each extraction, dried algae biomass was mixed with 7.5% ethanol as co-solvent and used with a CO2 flow rate of 0.35 g/min. In the end of the experiment the fish growth performance showed better values in all E10 groups. The comparison among ER groups showed no significant differences regarding growth performance parameters (SGR, PER). Results into the effects of algal extract on fish health is ongoing, but preliminary studies suggest promising benefits.

RE-THINKING THE GUT CORE MICROBIOTA DYNAMICS IN GILTHEAD SEABREAM FED ALTERNATIVE DIETS ACROSS THE PRODUCTION CYCLE

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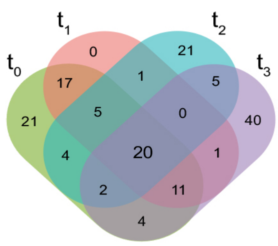
The term "intestinal core microbiota" refers to the set of microbial taxa that consistently occur in a particular host in which microorganisms interact with each other in a contiguous environment. This would require to explore bacteria taxa abundance and occupancy across multiple samples that are often reduced to a unique sampling point at the end of a given trial, which risks to mismatch the succession of microbial communities over time. Besides, it is well documented that nutrition and feeding is a major factor driving the changes in gut microbiota composition and function of living organisms, including farmed fish. Considering together temporal and nutritional aspects, the aim of this study was to rethink in a dynamic manner the core microbiota composition of farmed gilthead seabream fed with standard or alternative feed formulations through the production cycle, coinciding with the extreme 2022-2023 climate period.

Juveniles of gilthead seabream (~15g) of Mediterranean origin (Avramar, Burriana, Spain) grew from May 2022 to February 2023 in a flow-through system (3000 L tanks) operated with natural photoperiod and temperature conditions at the IATS infrastructure under standard rearing conditions. During the trial, fish were fed with a CTRL fish meal-based diet, or alternative feeds with processed animal protein (PAP), or insect meal + bacterial protein (ALT) as a main fish meal replacer. At initial (May 2022, t_0), intermediate (July 2022, t_1; November 2022, t_2) and final (February 2023, t_3) sampling points, 12-16 animals per time and diet were sampled for adherent intestinal microbiota. Bacterial DNA was extracted, and V3-V4 region of 16S rRNA gene was sequenced with Illumina MiSeq platform and taxonomically assigned against SILVA v138.1 database. The core microbiota was defined for each time point by setting an occurrence threshold of more than 50% of individuals within each dietary group.

Partial Least Squares Discriminant Analysis (PLS-DA) disclosed 116 taxa (filtered by ≥0.1% abundance) of discriminant value (VIP>1), driving the cyclic trend of gut microbiota following changes in season. Core microbiota analysis discerned always the consistent presence of 55, 58 and 83 taxa at t_1, t_2 and t_3, respectively. The overlapping of these taxa generated a reduced list of 20 taxa, which were also represented at the beginning of the trial (t_0). Overall, this constrained list represented >58% (45-72%) of the total intestinal microbiota regardless of sampling time and diet. Of note, 12 out of 20 core bacterial taxa exhibited a temporal discriminant role, while 8 persisted almost unaltered throughout the production cycle. Firmicutes and Actinobacteriota phyla are overrepresented in this bacteria fraction, becoming probiotic candidate markers for promoting robust fish in a warming challenging scenario. Such approach was complemented with the construction of causal Bayesian networks to define gut microbiota associations, and their practical implications to stablish what is the most adequate probiotic solution for a given strain, physiological condition and rearing system.

This work was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (THINKINAZUL/2021/024).



Core microbiota throughout production cycle.

EFFECT OF TEMPERATURE ON GROWTH PERFORMANCE OF THE MOON JELLYFISH *Aurelia sp.*

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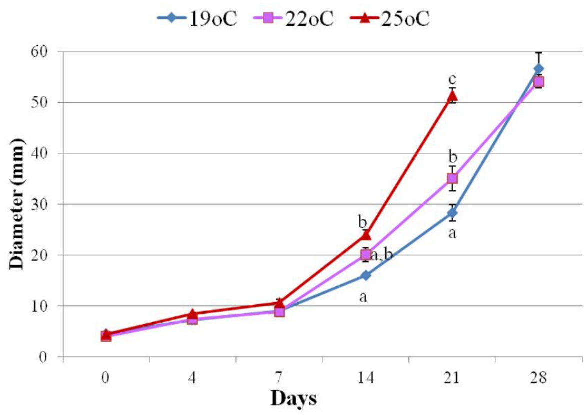
Aurelia sp., commonly known as the moon jellyfish, is a fascinating jellyfish species often exhibited in aquariums due to its mesmerizing appearance and graceful movements. However, maintaining moon jellyfish in an aquarium demands meticulous attention and specific conditions to ensure their welfare. A significant challenge in displaying moon jellyfish is their relatively short lifespan, typically ranging between 6 months to a year in captivity. Thus, it becomes imperative for aquariums to devise efficient cultivation methods to sustain their populations for exhibition purposes. Temperature regulation emerges a crucial factor in the cultivation process. Moon jellyfish thrive within specific temperature ranges, typically between 18°C to 25°C. In this study, three temperature regimes (19°C, 22°C and 25°C) were examined to assess their impact on accelerating jellyfish growth.

The experiments took place at Cretaquarium, a public aquarium operated by the Hellenic Centre for Marine Research (HCMR) in Crete. In total, 900 ephyrae of *Aurelia sp.* sourced spontaneouly, from the same polyp broodstock were distributed across three 150L cylindroconical tanks. Seawater from a borehole ensured consistent tank renewal. Each tank was heated to maintain temperatures of 19°C, 22°C, and 25°C respectively. Jellyfish were fed enriched *Artemia sp.* nauplii twice daily. Ten individuals from each temperature group were sampled weekly until reaching a size appropriate for transfer to the exhibition tank (>50mm). Ephyrae were photographed under a stereoscope, and larger jellyfish were documented using macrophotography. Jellyfish diameter (mm) was measured using tpsDIG2 software.

Elevated temperature accelerated jellyfish growth performance. Jellyfish cultivated at 25°C exhibited faster growth, reaching exhibition size (>50mm) a week earlier than those cultivated at 19°C and 22°C (Fig. 1). While no significant differences in growing performance were observed during the first week, from day 14 onwards, jellyfish cultivated at 25°C showed significantly larger sizes compared to those at 19°C. By day 21, all populations displayed distinct differences, with the largest individuals consistently found in the group of 25°C.



1: Jellyfish diameter (mm). Values represent means ± S.E., values scored with different letter are significantly different ($p<0.05$)

SEASONAL VARIABILITY IN DIETARY PHOSPHORUS REQUIREMENTS IN FARMED ATLANTIC SALMON *Salmo salar*

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Introduction
Phosphorus (P) is essential for systemic functioning and bone mineralisation. The recommended level of total P for farmed Atlantic salmon (*Salmo salar*) is 10.0 g/kg. The requirements of dietary P are, however, life stage dependent. Early freshwater stages require increased levels of dietary P (up to 11.4 g/kg total P) due to high metabolic demand and fast growth. The P demand in seawater is reduced. Still, within the seawater phase, periods of prolonged day light and elevated water temperatures result in a faster growth potentially increasing the P demand. The current study provides knowledge about the P requirement during the final grow-out phase in seawater as an attempt to increase sustainability.

Materials and Methods
Seawater stages of Atlantic salmon were fed mono-ammonium phosphate supplemented diets with different P levels, 6.1 g/kg (A), 8.0 g/kg (B), 8.7 g/kg (C), 9.5 g/kg (D), 10.4 g/kg (E), and 11.2 g/kg (F) total P on dry matter basis, from December (average weight (a.w.): 1.7 kg) to July (a.w.: 4.1 kg). Animals (4 net pens/ diet, 90 fish/ net pen) were reared under natural photoperiod and sampled in April (water temperature range: 4.6 - 8.4 °C) and in July (water temperature range: 6.7 - 13.3 °C). Analyses included growth performance (weight and length increase), deformities, vertebral centra strength, and bone mineralisation.

Results and Discussion
In April, the growth of animals in all diet groups was comparable (0.61 ± 0.05 mm/day). Yet, in diet A animals, the resistance of vertebral centra to deformation (elastic modulus, MPa/%) and bone ash content (%) were already reduced (0.36 ± 0.03 MPa/%, 26.39 ± 1.54 %) compared with the remaining diet groups (0.53 ± 0.04 MPa/%, 32.56 ± 1.19 %). Later in April-July period, the increased water temperatures and prolonged day light led to growth differences among groups. In July, diet A animals had a slower growth rate (0.77 ± 0.05 mm/day) compared with animals in group B, D, and E (1.01 ± 0.06 mm/day). Diet A animals' elastic modulus and ash content was further reduced (0.23 ± 0.02 MPa/%, 21.17 ± 1.54 %) compared to all groups (0.50 ± 0.03 MPa/%, 30.89 ± 1.24 %). Diet B animals began to lag behind in elastic modulus and ash content (0.43 ± 0.03 MPa/%, 29.14 ± 1.14 %) relative to diet D, E, and F animals (0.52 ± 0.04, 31.42 ± 1.27 %). Still, on x-ray images, low-mineralised vertebrae were only observed in diet A animals (Fig. 1). To conclude, the increased growth altered the dietary P requirements from 8.0 g/kg total P during December-April period to 8.7 g/kg total P during April-July period. Both values represent a significant reduction compared to the value of total P recommended for commercial diets. Optimisation of dietary P levels can lower the P effluent from fish farms, aids in sensible use of limited inorganic phosphate resources, and can help the industry to economise feed production.

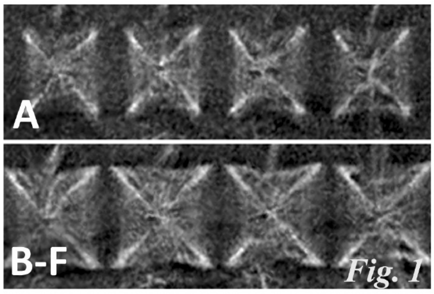

Fig. 1

CHARTING SUSTAINABLE PATHWAYS: A REAL-LIFE CASE STUDY OF GREEN INITIATIVES IN A CHALLENGING INDUSTRY

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Marine sources such as omega-3, proteins, and other nutrients are in high demand, but the supply struggles to keep up. Sustainable sourcing from oceans and other systems is crucial to meet this demand. Antarctic krill stands as the world's largest yet underutilized marine resource, with an established science-based fisheries management. With an anticipated shortage of proteins, lipids, and essential nutrients for feed, utilizing all available nutrient sources becomes imperative for meeting industry demands. While the shortage of omega-3 and proteins creates the need for sourcing new ingredients, the flexibility also empowers fish nutritionists and feed formulators to maximize ingredient and tool usage. AkerBiomarine leads in the sustainable harvest and production of marine ingredients from Antarctic krill, while continuously advancing krill fisheries and production methods to ensure sustainability. The present poster showcases AkerBioMarine' s approach to sustainable harvesting and production methods, including demonstration cases of CO_2 hot spotting and GHG emission reduction targeting. It highlights initiatives for sustainable fishing and production, with examples of short and long-term actions to minimize impact. Despite the ongoing need for further efforts, the success of current initiatives demonstrate the feasibility of sustainable operations.

IMPROVING SHRIMP FEED PERFORMANCE WITH KRILL MEAL

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Krill meal, sourced from Antarctic krill (*Euphausia superba*) harvested in the Southern Ocean, is a nutrient-rich feed ingredient containing approximately 60% protein and 25% lipids high in phospholipids, omega-3 fatty acids, and astaxanthin esters. Additionally, it contains chitin, trimethylamine oxide, free amino acids, and nucleotides, serving as potential feeding stimulants. Partial replacement of fish meal with krill meal in diets has shown to stimulate feed intake and enhance growth performance in whiteleg shrimp (*P. vannamei*). Moreover, studies have explored its effects on immune gene expression and hepatopancreas function, highlighting its role in supporting shrimp health. Lastly, the inclusion of dietary krill meal was investigated in relation to *Enterocytozoon hepatopenaei* (EHP) infection in shrimp, demonstrating improved growth outcomes.

This presentation aims to summarize how krill meal functions as a feeding stimulant, growth accelerator, health promoter, and cost-effective solution in aquaculture, providing insights into its multifaceted benefits and potential for sustainable aquafeed formulation.

ANALYSING MICROPLASTICS IN AQUACULTURE: QUALITY ASSURANCE FROM COMMERCIAL TESTING

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The capabilities to monitor for the presence of microplastics in aquaculture in view of evaluating sources and protecting the industry from potential negative impacts reside in the implementation of methods which can be implemented at large scale. Comparability of results across studies is a necessity but is proven is difficult because different methods are used globally, and the focus of published studies often differ.

We used a combination of FTIR-ATR and microFTIR to evaluate the presence of microplastic potentially impacting aquaculture productions. To ensure comparability of our results with other published studies we made use of the freeware siMPle which is implemented in large number of organizations worldwide and allows to speed up the time spent for image analysis. We validated the development of our methods and include an evaluation of the recovery rates of microplastics through the process of density separation using NaCl and polytungstate and oxidation using H2O2 in view of preparing samples for microFTIR analysis.

PROTECTING AQUACUTLURE FROM PATHOGENS TRANSPORTED BY SHIPPING – AN UPDATE OF THE ONGOING BALLAST WATER CONVENTION REVIEW

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The International Maritime Organization (IMO) International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention) entered into force in 2017. The number of ships installing ballast water management systems (BWMS) to meet their compliance obligations has risen steeply in the last years. When BMWS are installed, "commissioning testing" occurs to determine the suitability of the installation and since June 2022 there is a requirement following the BWM Convention to include biological efficacy testing during all commissioning tests to verify that the installation allows for the ship to discharge water with organisms concentration below the "D-2 performance standard".

We present findings from >600 tests carried out in 28 countries on more than 25 technologies. Notably, nearly all failures to meet the D-2 performance standard occurred in the largest size class (≥50 µm) of organisms. Between November 2019 and March 2022, the failure to meet the D-2 standard during commissioning as decreased from >20% failure to <7% failure. However, regular compliance monitoring from authorities during normal operations reveals that more than 1/3 of ships do not meet the convention objectives. The data set suggests that experience (spurred by mandatory, third-party testing) has allowed all stakeholders involved in the selling, design, planning, installation and operations of BWMS to correct many issues over time. As part of the Ballast Water Management Convention, the IMO has agreed to carry our regular testing (every 2.5 years) to verify ships can meet the discharge standard of the Convention. Regular testing of discharges from ship to be carried out by independent testing organisations is the only way to improve the protection of aquatic environment from the transfer of harmful aquatic organisms and pathogens.

OPTIMIZATION OF A SEAWEED CULTIVATION SYSTEM ASSOCIATED TO A SOLE AQUACULTURE

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Fish aquaculture produces a nutrient-rich (nitrogen and phosphorus) water that may affect the surrounding environment. Aquacria Piscícolas S.A. is a company dedicated to an intensive aquaculture of *Solea senegalensis*, which operates in recirculating aquaculture system (RAS). Although these systems minimize negative impacts, part of the RAS water is still discharged in the environment. Integrating a seaweed cultivation unit with these fish farms may be an effective way to reduce nutrients levels from these waters but also to growth biomass for valorization – stimulating sustainability and circular economy.

To improve nutrient bioremediation by seaweed, the production factors culture density and flow rate were optimized during a 3-month trial (January to March). *Ulva* sp. was selected, based on its growth rate and nutrient removal. Three density levels (1, 2 and 3 kg m^{-2}) and two water flows levels (low–LWF and high–HWF) were tested. Physicochemical parameters were measured and seaweed growth rate (RGR) and productivity were assessed every week. Nutrient removal was measured monthly. Cultivation conditions are currently being optimized throughout the year, thus these are preliminary results.

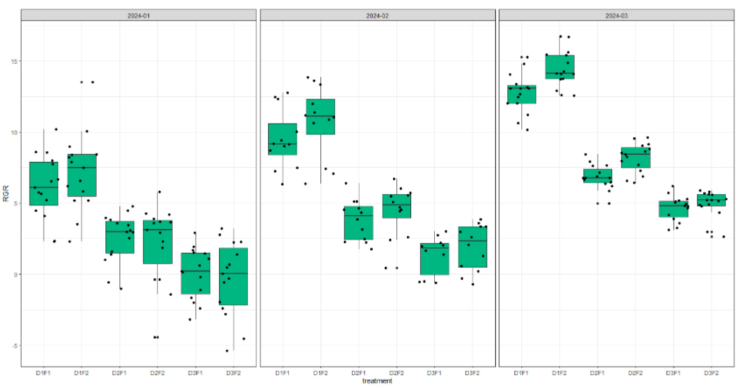
LWF was 57.1 ± 18.6 L h^{-1} and HWF was 165.0 ± 21.9 L h^{-1}. LWF led to lower temperatures and pH in the seaweed cultivation tanks than HWF. Salinity was similar for both water flows (table 1). In general, RGR and productivity increased from January to March at all densities. The highest RGR (13.6 ± 1.6% day^{-1}) was registered for the 1 kg m^{-2} of density. Productivity was similar for all densities, averaging 234.0 ± 61.4 g DW m^{-2} wk^{-1}. Considering N removal, the highest values were obtained in January, at the lowest density.

Results indicate that the best conditions to achieve higher growth rates and N removal are low cultivation density and higher flow. Increase overtime may be related to higher light availability. Since RAS water temperature is around 20 ºC, higher flows during this period (January to March) resulted in a more stable temperature, leading to higher growth.

Table 1. Physicochemical parameters measured from January to March (n=126)

Variable	Low water flow (LWF)	High water flow (HWF)
Salinity	23.2 ± 0.75	23.2 ± 0.77
Temperature (°C)	17.2 ± 1.7	19.2 ± 1.5
pH	7.65 ± 0.13	.35 ± 0.17

Figure 1. Growth rate (RGR) of *Ulva* sp. from January to March



EFFECTS OF ELEVATED TEMPERATURE ON GENE EXPRESSION, ENERGY RESERVES, AND CELLULAR ENERGY STATUS IN *Salmo trutta*

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Increasing water temperatures will pose great challenges for aquatic ecosystems in the future. Within the family of Salmonidae, *Salmo trutta* (brown trout) is the most vulnerable to higher water temperatures. Evidence of increasing water temperature in Austria includes measurements from 76 stations from 1984 to 2004, which indicated a rise of up to 1.8 °C. According to NOAA, as we approach 2100, water temperature will rise even faster, and by the year 2080, 64% of European streams may become uninhabitable for brown trout.

This study explored the acclimatisation potential of brown trout by gradually exposing them to a water temperature of 20 °C over a 21-day period. The acclimatisation potential was studied at three levels: molecular, metabolic and physiological. Results showed an increase in the expression of heat shock proteins (*hsp90*) as well as genes related to oxidative stress (*cat*) and apoptosis (*casp8*) in the treatment group (Fig. 1). The fish also exhibited energetic stress, indicated by a reduction in energy reserves (glycogen and triglycerides) and cell energy status (ATP) from the control (C) and treatment at the beginning (TB) to the treatment at the end (TE) (Fig. 1). Physiological stress was also evident, with the treatment group showing a suppression in growth compared to the control group. The findings of this study are important to evaluate the species' adaptive capacity in a warming world and assist in guiding targeted mitigation efforts.

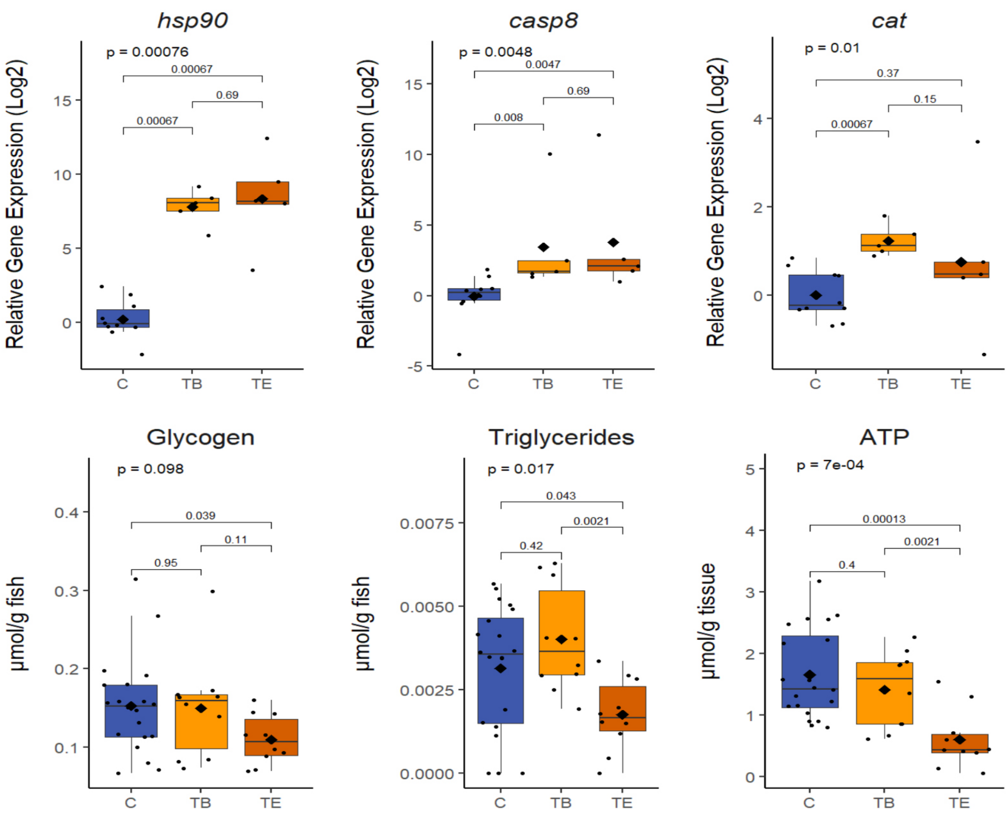

Fig 1: Relative gene expression of *hsp90*, *cat*, *casp8* in brown trout liver samples and metabolite concentration (glycogen, triglycerides, and ATP). C= control, TB = Treatment beginning - day 1, TE = Treatment end- day 21. P-values are indicated on the brackets above the box plots.

THE ANTIMICROBIAL AND VIRULENCE CHARACTERISTICS OF ZOONOTIC PATHOGEN *Myroides odoratimimus* ISOLATED FROM AQUARIUM FISH

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Myroides odoratimimus is an opportunistic bacterial pathogen predominantly found in environmental settings, particularly aquatic ecosystems. These species are usually regarded as low-grade opportunistic pathogens and are rarely isolated from clinical samples; however, they can occasionally become life-threatening. In immunocompromised individuals associated with aquatic ecosystems and fish carrying this bacterium, it poses significant health risks and exhibits zoonotic characteristics. Infections in humans are often overlooked due to their opportunistic pathogenic nature. The lack of extensive studies on the phenotypic and genotypic characterization of this environmental and opportunistic pathogen has meant that the virulence and antimicrobial properties of this agent have not been fully delineated.

In the present study, we aimed to detect bacterial pathogens in aquarium fish that have identified *M. odoratimimus* as a significant zoonotic risk to individuals involved in aquarium fish cultivation. For this purpose, conventional bacteriological sampling was employed using media such as Tryptic Soy Agar, Blood Agar, and Marine Agar, with samples taken from the internal organs of diseased fish. Following the isolation of the bacterial agents in pure culture, gram-negative, non-fermentative, and non-motile bacteria were isolated, and their genomes were characterized using next-generation sequencing technology. The genomes were assembled using online databases, and the antimicrobial resistance genes were analyzed using the antimicrobial resistance gene database (CARD), while the virulence genes were analyzed using the virulence gene database (VRDB).

The zoonotic potential of *M. odoratimimus* isolated from aquarium fish, along with the discovery that it carries the beta-lactamase gene (MUS-1), which is responsible for resistance to carbapenem, penam, and penem group antibiotics, was established. Detected virulence genes such as *pil*R, *htp*B, capsule *rml*C, Hemolytic phospholipase C (*plc*H), *bau*E, *hem*B, *hem*L, *clp*V1, *ure*B, *ure*G, *rml*A, *acp*XL, *gal*E, and *pan*D encode various virulence mechanisms of *Myroides* to the host cell (Table 1). Compared to other pathogens like *Aeromonas, Pseudomonas*, and *Lactococcus,* known for causing disease in fish and possessing zoonotic features, the antimicrobial resistance gene carriage of *M. odoratimimus* was found to be significantly lower, indicating a reduced risk of antimicrobial resistance spread. The virulence genes identified in this study highlight the pathogenicity features in humans, such as hemolytic activity, biofilm formation and urea degradation, demonstrating the opportunistic pathogenic nature of the isolate.

Table 1. Virulence genes and functions detected on the *M. odoratimimus* genome

Gene	Gene function
*pil*R	expression of type IV pili
*htp*B	bacterial invasion and survival
*rml*C	bacterial capsule
*plc*H	Hemolytic phospholipase
*hem*B	biosynthesis of heme
*hem*L	synthesis of tetrahydrofolate
*clp*V1	ATPase activation
*ure*B, *ure*G	urease enzyme complex that hydrolyzes urea into ammonia and carbon dioxide
*rml*A	biosynthesis of dTDP-L-rhamnose
*acp*XL	acyl carrier protein on very-long-chain fatty acid
*gal*E	Lipopolysaccharide synthesis
*pan*D	Aspartate-α-Decarboxylase

USING MACHINE LEARNING IN MOBILE RESPONSIVE CLINICAL FISH HEALTH DATABASE TO RECOGNIZE DISEASE DEVELOPMENT

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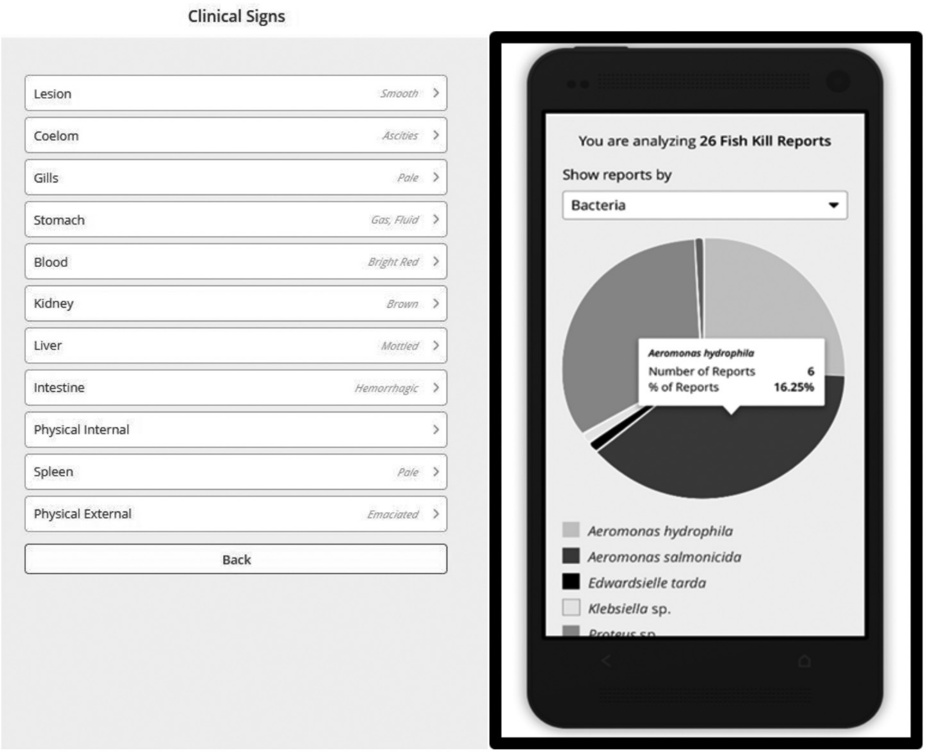
Kentucky State University's digitized Mobile Responsive Clinical Fish Health Database (for computers and mobile devices) enables more accurate disease case record-keeping, data mining, and timely identification and remediation of fish pathogens by fish health professionals, enhancing the effectiveness of fish health services to the aquaculture industry. The Database also serves as a reference source and teaching tool for fish disease diagnosticians and students. A current goal is to incorporate machine learning capabilities into the Database by uploading photographs of fish during the development phases of a disease (columnaris disease, *Flavobacterium columnare*, development will be used initially). Use of a monitoring camera and artificial intelligence to identify early stages of this disease in a fish population will be used when comparing the appearance of these fish to the uploaded photographs in the Database. It is anticipated that initial stages of columnaris will be detectable by a.i. before it is noticeable by those working with the fish, thus enabling earlier treatment of the disease, which would increase the chances of successfully treating the disease.



SELECTIVE BREEDING OF COPEPODS FOR HIGH FECUNDITY

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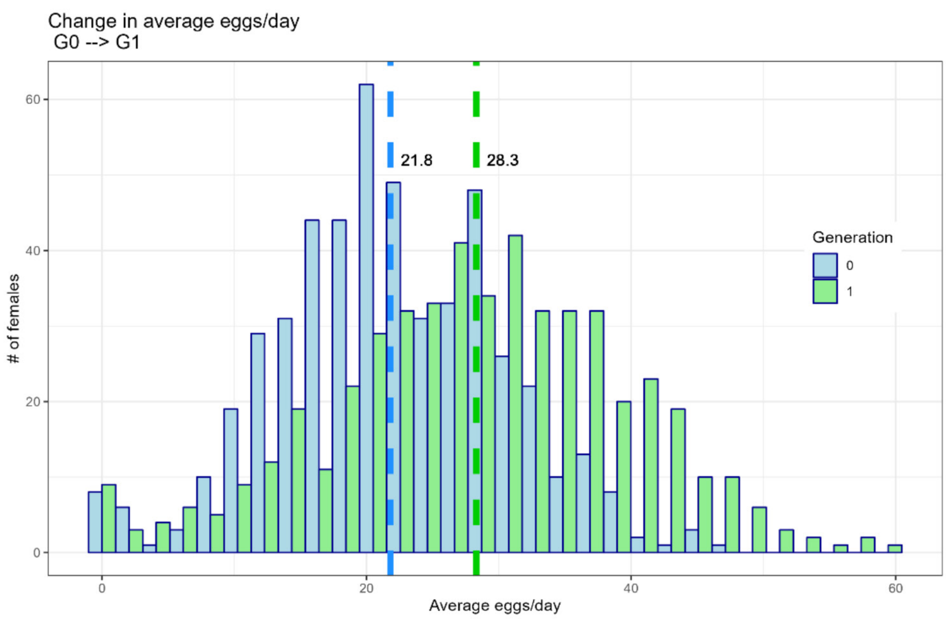
Copepods are a highly valued live prey item for larvae of marine finfish but until recently the controlled production of copepods for use in aquaculture has been scarce and small in scale. CFEED, a private company headquartered in Trondheim, Norway, has pioneered larviculture techniques to effectively produce *Acartia tonsa* year-round and offer a steady supply of eggs which can be delivered to hatcheries around the world. The population of copepods at CFEED, however, remains essentially wild and less effort has been made to improve production through selective breeding. Fecundity in calanoid copepods has been reported to be a highly heritable trait but there exist few examples of multi-generational breeding for increased egg production in these species.

In order to explore the potential of breeding to increase the egg production of female copepods, a selection program was initiated that systematically measured daily egg production for females, individually, over 7 days. Females with the highest average daily production of eggs were then selected for crossing into the next generation via a partitioned mass spawn design. Subsequent generations were reared in a similar fashion, selecting females at random from the mass spawned pool of juvenile copepods.

Initial results suggest that selection for fecundity produced rapid changes in the mean egg production of copepods between generations. The extent to which these substantial gains can continue to be improved at this rate remains to be determined, as well as whether increased egg production is a physiological trait with trade-offs in other performance metrics. Nevertheless, these findings suggest that selective breeding could be a valuable tool to increase the efficiency of commercial copepod farming. After several generations, egg production traits will be compared to an unselected population of copepods in a common environment to evaluate estimates of genetic gain.



PREVENTION OF FOOD CONTAMINATION BY A POLYSACCHARIDE – ZINC COMPLEX DERIVED FROM RED MICROALGAE

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The potential applicability of algae as a bioresource for the sustainable production of foods, cosmetics and pharmaceutical products is virtually unlimited. The exploitation of algal bioresources is timely in light of the current market trend toward a greater reliance on natural products. Among the principal algal sources are red microalgae, which produce unique biochemicals including novel sulfated polysaccharides (PS). In recent years, Arad laboratory has developed the biotechnology for the production of valuable products based on red microalgae with an emphasis on isolated sulfated polysaccharides found in the algal cell wall. The combination of the diverse biological activities of these novel molecules (e.g. antiviral, antioxidant, anti -inflammatory and soothing properties), with their distinctive properties (i.e., composition, structure, rheology and extreme stability), can be exploited across a vast range of applications in the pharmaceutical, cosmetics, and food industries.

The red microalga, *Porphyridium* sp., is encapsulated within a negatively charged PS that has unique rheological characteristics which make it an excellent emulsifier. The PS was shown to act as a platform for metal incorporation, taking advantage of its ion-exchange capabilities and its negative charge. In the current study we investigated the combination of emulsifying and antibacterial activities of a Zn-PS complex. It was shown that dairy emulsions and oil-in water emulsions were stable in low concentrations of Zn-PS complex (<0.2% and <500 ppm Zn). The Zn-PS complex was also shown to have higher effect on inhibition of bacterial growth when compared with the algal polysaccharide alone. These results suggest that the Zn-PS complex has significant potential as a novel emulsifier that also inhibits food contamination. Overall, the data support the potential of using functional sulfated polysaccharides from red microalgae to stabilize emulsions and to act as an antibacterial agent in food applications. As such, the sulfated polysaccharide of the red microalga *Porphyridium* sp. is of particular interest. The results of this study may hold important implications for the possible utilization of red microalgal polysaccharides as a novel additive in food manufacturing.

EUGENOL SELF-ASSEMBLED NANOPARTICLE AS AN ALTERNATIVE TO TREAT AHPND-INFECTED SHRIMP *Litopenaeus vannamei*

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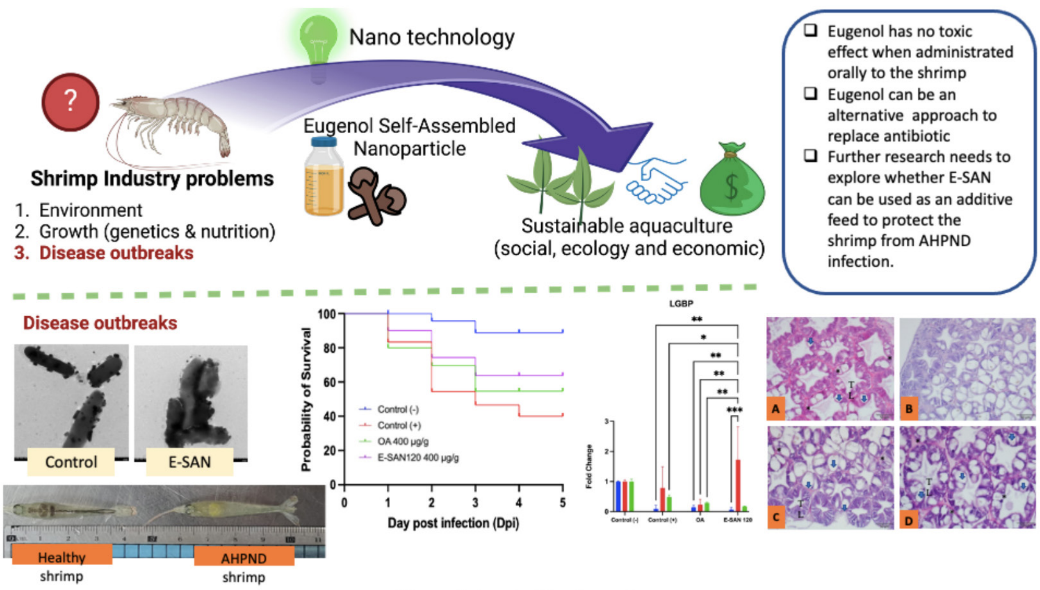
Eugenol has demonstrated a diverse array of beneficial properties, underscoring its importance as a bioactive phytoconstituent with a range of health-promoting effects, such as antioxidant, anti-inflammatory, and antimicrobial activity against a variety of pathogens, including Gram-positive and Gram-negative bacteria. In shrimp industry, acute hepatopancreas necrosis disease (AHPND) is the main bacterial disease which infected both hatchery and grow out pond. Eugenol self-assembled nanoparticle (E-SAN) was produced by heating in 120 °C and proceed with solvent injection method. The size of particles is around 60-100 nm and MIC_{50} against *Vibrio parahaemolyticus* is around 40-80 μg/mL. The E-SAN has been proven no toxic effect based on MTT assay in hemocyte at concentration 400 μg/mL and has antioxidant activity that able to inhibit ROS when hemocyte was induced with bacterin. The acute toxicity assay was tested in several dosages of E-SAN 40, 400, and 4000 μg/g to the shrimp for 96 hours. There was no mortality occurred during observation and also no significant different in histology of hepatopancreas. Moreover, as an alternative to replace antibiotic, E-SAN can be used as curative agent to infected-AHPND shrimp at 400 μg/g in dose by administrated orally. Further research needs to explore whether E-SAN can be used as an additive feed to protect the shrimp from AHPND infection.



Graphical Abstract

UNIVERSITY OF MAINE AQUACULTURE RESEARCH INSTITUTE: AQUACULTURE EXPERIENTIAL OPPORTUNITIES FOR UNDERGRADUATE STUDENTS (AQUEOUS) PROGRAM

PIs: Darren Ranco, tish carr, M. Scarlett Tudor and Meggan Dwyer

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The Aquaculture Experiential Opportunities for Undergraduate Students (AquEOUS) program is at the forefront of bridging aquaculture research with Indigenous knowledge while fostering diversity and inclusivity in the field. AquEOUS's multifaceted approach, addresses an overarching goal is to establish sustainable partnerships with Native communities and recruit a diverse cohort of undergraduate students, ensuring broad participation in aquaculture initiatives. To achieve this, the program employs targeted outreach strategies, including meetings with Wabanaki representatives and partnerships with organizations like the the Wabanaki Center and the Wabanaki Youth in Science (WaYS) Program.

Central to AquEOUS's approach is cohort building and peer-to-peer mentorship, fostering a collaborative and supportive learning environment. The program facilitates interdisciplinary interactions through field trips to aquaculture sites, seminars on Indigenous knowledge, and workshops on inclusive science communication. Moreover, AquEOUS prioritizes the incorporation of Indigenous Cultural and Ecological Knowledge into student projects, promoting a holistic approach to aquaculture.

In addition to academic learning, AquEOUS provides hands-on skills development in aquaculture research and science communication. Students engage in fieldwork, laboratory experiments, and extension activities, gaining practical experience in sustainable aquaculture practices. Professional development opportunities, including workshops and seminars, further enhance students' skills in science communication and community engagement. AquEOUS's innovative approach to aquaculture education highlights a commitment to diversity, sustainability and hands-on learning experiences.

INFECTION WITH NERVOUS NECROSIS VIRUS DIFFERENTIATES THE NEURONAL AND ASTROGLIAL GENE EXPRESSION OF DISEASE RESISTANT AND SUSCEPTIBLE EUROPEAN SEA BASS FAMILIES (*Dicentrarchus labrax*)

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Aquaculture is essential to cover fish-product demands, providing seafood in high quantities, and covering the half amount of fish consumed worldwide. Greece is one of the leading producers of sea bream (*Sparus aurata*) and European sea bass (*D. labrax*) in the Mediterranean. As fish health and welfare is a prerequisite for sustainable and profitable production in the Mediterranean area, combating diseases is highlighted as priority for the development and improvement of aquaculture sector. The most significant disease in terms of severity, economic impact and spread, is viral nervous necrosis (VNN). VNN is a devastating disease, which induces cell necrosis accompanied by vacuolation in fish retina and brain. The disease is caused by nervous necrosis virus (NNV), affecting more than 30 different fish species, worldwide. The first step to move forward on the battle against the NVV disease is to fully understand its progression and its effect on the host. Aim of the present study is to identify which cell brain population is attacked by the NNV and how it affects the neuronal and astroglial gene expression during the disease progression.

Hence, we studied the gene expression of neuronal and astroglial markers in brain tissues of experimentally infected *D. labrax* upon 0 and 3 hours of infection, 2 and 14 days of infection and upon 7 days following re-infection of the fishes that survived. Two different families of *D. labrax* were included in the current study: a sea bass family which exhibits resistance to NNV and an NNV-susceptible family. Comparison of the brain cell types' gene expression in these two families upon infection with NNV, showed different expression patterns for neuronal (rbfox3, map), astroglial (coro1a, gfap) and whole brain markers (cart1, pomc1) between the two families, highlighting probable molecular mechanisms that are triggered in the brain for managing the infection more effectively.

Moreover, in brain primary cell cultures derived from uninfected *D. labrax*, we evaluated the expression of neuronal and astroglial markers using specific antibodies in an immunofluorescence before and after NNV infection of the primary cell cultures. Both neuronal and astroglial expressing cells were developed in the cultures and upon NNV infection, glial fibrillary acidic protein (GFAP- astroglial marker) became condensed while vacuoles and disrupted structures were observed in Tuj- expressing cells (neuronal marker).

Overall, the results of the present study will provide a better understanding of the impact of NNV in the fish brain cell populations and it will give us the tools to further investigate potential signaling pathways involved in the progression of a nervous necrosis infection.

Funding: The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: 03990)

THE EFFECT OF DIETARY CHITIN ON NUTRIENT DIGESTIBILITY IN RAINBOW TROUT FEED

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As the investigation for sustainable aquafeed ingredients intensifies, alternative ingredients such as shellfish waste, fungi, and insects have received increasing attention. Despite the moderate to high protein content in these raw materials, chitin might potentially limit their applications in fish feed. In this study, we investigated the effect of dietary chitin on nutrient digestibility in juvenile rainbow trout (*Oncorhynchus mykiss*). Trout were acclimated for two weeks followed by a two-week digestibility period. Triplicate groups of trout were fed either a control diet or a control diet where 1.0, 2.5, or 5.0% of wheat meal was replaced by a commercial grade chitin powder from shrimp shells. It was found that the feed conversion ratio gradually increased with increasing dietary chitin inclusion levels. The apparent digestibility of dry matter, organic matter, protein, lipid, and energy showed a strong linear negative correlation to dietary chitin inclusion levels. These findings suggest that even at dietary inclusion levels as low as 1%, chitin exerts adverse effects on both fish performance and nutrient digestibility.

EFFECT OF RED MICROALGA *Rhodomona salina*-BASED EXPERIMENTAL FEED ON ATLANTIC SALMON (*Salmo salar*) FILLETS IN RECIRCULATING AQUACULTURE SYSTEMS: CAN MARINE FLAVOURS BE ENHANCED WHILE MITIGATING OFF-FLAVOURS?

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Recirculating aquaculture systems (RAS) offer many production and environmental advantages, yet present challenges in ensuring high-quality seafood flavour due to the presence of off-odour compounds. Specifically, compounds like geosmin and 2-methylisoborneol (2-MIB) are associated with an "earthy" off-flavour, which can diminish palatability and reduce consumer demand for RAS-grown fish (Abd El-Hack et al., 2022; B. C. Jones et al., 2022). Recent studies examined correlations between off-odour taints in aquaculture fish and the respective feeds, suggesting a potential absorption of such odorous compounds through ingestion (Mahmoud et al., 2018; Podduturi et al., 2017). At the same time, the accumulation of palatable feed-borne odours in seafood may offer a beneficial masking effect on the RAS waterborne "earthy" off-odours as well as enhancing "marine" odours, which are notoriously weaker or absent in aquaculture seafood (B. C. Jones et al., 2022). The red microalga *Rhodomona salina*, for example, is rich in volatile organic compounds eliciting "marine" odours (Coleman et al., 2022; Van Durme et al., 2013), and algae-based aquaculture feeds have proved to enhance "marine", "crustacean-like" and "algae-like" flavours in different fish species (Guevara et al., 2011; B. Jones et al., 2016). To explore this opportunity in detail, the present work reports on a dose-response feeding trial that was conducted in a semi-commercial RAS to enhance *R. salina*-sourced "marine" odours in Atlantic salmon (*Salmo salar*) and assess the potential mitigation of "earthy" off-odours (B. Jones et al., 2016). Four experimental feeds were formulated and manufactured with increasing inclusion levels of *R. salina* (Control, 10%, 20% and 30%) and fed to 100 Atlantic salmon reared in two RAS systems for 22 days. Salmon fillets and water were sampled on day 0, 7, 14 and 21. Several algal compounds, were targeted in this study, as were waterborne RAS off-odours, including geosmin, 2-MIB, benzaldehyde, dimethyl sulfide, methional, dimethyl disulfide, dimethyl trisulfide, 3-methyl butanal, a-ionone, furfural, 2,3-octanedione, pentanal, 1-octen-3-ol, (3E,5Z)-octa-3,5-dien-2-one, (3E,5E)-octa-3,5-dien-2-one, (2E,4E)-hepta-2,4-dienal and 6-methyl-5-hepten-2-one. These compounds were traced and quantified with sensory evaluations combined with analytical methods (e.g., solvent assisted flavour evaporation (SAFE), gas chromatography-mass spectrometry/olfactometry (GC-MS/O), aroma extraction dilution analysis (AEDA), and proton transfer reaction-time of flight-mass spectrometry (PTR-TOF-MS). Comparative sensory profiling of salmon fillets was also conducted to verify the absorption of "marine" odours, examine the masking of "earthy" off-odours and identify the optimum concentration and feeding duration of *R salina*. This research project offers valuable insights and strategies for mitigating off-odours in aquaculture seafood, enhancing the flavour quality, and optimizing cost-efficiency in RAS production.

References

Abd El-Hack M. E., El-Saadony M. T, Elbestawy A. R., Ellakany H. F., Abaza S. S., Geneedy A. M, Salem H. M., Taha A.E., Swelum A. A., Omer F. A., AbuQamar S.F., El-Tarabily K. A., (2022). Undesirable odour substances (geosmin and 2-methylisoborneol) in water environment: Sources, impacts and removal strategies, Marine Pollution Bulletin, Volume 178, 2022 113579, ISSN 0025-326X, https://doi.org/10.1016/j.marpolbul.2022.113579.

Coleman, B., Van Poucke, C., Dewitte, B., Ruttens, A., Moerdijk-Poortvliet, T., Latsos, C., De Reu, K., Blommaert, L., Duquenne, B., Timmermans, K., van Houcke, J., Muylaert, K., & Robbens, J. (2022). Potential of microalgae as flavoring agents for plant-based seafood alternatives. Future Foods, 5. https://doi.org/10.1016/j.fufo.2022.100139

Guevara, M., Bastardo, L., Cortez, R., Arredondo-Vega, B., Romero, L., & Gómez, P. (2011). Pastas de Rhodomonas salina (Cryptophyta) como alimento para Brachionus plicatilis (Rotifera). Revista de Biologia Tropical, 59(4), 1503–1515. https://doi.org/10.15517/rbt.v59i4.3415

(Continued on next page)

Jones, B. C., Rocker, M. M., Keast, R. S. J., Callahan, D. L., Redmond, H. J., Smullen, R. P., & Francis, D. S. (2022). Systematic review of the odorous volatile compounds that contribute to flavour profiles of aquatic animals. Reviews in Aquaculture. https://doi.org/10.1111/raq.12657

Jones, B., Smullen, R., & Carton, A. G. (2016). Flavour enhancement of freshwater farmed barramundi (Lates calcarifer), through dietary enrichment with cultivated sea lettuce, Ulva ohnoi. Aquaculture, 454, 192–198. https://doi.org/10.1016/j.aquaculture.2015.12.017

Mahmoud MAA, Tybussek T, Loos HM, Wagenstaller M and Buettner A (2018). Odorants in Fish Feeds: A Potential Source of Malodors in Aquaculture. Front. Chem. 6:241. doi: https://doi.org/10.3389/fchem.2018.00241

Podduturi R., Petersen M. A, Mahmud S., Rahman Md. M, and Jørgensen N.O. G., (2017). Potential Contribution of Fish Feed and Phytoplankton to the Content of Volatile Terpenes in Cultured Pangasius (Pangasianodon hypophthalmus) and Tilapia (Oreochromis niloticus). Journal of Agricultural and Food Chemistry 2017 65 (18), 3730-3736. DOI: https://doi.org/10.1021/acs.jafc.7b00497

Van Durme, J., Goiris, K., De Winne, A., De Cooman, L., & Muylaert, K. (2013). Evaluation of the volatile composition and sensory properties of five species of microalgae. Journal of Agricultural and Food Chemistry, 61(46), 10881–10890. https://doi.org/10.1021/jf403112k

Schram, E., Kwadijk, C.J.A., Hofman, A., Blanco, A., Murk, A.J., Verreth, J.A.J., Schrama, J.W., 2021. Effect of feeding during off-flavour depuration on geosmin excretion by Nile tilapia (Oreochromis niloticus). Aquaculture 531 (2021) 735883 https://doi.org/10.1016/j.aquaculture.2020.735883

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QUANTIFICATION OF WATERBORNE GEOSMIN ABSORPTION BIOKINETICS IN RAINBOW TROUT (*Oncorhynchus mykiss*) TISSUES AND ASSESSMENT OF HORMONAL STRESS RESPONSE: ENHANCEMENTS TO THE ONE-COMPARTMENT MODEL

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Recirculating aquaculture systems (RAS) provide various production and environmental benefits yet introduce challenges in maintaining high seafood flavour quality. Specifically, the microbial compound geosmin is frequently associated with an 'earthy' off-flavour in RAS-grown fish. Due to its moderate lipophilicity, geosmin readily accumulates in fish tissue, thereby diminishing palatability and lowering consumer demand for RAS-cultivated fish (Abd El-Hack et al., 2022). There is currently a lack of comprehensive description of the waterborne geosmin biokinetics in fish tissues and only a few models partially describe the absorption and excretion processes (Tucker & Schrader, 2020). Of note is the one-compartment model developed by Howgate (2004), which conceptualises fish as a single compartment consisting of lipids and water, wherein passive uptake and elimination rates are governed by the octanol-water partitioning coefficient between the organism and its surrounding water. Several studies by Schram et al., (2016, 2017, 2018; 2021a; 2021b) advanced the one-compartment model across various aquaculture species. Their findings revealed the non-significant influence of the renewal rate of depuration water on geosmin excretion, contradicting the one-compartment model's assumption of passive diffusion and implying the involvement of alternative biotransformation mechanisms. Fish liver was proposed as a site for geosmin biotransformation, and faster elimination rates were observed in fed fish compared to starved ones, thereby implicating blood lipid content in improving geosmin excretion. Few recent studies suggest the involvement of cytochrome P450 and UDP-glucuronosyltransferase in geosmin biotransformation in the liver as well as causing oxidative stress in fish (Zhang et al., 2024). The objective of this study was to quantify the absorption kinetics and dynamics of geosmin across three biological tissues: fillet, blood, and liver. Additionally, the study aimed to evaluate the excretion/breakdown of geosmin in the liver and the presence of hormonal stress response. During the trial, rainbow trouts (*Oncorhynchus mykiss*) were unfed and kept in static water conditioning tanks. Pure geosmin standard was spiked at 400 ng/L and the respiratory uptake and metabolism in juvenile rainbow trout was assessed over time (144h), applying the Before-After-Control-Impact design, hence sampling animals before spiking geosmin and fishing out one entire treatment and one control tanks at five time points. The water, fish blood, fish fillets and livers were collected at each sampling event. A subsample of blood was set aside for biochemical profiling of the blood plasma. Geosmin was traced and quantified with sensory evaluations combined with analytical methods (gas chromatography-mass spectrometry/olfactometry (GC-MS/O) and proton transfer reaction-time of flight-mass spectrometry (PTR-TOF-MS). This research offers valuable insights on the biological mechanism governing geosmin absorption and excretion/breakdown in aquaculture fish and suggests strategies for mitigating off-odours in aquaculture seafood.

References

Abd El-Hack, M. E., El-Saadony, M. T., Elbestawy, A. R., Ellakany, H. F., Abaza, S. S., Geneedy, A. M., Salem, H. M., Taha, A. E., Swelum, A. A., Omer, F. A., AbuQamar, S. F., & El-Tarabily, K. A. (2022). Undesirable odour substances (geosmin and 2-methylisoborneol) in water environment: Sources, impacts and removal strategies. Marine Pollution Bulletin, 178, 113579. https://doi.org/10.1016/j.marpolbul.2022.113579

Howgate, P. (2004). Tainting of farmed fish by geosmin and 2-methyl-iso-borneol: A review of sensory aspects and of uptake/depuration. Aquaculture, 234(1–4), 155–181. https://doi.org/10.1016/j.aquaculture.2003.09.032

Schram, E., Kwadijk, C., Blom, E., Verreth, J. A. J., Murk, A. J., & Schrama, J. W. (2021). Interactive effects of temperature and water exchange of depuration tanks on geosmin excretion by Atlantic salmon (Salmo salar). Aquaculture, 535. https://doi.org/10.1016/j.aquaculture.2021.736358

(Continued on next page)

Schram, E., Kwadijk, C., Hofman, A., Blanco, A., Murk, A., Verreth, J., & Schrama, J. (2021). Effect of feeding during off-flavour depuration on geosmin excretion by Nile tilapia (Oreochromis niloticus). Aquaculture, 531. https://doi.org/10.1016/j.aquaculture.2020.735883

Schram, E., Schrama, J., Kusters, K., Kwadijk, C., & Palstra, A. (2016). Effects of exercise and temperature on geosmin excretion by European eel (Anguilla anguilla). Aquaculture, 451, 390–395. https://doi.org/10.1016/j.aquaculture.2015.09.032

Schram, E., Schrama, J. W., van Kooten, T., Kwadijk, C. J. A. F., Kampen, H., van de Heul, J. W., Verreth, J. A. J., & Murk, A. J. (2018). Experimental validation of geosmin uptake in rainbow trout, Oncorhynchus mykiss (Waldbaum) suggests biotransformation. Aquaculture Research, 49(2), 668–675. https://doi.org/10.1111/are.13496

Schram, E., van Kooten, T., van de Heul, J. W., Schrama, J. W., Verreth, J. A. J., & Murk, A. J. (2017). Geosmin depuration from European eel (Anguilla anguilla) is not affected by the water renewal rate of depuration tanks. Aquaculture Research, 48(9), 4646–4655. https://doi.org/10.1111/are.13287

Tucker, C. S., & Schrader, K. K. (2020). Off-flavors in pond-grown ictalurid catfish: Causes and management options. In Journal of the World Aquaculture Society (Vol. 51, Issue 1, pp. 7–92). Blackwell Publishing Inc. https://doi.org/10.1111/jwas.12672

Zhang, J. ming, Han, H., Li, Y. chao, Fu, B., Kaneko, G., Li, K., Jin, X. chen, Ji, S., Yu, E. meng, & Liu, L. ping. (2024). Comprehensive mRNA and microRNA analysis revealed the effect and response strategy of freshwater fish, grass carp (Ctenopharyngodon idella) under geosmin exposure. Ecotoxicology and Environmental Safety, 269. https://doi.org/10.1016/j.ecoenv.2023.115775

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FERMENTED SUNFLOWER MEAL PROMOTES GUT LACTIC ACID BACTERIA AND IMPROVES GUT HEALTH OF FARMED ATLANTIC SALMON UNDER COMMERCIAL-LIKE FIELD CONDITIONS

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Increasing production volumes of fed aquaculture have caused an escalating demand for alternative protein raw materials in fish feed. Agro-industrial by-products such as sunflower meal are relatively abundant and cheap, but inclusion levels are limited due to the presence of antinutritional factors, an imbalanced amino acid profile and fiber. Solid state fermentation is a processing method which intends to reduce the content of fiber and antinutritional factors, increase protein content and nutrient digestibility to make plant raw materials more suitable in aquafeeds. The aim of this study was to evaluate the effect of fermented sunflower (FSF) meal in diets for Atlantic salmon farmed under field conditions at two commercial-like fish farms in a coastal and fjord location with 309 500 and 235 800 fish, respectively.

Atlantic salmon were fed diets containing 5% and 10% FSF meal and a reference diet containing 5% non-fermented sunflower (SF) meal (Table 1). The field experiment lasted for eight and eleven months at the coast and fjord location, respectively. The technical quality of the pellets was assessed based on expansion, hardness, durability and sinking velocity. Digesta samples were collected from the distal intestine (DI) for microbiota analysis with 16s ribosomal rRNA sequencing, and DI tissue was used for RNA sequencing and histological analysis with H&E, periodic acid-Schiff and Alcian blue staining.

All diets showed high physical pellet quality. There were no significant differences between dietary groups related to feed intake, feed conversion ratio or growth rate. Histological analyses revealed that fish at the coast location had significantly less ectopic goblet cells in the DI, when fed 10% FSF meal. At the fjord location, fish fed 10% FSF meal had a significantly reduced prevalence of prominent DI inflammation (Figure 1). 16s RNAseq of DI digesta showed that *Lactiplantibacillus (p = 0.0001)* and *Lactobacillaceae (p = 0.006)* were significantly more abundant in fish fed 10 % FSF meal, and beta diversity increased significantly (Figure 2).

The results of this study indicate that FSF meal promotes gut lactic acid bacteria and improves gut health in Atlantic salmon farmed under commercial-like conditions.

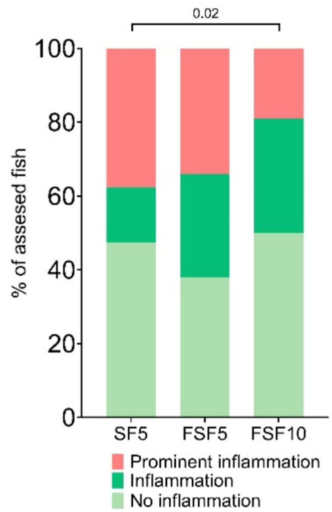

Figure 1: Histology scores of the distal intestine of fish fed experimental diets.

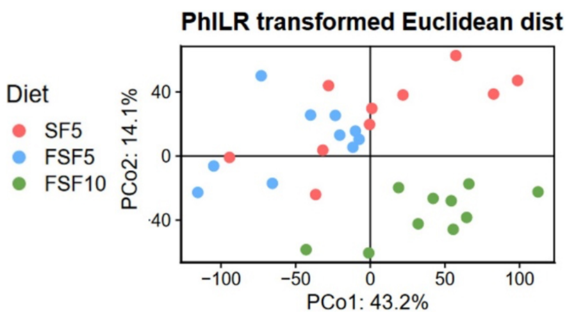

Figure 2: Beta diversity of distal intestine microbiota of fish fed experimental diets, assessed with the PhILR distance metric.

ONSET OF CIRCADIAN RHYTHMICITY IN THE BRAIN OF ATLANTIC SALMON IS LINKED TO EXOGENOUS FEEDING

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Biological processes are adapted to and driven by rhythmicity in the natural environment and periodicity of light is one of the most influential factors. In the aquaculture of Atlantic salmon, artificial light conditions are widely used, even though the life history transitions of salmon are closely linked to the seasonally changing light environment. The onset of circadian rhythmicity might indicate the time point of functional necessity for aligning processes to the environment, and here, the circadian clock mechanism has been studied in the developing brain of Atlantic salmon. By a transcriptomic approach, the endogenous feeding alevin, independent on the environment for nutritional supply, has been compared to the exogenous feeding fry, dependent on the light period for detecting and catching prey. The results revealed that circadian rhythmicity becomes established during the first feeding period in the salmon brain, when the main components of the molecular clock mechanism start to cycle.

RNA sequencing has been done to analyze 48 h circadian sampling series of 104 Atlantic salmon brains, in the endogenous feeding alevins and exogenous feeding fry, to study the onset of circadian rhythmicity.

The results show that many of the circadian clock genes have started to cycle with a period of 24 h in the exogenous feeding salmon fry, while few clock genes were cyclic in the exogenous feeding yolk sac alevin. Fig 1 illustrates the expression profile of clock genes, revealing a significantly higher and cyclic expression in the fry, compared to the non-cyclic alevin. Few genes were differentially expressed between timepoints in the circadian sampling series before feeding, but in the exogenous feeding salmon fry, several hundred were differentially expressed. In the KEGG pathway circadian rhythm many genes were cyclic in the exogenous feeding fry and analyzes of the KEGG pathway cell cycle indicated a clock-controlled cell cycle at exogenous feeding.

Taken together, the life history transition related to onset of exogenous feeding is linked to the establishment of a persistent circadian rhythmicity in the salmon brain, which needs to be synchronized to light-dark cycles to enable the fry to search and capture feed.

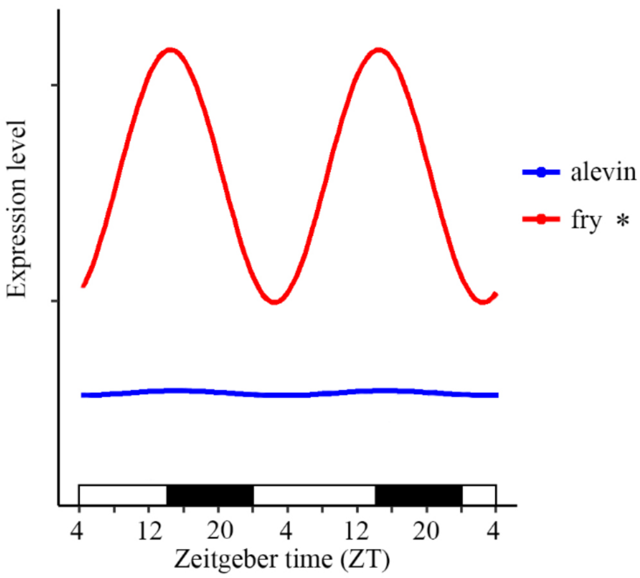

Fig. 1 Brain expression profile. In salmon alevin the expression was non-cyclic, while in the fry several clock genes were significantly cyclic with an acrophase early in the dark phase. * 24 h cycling period with $p < 0.05$.

THE DIGESTION TIME FOR SALMON LOUSE *Lepeoptheirus salmonis* IN LUMPFISH *Cyclopterus lumpus* IN RELATION TO SAMPLING METHOD, DEVELOPMENTAL STAGE, AND TEMPERATURE

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Cleaner fish are widely used to combat sea lice on farmed Atlantic salmon. Unlike wrasse, lumpfish are native to the Faroe Islands, making them the only cleaner fish species used in the local salmon production. The number of lumpfish used in the Faroe Islands has been gradually decreasing since 2020. Mostly due to high mortalities and difficulties in verifying their effect. Routine welfare monitoring of lumpfish includes stomach content analysis to assess their consumption of sea lice. However, estimating lumpfish efficiency based on this method is challenging due to uncertainties about the digestion time for salmon lice in lumpfish, complicating the assessment of lumpfish efficacy.

Two 12-day trials were conducted at two different temperatures (6°C and 9°C), each involving 64 lumpfish in batches of eight. Lumpfish were fed a single salmon louse, with six receiving a preadult II or adult male louse and two receiving an adult female louse. Lumpfish were fed lumpfish feed once or twice daily throughout the experiment.

The results showed no apparent difference in the proportion of lice recovered at different temperatures (Figure 1). However, lice were still detectable in the stomachs after 12 days. Notably, while all preadult II/adult males were fully digested by day 12, adult female lice were still present (Figure 1). This suggests that estimating lumpfish efficacy based on stomach content analysis should consider the developmental stages of lice.

These findings contrast with recently published results on the same subject, particularly regarding digestion time and differences in digestion rates among developmental stages of salmon lice. This emphasizes the complexity of accurately estimating the efficacy of cleaner fish in controlling sea lice infestations. Further research is needed to better understand and optimize the use of cleaner fish in aquaculture practices.

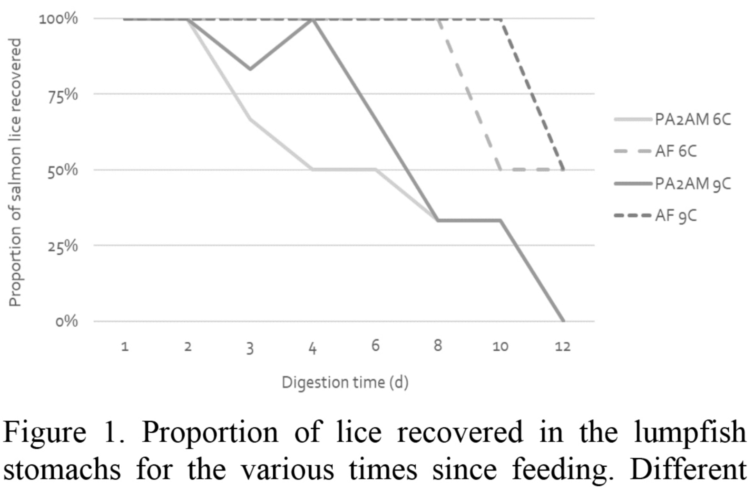

Figure 1. Proportion of lice recovered in the lumpfish stomachs for the various times since feeding. Different colours indicate different temperatures. Dashed and solid lines indicate adult females and preadult II/adult males, respectively.

CO-FARMING WATERCRESS (NASTURTIUM OFFICINALE) AND DUCKWEED (LEMNA MINOR) TO ENHANCE COMMON CARP (*Cyprinus carpio*) PRODUCTION

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Implementation of eco-intensification of common carp (*Cyprinus carpio*) aquaculture by improved nutrient management has the potential to diversify production strategies that are much needed in carp farming. Sequestration of nutrients, as well as other compounds, by aquatic plants is a common method to improve water quality. However, common carp aquaculture seeks for low-cost and adaptable solutions to co-produce plants that might be further sold a as a food or feed-grade products to increase overall profitability of the farm. One of the most promising aquatic plants is watercress (*Nasturtium officinale*), a semi-aquatic perennial plant known for its nutrient-rich leaves and crisp, peppery flavour. Another widespread aquatic plant is duckweed (*Lemna* sp.), a small floating aquatic plant that may holds immense promise in pond ecosystems. Duckweed offers several benefits when used in carp ponds. For example, duckweed can efficiently remove nutrients such as nitrogen and phosphorus from the water, contributing to improved water quality. The aim of the present study was to assess the production yield of watercress in recirculated aquaculture system (RAS), earthen ponds, and artificial channels, as well as duckweed in pond connecting channels. Additionally, the work evaluated the chemical composition of the plants produced in different systems and analysed possible further directions to use the biomass as food or feed.

The research findings revealed significant variations in water parameters among the experimental points where duckweed and watercress were cultivated. The RAS water showed distinct characteristics compared to other experimental sites, including the lowest pH, dissolved oxygen (DO), and total hardness, along with higher oxidoreductive potential (ORP) and the highest conductivity, alkalinity, and total dissolved solids. Water discharged from the pond with growing watercress had higher levels of DO (approximately 37-38%), temperature (around 3% higher), and reduced phosphorus content (decreased by approximately 33%, compared to inflow). Conversely, in the water at the end of the artificial channel with watercress, there were observed higher levels of pH (approximately 1%), ORP (by roughly 34%), and DO (approximately 5%). Analysis of water from the duckweed channel showed significant variability in parameters such as pH, ORP, DO, temperature, nitrogen, phosphorus, potassium, and inorganic carbon, showing great bioremediation potential.

Furthermore, the results underlined the viability and scalability of watercress and duckweed production within a common carp farm environment. Additionally, the chemical composition analysis revealed that both watercress and duckweed contained high levels of macro and micronutrients (excluding heavy metals), suggesting their potential for utilization in agriculture or related sectors.

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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 SNPtype Assays and the X9 Real-Time PCR System have addressed these barriers with a custom assay design service, cost-effective and high quality SNP assays, and a high-throughput workflow minimizing hands-on time. The salmon research community has been specifically hampered by the cost barriers, and would 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 SNPtype Assays, 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, SNPtype Assays provide significant cost savings for high-throughput, routine testing post panel development.

SNPtype Assays and the X9 System provided a rapid and cost-effective way to develop and implement a SNP genotyping panel. For the chum salmon panel development, a candidate list of 143 SNPs was selected based on their ability to provide population structure and harvest composition information within the chum species of salmon. The sequences around these SNPs were submitted to the Standard BioTools Assay Design Group for design and manufacture of allele-specific PCR primers. TaqMan® assays were also ordered for the same SNPs for comparison. Assays were run with 95 samples from three different locations in Washington State (Hamma Hamma River – 24, Kalama River – 24, Skookum River – 23), and one from British Columbia (Squakum Creek – 24). The Standard BioTools SNP Genotyping User Guide (PN 68000098) was followed, including specific target amplification (STA), or preamplification.

SNPtype Assays greatly reduced routine running costs of SNP genotyping panels for non-model organisms that previously were inhibited by the high cost of TaqMan assays. Recent developments in DNA sequencing technology greatly increased the numbers of SNPs available for non-model organisms. More SNPs can be tested using SNPtype Assays due to significant cost savings over TaqMan assays and researchers now have the option to develop multiple sets of high-quality 96 SNP panels for increased discrimination power. Additionally, Standard BioTools X9 System provided a rapid, high-throughput, low hands-on workflow that accelerated the panel development process and routine run time.

IMPACT OF FRESHWATER TREATMENT OF AMOEBIC GILL DISEASE ON ATLANTIC SALMON, *Salmo salar*, GILL HEALTH AND MICROBIOME DYNAMICS

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Amoebic gill disease (AGD) affects many different species across a broad geographical range within the context of commercial finfish mariculture. Associated with high economic losses, 100 million USD from two outbreaks alone, considerable scientific endeavour has been undertaken to identify and mitigate the impact of this disease. Caused by the ectoparasite *Neoparamoeba perurans,* significant effort has been made to understand the disease and inform approaches to control outbreaks and reduce loss. Currently, freshwater treatment is the method of choice for the treatment of AGD in Atlantic salmon farms however little is known about the impact of freshwater treatment on gill health and microbiome dynamics.

In this study, we sought to examine the impact of freshwater treatment of AGD on gill health. Several methods were used to characterise gill health and response to freshwater treatment including a detailed examination of the gill surface for microparasites using both histology and qPCR and targeted expression analysis of key markers of the Atlantic salmon gill immune response. Additionally, we analysed the bacterial communities present on the gill surface using a non-invasive sampling approach. Microbiome analysis was performed by developing a novel quantification protocol that included a titration step before building 16S rRNA amplicon libraries in this challenging tissue.

Results showed freshwater treatment of AGD had a notable impact on the Atlantic salmon gill microbiome and inflammatory responses. Gill bacterial community structure changed significantly after freshwater bath application (Figure 1). Our data indicated that the freshwater treatment not only decreased levels of *N. perurans* but also decreased the bacteria related to AGD. Parallel analyses of gene expression revealed that MHCII, IL-1β and TNF-α mRNA transcripts were downregulated after freshwater treatment, whereas TGF- β and IL-10 mRNA abundance was not impacted.

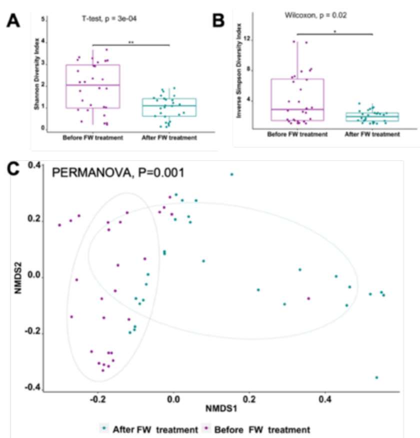

Figure 1. Impact of freshwater treatment of amoebic gill disease (AGD) on Atlantic salmon gill microbiome (n=30). Alpha diversity metrics, Shannon (A) and Inverse Simpson (B), of salmon gill microbial communities before and after freshwater treatment. (C) Non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarity of gill microbial communities.

GENETIC PARAMETERS FOR RED MUSCLE DEVELOPMENT MEASURED USING ARTIFICIAL INTELLIGENCE AND GENETIC CORRELATIONS WITH GROWTH AND PROCESSING TRAITS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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The production of rainbow trout (*Oncorhynchus mykiss*) in France is increasingly focused on large-sized fish intended for smoking. The presence of red muscle (RM), easily identifiable by its brown color, leads to flavor defects. Some specifications require processors to remove this portion of fillet, resulting in a direct economic loss. One of the objectives of the study is to quantify the genetic component associated with red muscle, through the estimation of genetic parameters including correlations with growth and processing traits and comparison of performances between crosses of two distinct strains.

An experimental cohort was produced by crossing 40 neomales and 40 females from the 2 commercial lines of rainbow trout from Aqualande group, according to 4 genotypes (AA, AB, BA and BB). At 18 months, 1436 fishes were slaughtered and measured. A steak before the dorsal fin and the caudal part of each individual were frozen and a DNA sample was collected for genotyping. The relative area of subcutaneous fat was estimated by Magnetic Resonance Imaging (MRI) on the steak. A thin transversal slice of the caudal part was photographed to quantify the area of red muscle using artificial intelligence (AI). After quality control for phenotypes, genotypes, and pedigree reconstruction through parentage assignment, data from 1415 individuals were finally used for analysis. An animal model with genomic relationship matrix was used to estimate the genetic parameters related to the area and proportion of RM. Performances of the 4 genotypes were compared with Tukey test to investigate heterosis and dominance effects.

The mean proportion of RM relative to the area of the steak was 8.52% in the study dataset. Heritability estimates were 0.24 for RM area and 0.32 for RM proportion. The genetic correlation between the two traits was positive but moderate (0.52). The proportion of RM measured by AI was genetically uncorrelated of other processing traits, whether it was with weight (0.09), carcass yield (0.09), eviscerated headless carcass yield (0.08) and relative surface of subcutaneous fat (0.09). Mean performances of hybrid crosses were systematically positioned between performances of pure lines. No dominance or heterosis effect was observed.

In brief, a new phenotyping method to measure RM surface by IA was developed in this study. The proportion of red muscle is heritable and genetically uncorrelated to growth and main processing traits. Reducing red muscle proportion in commercial stocks through its integration into breeding program is therefore feasible.

European Maritime and Fishery Fund and the French Ministery of Agriculture co-funded this work within the RedOUT project (FEAMP PFEA470019FA1000007).

Figure: Red muscle segmentation using artificial intelligence.

TAKE A CHILL PILL: REDUCING STRESS LEVELS OF SMOLT SALMON *(Salmo salar)* USING A COMMERCIAL BLEND OF *Melissa officinalis* EXTRACT DURING TRANSPORTATION REDUCES EARLY MORTALITY AT SEA

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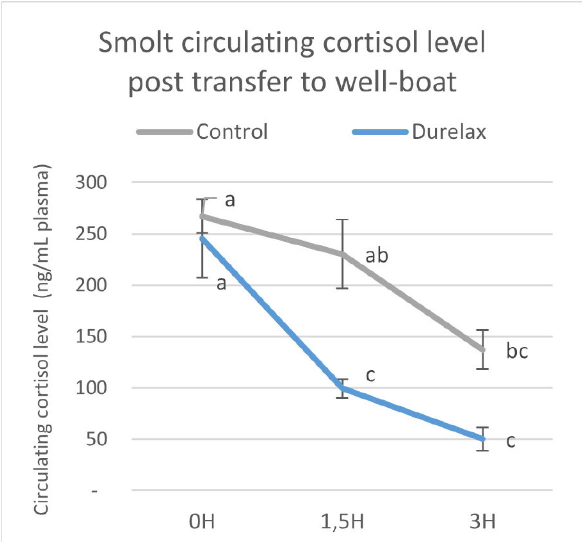
Stress is a critical aspect of fish farming since it can trigger metabolic cascades resulting in immunosuppression and weakening of the fish. Previous research showed that the use of a commercial blend of *Melissa officinalis* and soluble magnesium (Durelax® Liquid, DLX, Nor-Feed, France) in the water of juvenile Atlantic salmon (*Salmo salar*) led to the reduction of their circulating cortisol levels.

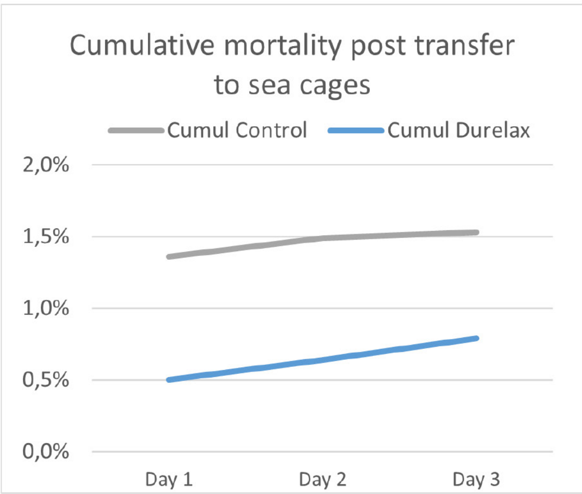
The aim of the present field study was to evaluate the effect of the use of this solution in the transfer water of smolt salmon from the RAS farm to sea cages in challenging conditions, and to follow the mortality events in the first few days at sea.

To do so, 2 field trials were set up. In the first one, 250 mL of DLX were added per cubic meter of water in the well-boat tanks. Blood samples were drawn from 4 fish per tank 0h, 1.5h and 3h after loading the fish in the tanks to quantify their circulating cortisol levels using Bioplasma Stress kits. The second trial was carried out in duplicate, DLX was added in the smolt tank at the RAS farm 30 minutes before the loading, as well as in the well-boat tanks, 30 minutes before the unloading (250mL/m3). After fish stocking in cages, the daily mortality was recorded for the first 3 days at sea.

Circulating levels of cortisol in the smolt from the DLX group showed a faster reduction of circulating cortisol than the control group, with significantly lower levels 1.5h after loading ($p<0.05$, fig. 1). In addition, the second trial showed a drastic reduction of mortality at sea within the first 3 days post transfer in both replicates (-48.0% in both cases, fig 2: cumulative mortality of smolt at sea in duplicate 2).

The results confirm previous findings on the use of DLX to reduce stress in smolt salmon as evidenced by the reduction of circulating cortisol levels. This better management of the fish stress around this critical period, in turn, leads to decrease in mortality post transfer to sea cages, thus providing positive economical return.





CURRENT POLICY CHALLENGES IN AQUACULTURE AND IMTA PRACTICES IN THE ATLANTIC REGION: FINDINGS FROM THE ASTRAL PROJECT

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There have been various efforts at regulating aquaculture practices around the world. This can be observed through the extensive regulatory documentation issued by many countries at different administrative levels, such as national/provincial/state laws, acts, codes of conduct, codes of best practices and practice guidelines. These can cover a wide range of related topics, from general concerns to more specific actions. Each regulation is important for building a broader understanding of aquaculture. Our aim was to provide a series of policy recommendations based on current legislation per country regarding aquaculture and IMTA practices.

This analysis is focused on the Atlantic Area, involving 5 case studies from the IMTA sites involved in the ASTRAL project: Argentina, Brazil, Ireland, Scotland, and South Africa. Thirty-five semi-structured interviews were performed, and workshops were held, in which key stakeholders from different fields participated. In parallel, an extensive literature review was undertaken. After data collection was completed and triangulated, a codification structure was developed to organize and analyse all the information gathered. Thus, four main themes emerged that played a major role in shaping the content of the analysis: 1) Policy dimension, 2) Environmental dimension, 3) Social dimension, 4) Communication dimension.

All contexts are diverse and each faces particular challenges. Yet, shared issues were identified on which the same policy recommendations could be developed. One of the main comments is the necessity for updated and flexible legislation and regulatory frameworks to ensure the insertion of novel technologies within aquaculture practices, such as IMTA systems. Moreover, improving the capacity and building the expertise of government staff, policymakers, and regulators, so that more informed decisions could be made was underlined. On the other hand, it was highlighted that the procedure for acquiring licences must be improved, for example, through the introduction of technical assistance for industry and especially for new and small producers. Lastly, it was emphasized that further collaboration, involving the active participation of industry, small producers, researchers, scientists, academia, policymakers, and regulators, is key to enhance the development of a more sustainable aquaculture sector, highlighting the challenges in implementing IMTA practices.

TORULA YEAST CAN BE USED AS AN ALTERNATIVE INGREDIENT IN DIETS FOR POST-SMOLT ATLANTIC SALMON *Salmo salar* TO REDUCE THE RELIANCE ON SOY PROTEIN CONCENTRATE

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The utilization of plant-based ingredients in aquaculture feeds has been beneficial in augmenting the production of aquaculture by decreasing the dependency on marine components. However, these feeds now account for almost 80% of the scope 3 greenhouse gas emissions in commercial salmon farming. Furthermore, as consumers increasingly recognize the significance of sustainability, there is a demand to explore more environmentally friendly and sustainable alternatives that can minimize greenhouse gas emissions and broaden the array of ingredients utilized in feed formulation. Single-cell proteins are a promising substitute for plant-based ingredients, although their nutritional efficacy and safety in diets for salmonid fish need to be thoroughly scrutinized. Consequently, this study aims to evaluate the effects of replacing up to 20% of plant-based ingredients with torula yeast in commercially relevant diets for Atlantic salmon.

A dose-response experimental design was implemented to formulate five diets that contained increasing levels of torula yeast (0%, 5%, 10%, 15%, and 20%), and fed to quadruplicate groups of post-smolt Atlantic salmon over a 12-week period to evaluate their effects on performance. The fish, with an initial weight of 92.4 ± 7.3g, reached a final weight of 435.1 ± 52.7g. The diets' impact on the fish was assessed through various growth performance indicators, nutrient utilization, blood chemistry, histology, gene expression in the liver and spleen, and metabolomic responses in the liver.

The research findings indicate that there were no significant differences between the experimental groups in terms of specific growth rate, thermal growth coefficient, or feed conversion ratio. However, there were notable changes observed in the plasma metabolites of fish fed with diets containing yeast. These changes included a reduction in total bile acids and indications of increased alkaline phosphatase levels, which could be related to alterations in liver metabolism. Moreover, there was an increase in calcium levels in the plasma of fish in the experimental diets compared to the control group. The initial histological evaluation of the anterior and distal intestines of the fish revealed some variations among the groups. The fish fed with 10 and 20% yeast exhibited a reduction in intestinal fold length in the anterior intestine. In contrast, the submucosa width was reduced in the diets containing 10, 15, and 20% inclusion of torula yeast. In the distal intestine, there was no difference in villus length between the dietary treatments, but the submucosa was reduced at 15 and 20% of yeast inclusion. Furthermore, a reduction in the width of the lamina propria was evident from 15% yeast inclusion. The gene expression analysis of the liver and spleen did not indicate any adverse effect of the dietary treatments, nor was there any evidence that the fish were stressed. However, several genes related to carbohydrate metabolism were altered in the liver, which will be further investigated with metabolomics.

INTERNALIZATION OF EXTRACELLULAR VESICLES FROM FISH PATHOGENIC *Streptrococcus parauberis* IN RAW264.7 CELLS

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Bacterial extracellular vesicles (BEVs) are small, membrane-bound particles released by both Gram-positive and Gram-negative bacteria, playing crucial roles in intercellular communication, pathogenicity, and transfer of cargo between bacteria and hosts. *Streptococcus parauberis*, a Gram-positive pathogenic bacterium, causes significant mortality among various fish species. In our previous study, we isolated and characterized BEVs derived from *S. parauberis* (*Sp*EVs) and demonstrated their immunomodulatory activity. In this study, we focus on the interaction of *Sp*EVs with receptor proteins in Raw 264.7 cells and their mechanism of internalization.

Real-time quantitative reverse transcription PCR (qRT-PCR) was conducted on Raw 264.7 cells treated with *Sp*EVs (5 and 10 µg/mL) to determine which receptors were activated. The results showed that the cell surface receptor *Tlr2*, peptidoglycan receptors *Nod1* and *Nod2*, and endolysosome receptors *Tlr3* and *Tlr9* interacted with *Sp*EVs. To confirm the internalization mechanism of *Sp*EVs, Raw 264.7 cells were pretreated with various EV internalization pathway inhibitors, including chlorpromazine (Clathrin inhibitor), dynasore (Dynamin inhibitor), methyl-□-cyclodextrin (Lipid sequestrant), cytochalasin D (actin polymerization inhibitor), and 5-(N-ethyl-N-isopropyl)-amiloride (macropinocytosis inhibitor). The cells were then treated with fluorescence labeled *Sp*EVs. The results showed that cells treated with chlorpromazine and dynasore significantly reduced fluorescence levels compared to negative control (NC). Finally, the internalization pathway was validated by performing qRT-PCR with selected highly expressed genes in Raw 264.7 cells pretreated with the inhibitors and then treated with *Sp*EVs. A significant reduction in the fold expression of *Tnf*□ and *Il10* was observed in chlorpromazine and dynasore-pretreated cells compared to the NC. This validates that *Sp*EV internalization occurs through a clathrin and dynamin-dependent endocytic pathway.

In conclusion, *Sp*EVs interacted with various cell receptors sensitive to BEVs membrane peptidoglycan and other BEVs cargos. These interacted *Sp*EVs were internalized into Raw 264.7 cells through clathrin and dynamin-dependent endocytic pathways and subsequently induced immunomodulatory activity.

EFFECTS OF COMMERCIAL MICROALGAL FEEDS ON GROWTH, AMINO ACID AND FATTY ACID COMPOSITIONS OF MEALWORM (*Tenebrio molitor*)

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In order to sustainable aquaculture production, fishmeal and fishoil must be reduced and/or replaced by several terrestrial and marine sources. Among alternative protein sources, mealworm (*Tenebrio molitor*) is one of the most promising animal with including high protein contents. In this study, we investigated the effect of different microalgae powder products (Algamac3050® (dried *Schizocthytrium* sp.), Algome® (dried *Schizocthytrium* sp.), Naturiga® (dried *Spirulina platensis*), ProteinPlus®, AlgomeGrow® (dried *Chlorella* sp.)) on culture performance, fatty acids and amino acids compositions of mealworm. Mealworms were fed during 21 days under laboratory conditions. The culture box were filled one cm of feed depth and kept constant dark (0 h L, 24 h D) conditions. Total lenght and wet weight were measured every 7 days of culture. Mealworm group fed control diet showed similar total lenght with mealworm fed Algome® diet and significantly higher than other groups at the end of the experiment (p<0.05) (Fig.1). Wet weight of mealworms were enhanced by all diets except ProteinPlus® diet at 7 days of culture, however, this trend was changed at the end of the experiment (Fig.2). On the other hand, Σ n-3 HUFAs (ARA, EPA and DHA) levels were increased by Algamac3050® (1.39%), Algome® (1.36%) and ProteinPlus® (1.02%) diets. Whereas, total essential amino acid compositions were enhanced by AlgomeGrow® diet (244.7 mg. g^{-1}). In conclusion, AlgomeGrow® diet can be good option for mealworm culture resulting both amino and fatty acid accumulation.

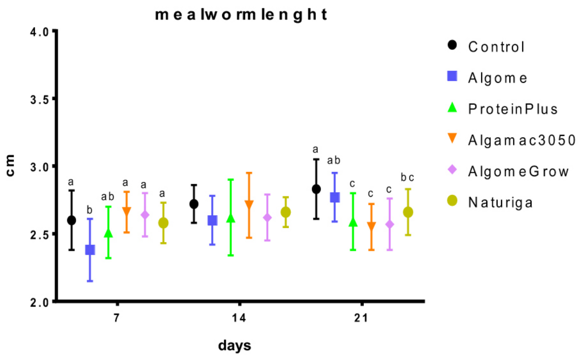

Figure 1. Total length of mealworm during experiment.

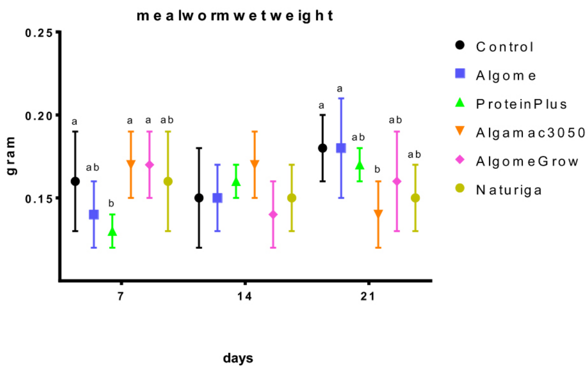

Figure 2. Wet weight gain of mealworm fed different diets.

INDUCED SWIMMING ACTIVITY MODULATES THE ANTIOXIDANT STATUS IN LIVER AND SKELETAL MUSCLE OF EUROPEAN EEL *Anguilla anguilla*

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Swimming activity promotes several biochemical and enzymatic adaptations at a systemic level, mainly affecting liver and skeletal muscle functions. Inadequate exercise loads can generate excessive reactive oxygen species in these tissues, although it has been described that optimal swimming conditions can lead to increased growth and robustness. However, these responses vary greatly from species to species. In this study, we focused on a migratory fish species, the European eel (*Anguilla anguilla*), a highly efficient swimmer of great importance for aquaculture. Sixteen fish were divided into two groups, one was subjected to continuous swimming at 0.3 BL (body-lengths) s^{-1} (S, n = 8), while the other group was not induced to swim, and served as a control (NS, n = 8). These conditions were applied to individual fish in swimming tunnels for 7 hours. Thereafter, liver, posterior and anterior muscles were sampled for oxidative stress analyses.

Interestingly, European eels induced to swim showed lower levels of lipid peroxidation in the liver and posterior muscle, as well as increased levels of reduced glutathione (GSH) and consequently a higher reduced/oxidized glutathione ratio (GSH/GSSG) (Figure 1). Nevertheless, oxidative stress markers analyzed in the anterior skeletal muscle were similar in both groups. These results contribute to the understanding of swimming physiology in anguilliform species and emphasize the importance of suitable swimming conditions for highly-efficient swimming species in aquaculture to promote welfare.

This research was supported by national funds through FCT – Foundation for Science and Technology (Portugal) within the scope of UIDB/04423/2020 and UIDP/04423/2020. C.E-S was supported by the Blue Young Talent PhD program (BYT-PhD) grant (UI/BD/150911/2021) funded by FCT.

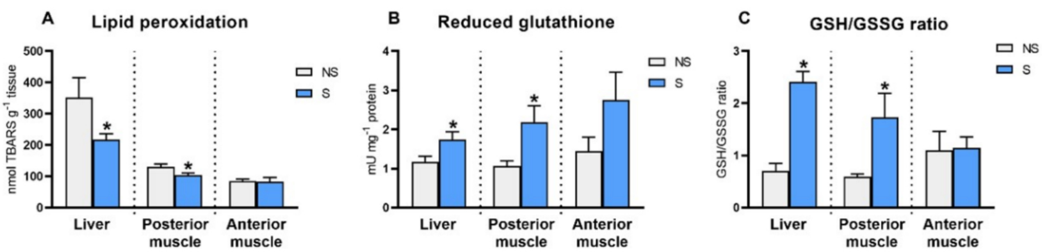

Figure 1. Lipid peroxidation levels (A), reduced glutathione content (B) and reduced/oxidized glutathione ratio (GSH/GSSG) in liver, posterior and anterior skeletal muscle of European eel in non-swimming (NS) and induced to swim (S) conditions. Data represent mean ± SEM (n = 8; unpaired Student's *t*-test).

THE PHYSIOLOGICAL EFFECTS OF INDUCED SWIMMING ACTIVITY IN STRESS RESPONSE, IMMUNE AND ANTIOXIDANT STATUS OF EUROPEAN SEABASS *Dicentrarchus labrax*

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Swimming activity is a fundamental trait in the life cycle of most fish species. Adequate exercise has been shown to promote growth through muscle development, improve feed efficiency and reduce stress in some species. Although some studies have paved the way for a possible link between swimming activity and improved immunity and antioxidant status, the effects of induced swimming appear to vary greatly between species. In this study, several immune and oxidative indicators were analyzed in plasma, skin mucus, gills and skeletal (red and white) muscle of European seabass (*Dicentrarchus labrax*) after induction of different swimming conditions. Thirty-two specimens (total length: 11.5 ± 0.1 cm; body weight: 15.5 ± 0.6 g) were induced to swim for 6 h in individual swimming flumes at low (L, 0.8 BL (body-lengths) s^{-1}); high (H, 2.2 BL s^{-1}) and oscillating (O, 0.8–2.2 BL s^{-1}) velocities. A non-exercised group was used as a control group (C). The swimming velocity applied for fish in the H group was near 70% of the critical swimming speed described for European seabass, close to the optimal swimming speed defined for this species.

The fish swimming in the H condition had a higher white blood cells (WBC) count and increased plasma cortisol concentrations compared to the C and L groups. No differences were found in the innate immune parameters in plasma and skin mucus among the experimental groups. Gene expression analyses revealed an up-regulation of inflammatory cytokines (tumor necrosis factor alpha, *tnf*□ and interleukin 1 beta, *il1*□) in gills from H group. In addition, the activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were increased in the red muscle of fish swimming under the H condition, as were the lipid peroxidation (LPO) levels. Nevertheless, fish swimming in the L group showed a higher ratio of reduced/oxidized glutathione (GSH/GSSG) in the red muscle. Interestingly, the GSH/GSSG ratio was increased in white muscle under all swimming conditions compared to the control. These results suggest a possible inflammatory response in the gills of fish swimming in the H group, as inflammatory cytokines are up-regulated, and plasma cortisol and WBC concentrations are increased. The higher activities of SOD and CAT as well as LPO levels also indicate a susceptibility to oxidative stress in the red muscles of fish in the H group. Nevertheless, the increased GSH/GSSG ratio in white muscle under all swimming conditions indicates an improvement in antioxidant status associated with swimming activity in this tissue. These results emphasize the need for appropriate swimming conditions for farmed European seabass, one of the most commonly farmed species in Southern Europe, which could prove to be an innovative, non-invasive method to promote fish welfare.

This research was supported by national funds through FCT – Foundation for Science and Technology (Portugal) within the scope of UIDB/04423/2020 and UIDP/04423/2020. C.E-S was supported by the Blue Young Talent PhD program (BYT-PhD) grant (UI/BD/150911/2021) funded by FCT.

COMPARATIVE TRANSCRIPTOMICS ACROSS ATLANTIC SALMON *Salmo salar* SMOLTS, EUROPEAN SEABASS *Dicentrarchus labrax* AND RAINBOW TROUT *Oncorhynchus mykiss* UNDERGOING ACUTE STRESS INDUCED BY TRANSPORT

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Unravelling new knowledge on molecular markers signalling acute stress in farmed fish is essential for better monitoring, improve animal welfare and health, and reduce mortality. This study aimed to evaluate physiologic and metabolic effects of acute stress induced by transport in three fish species: rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), and European seabass (*Dicentrarchus labrax*). Trials were conducted at CIIMAR, Riasearch and University of Cádiz facilities, respectively.

After acclimatisation, fish were divided in one group of fish that was immediately sampled to serve as a non-stressed control. Additionally, two groups of fish were transferred each to three oxygen saturated containers and transported by a transport van for 2 (trout), 6 (salmon) or 4 (seabass) hours. Upon arrival, half were immediately sampled while the remaining fish were put in the original system and sampled at 24h post-arrival (n=18 per sampling time, except seabass n=12).

Total RNA was extracted from skin tissue and pooled (n=3-4 samples per treatment) for RNAseq on an Illumina sequencer. Raw sequence reads were trimmed and filtered using fastp, to remove adapter sequences and low-quality reads. Transcripts were quantified using the pseudoalignment method implemented in kallisto. Differentially expressed genes (DEGs) between the different treatments and the control samples at time 0 (i.e., before transport) were determined using DESEQ2.

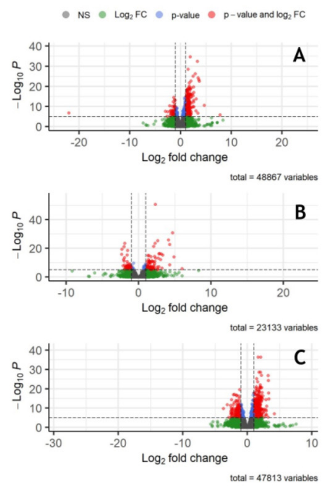

Figure 1 - Volcano plot of differentially expressed genes in A - Rainbow trout 2h after transport; B -Atlantic salmon smolts 6h after transport; C - European seabass 4h after transport.

Preliminary data showed different gene pathways regulated during acute stress (Figure 1), with the two salmonids sharing more genes. In addition, the European seabass seems to be nearly recovered by 24h, while both salmonid species still showed many DEGs at 24h highlighting interspecific variability in stress recovery.

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NATURAL ANTIBODIES OF EUROPEAN SEA BASS: FEATURES, HERITABILITY AND GENETIC CORRELATIONS WITH NODAVIRUS-SPECIFIC ANTIBODY TITER, NODAVIRUS RESISTANCE AND BODY WEIGHT

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Natural antibodies (NAbs) are immunoglobulins found in animals without prior antigenic experience. NAbs are believed to provide an immediate protection against a wide variety of pathogens. In teleost fish, innate and adaptive immune responses are species-specific; characteristics of innate immune system in European sea bass (*Dicentrarchus labrax* L.) have never been studied. Moreover, hypothesizing the use of innate immunity traits in indirect selective breeding approaches, we investigated genetic variation of NAbs and their relationships with: i) antibody titer against nodavirus antigens (NNV-AT), ii) mortality caused by nodavirus (MORT), and iii) body weight at a fixed age (BW).

The experimental population was produced by artificial fertilization using a commercial breeding stock. 650 randomly chosen fish (age: 548 d post-hatching; average BW (CV): 146.4 g (0.29)) were individually PIT-tagged, blood sampled and then subjected to a 29 days-NNV challenge test. MORT was recorded as 1 (dead) or 0 (survived). The survival rate at the end of the challenge test was 52.2%. Indirect ELISA assay for NAbs and NNV-AT was performed on the blood serum of each fish. All the experimental fish and their parents were genotyped to reconstruct a high-likelihood pedigree for each tested individual. Genetic parameters, heritability, genetic and phenotypic correlations between traits were estimated using Bayesian procedures and bivariate animal models (gibbsf90+/BLUPf90+ software suite).

Mean (CV) of the sample-to-positive ratio of the optical density values 450 nm of NAbs titer was 15.86 (1.50). The frequency distribution was skewed and it was normalized through Box-Cox transformation of the original data (mean (CV) of the transformed data: 2.29 (0.60)). No significant differences between means of NAbs titer of dead and survivor animals (2.28 (0.61) vs. 2.30 (0.60)) were detected ($|\hat{\delta}|\hat{\delta}$ posterior difference = 0.02, $\log_e$ (bayes factor) = 2.34). Heritability, genetic and phenotypic correlations as medians of the marginal posterior density distributions are reported in Table 1. The results evidenced the presence of a genetic basis for NAbs. Genetic correlations between NAbs and both NNV-AT and BW were significantly positive. The high negative genetic correlation between NAbs and MORT suggests the possibility to develop indirect selective strategies to enhance VNN disease resistance using NAbs measures.

Table 1. Estimates of heritability (h^2), genetic (r_a) and phenotypic (r_p) correlations obtained in the bivariate analyses; standard deviations (SD) of the marginal posterior density distributions are reported in parentheses

Trait 1	Trait 2	h^2 (± SD) Trait 1	h^2 (± SD) Trait 2	r_a (± SD)	r_p (± SD)
NAbs	NNV-AT	0.19 (0.08)	0.38 (0.12)	0.70 (0.22)	0.33 (0.05)
NAbs	MORT	0.15 (0.07)	0.15 (0.08)	-0.89 (0.23)	-0.13 (0.06)
NAbs	BW	0.19 (0.09)	0.61 (0.14)	0.61 (0.24)	0.16 (0.06)

ANTIOXIDANT POTENTIAL OF ARTEPILLIN C AND CURCUMINOIDS MIXTURE THROUGH KRL TEST AND THEIR DIETARY IMPACT ON GROWTH IN GILTHEAD SEA BREAM (*S. Aurata*) UNDER COLD STRESS

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Low temperature is a contributing factor to winter syndrome in gilthead sea bream (*Sparus aurata*). This syndrome results in reduced antioxidant defense, growth performance and compromised welfare. Artepillin C from green propolis and curcuminoids from turmeric are known for their potent antioxidant activity, anti-inflammatory responses and gut health benefits. This study has two primary objectives. The first is to assess the potential of a mixture of these bioactive molecules, named Phyto AquaNity, to enhance blood's resistance to oxidation using the OXYLAB-KRL test. The second objective is to evaluate the effect of this mixture on growth performance under low-temperature conditions (13°C).

To achieve the first objective, we prepared dilutions of the various solutions in micro-well plates. The analysis was then conducted at 37°C using a multi-well plate reader equipped with agitation calibrated specifically for KRL analysis. The reader was connected to KRL software for data collection. Vitamin C, a well-known water-soluble antioxidant, was included as a control in the analysis. For each group, we determined the time of resistance (lag time) and the time it took for oxidation to reach 50%.

For the second objective, seabream were fed two commercially available diets: one without Phyto AquaNity and another containing 1g/kg. The experiment was conducted at two different temperatures: 23°C for 55 days (initial weight: 71g) and 13°C for 40 days (initial weight: 176g). The trial was performed in triplicate within a recirculating aquaculture system (RAS).

The results (Figure 1), demonstrated that Phyto AquaNity exhibited a higher antioxidant capacity compared to Vitamin C in the range of 35 to 100 mg/L. Furthermore, both Phyto AquaNity and Vitamin C displayed an increase in activity with increasing concentration. Daily weight gain (DWG) and specific growth rate (SGR) were both significantly higher at the optimal temperature of 23°C ($p < 0.1$) (Table 1). Notably, Phyto AquaNity supplementation significantly improved DWG and SGR even at the lower temperature of 13°C ($p < 0.05$). There was no significant difference observed in feed conversion ratio (FCR) between the two tested temperatures ($p > 0.05$). No mortality was recorded over the trial.

In conclusion, Phyto AquaNity demonstrated a strong antioxidant activity which likely explains the improved growth performance observed in sea bream at the low temperature of 13°C. Further analysis are underway to investigate its effects on *in vivo* antioxidant, immune status and fish welfare.

Figure 1: Antioxidant capacity of Phyto AquaNity vs Vitamin C through KRL test.

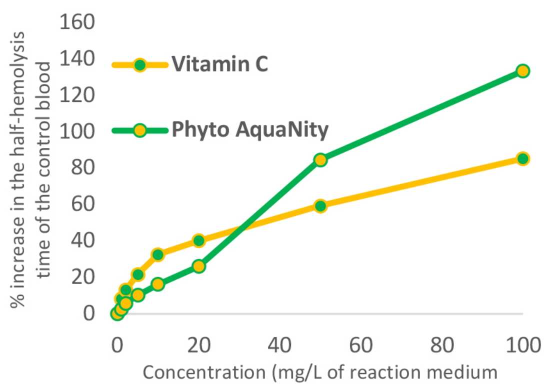

Table 1: Growth and feed utilization efficiency of Gilthead Sea bream fed with Phyto AquaNity..

	23°C		13°C	
	Control	**Phyto AquaNity**	**Control**	**Phyto AquaNity**
IW	71.01±0.34	71.02±0.46	174.43±0.67	177.64±2.31
FW	172.5±0.76	179.23±1.32	203.8±1.75	212.7±0.96
DWG	1.83±0.02	1.97±0.02	0.74±0.04^a	0.88±0.04^b
SGR	1.62±0.01	1.68±0.01	0.39±0.02^a	0.45±0.03^b
FCR	1.11±0.02	1.13±0.01	1.32±0.04	1.33±0.04

IW: Initial weight (g), FW: Final weight (g), DWG: Daily weight Gain per day, SGR: Specific Growth rate (%/day) and FCR: Feed Conversion rate. Different letters for each temperature in the same row indicates significant difference between experimental diets ($p<0.05$)

VALORISATION OF NON-COMMERCIAL BY-PRODUCTS FROM MEDITERRANEAN MUSSEL *Mytilus galloprovincialis* FOR THE OBTENTION OF ANTIMICROBIAL EXTRACTS WITH AQUACULTURE APPLICATIONS

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Aquaculture plays a crucial role in global food security prospects, providing an alternative to over-exploitation of marine resources. However, the industry faces several sustainability challenges. One of the most critical issues is the incidence of bacterial diseases, exacerbated by climate change, which often leads to excessive use of antibiotics with serious environmental impacts. Additionally, managing non-commercial by-products is another critical issue that needs to be addressed in this industry. The high resistance of Mediterranean mussels (*Mytilus galloprovincialis*) to infectious diseases and the surpluses generated from their farm production provide an opportunity for the reuse and valorisation of non-commercialized specimens through the development of antimicrobial extracts for their potential use as feed additives for aquaculture. Here it is presented the optimization of the organic extraction process guided by a Box-Behnken statistical model to reduce solvent use while maximizing antimicrobial activity.

A Box-Behnken statistical model was employed to comprehensively evaluate the impact of three extraction parameters, including the ratio (g analyte/ml solvent), ultrasonic power (J/ml), and maceration time (h). Fifteen extract conditions determined by the model were obtained and optimised based on their protein content and their antibacterial and antioxidant capacity. Subsequently, the model's predictions with the best antibacterial activity (ratio: 1/10 g/ml, ultrasonic power: 50 J/mL, maceration time: 2h) were validated through an experimental comparison with the model results.

Furthermore, additional analysis was carried out to characterise the molecular composition of the extracts using ultraviolet-visible (UV-Vis) absorbance spectroscopy and high-performance liquid chromatography with a triple quadrupole mass spectrometer coupled to an electrospray ionisation source (HPLC-ESI-QqQ-MS). However, the precise identification of the antimicrobial peptides that may be responsible for the observed antibacterial activity was hindered by technical limitations associated with the extraction methods used and the biological complexity of the mussel matrix.

The results of this research represent a preliminary study for future investigations aimed at finding innovative alternatives to antibiotics and improving the efficiency and sustainability of the aquaculture industry.

Table 1. Levels of variables and conditions used in Box-Behnken model.

Extract	Ratio (g/ml)	Time (h)	Ultrasound power (J/ml)
1	-1 (1/10)	-1 (2)	0 (100)
2	1 (1/40)	-1 (2)	0 (100)
3	-1 (1/10)	1 (24)	0 (100)
4	1 (1/40)	1 (24)	0 (100)
5	-1 (1/10)	0 (8)	-1 (50)
6	1 (1/40)	0 (8)	-1 (50)
7	-1(1/10)	0 (8)	1 (150)
8	1 (1/40)	0 (8)	1 (150)
9	0 (1/25)	-1 (2)	-1 (50)
10	0 (1/25)	1 (24)	-1 (50)
11	0 (1/25)	-1 (2)	1 (150)
12	0 (1/25)	1 (24)	1 (150)
13	0 (1/25)	0 (8)	0 (100)
14	0 (1/25)	0 (8)	0 (100)
15	0 (1/25)	0 (8)	0 (100)

CHANGES IN QUALITY OF CULTURED AND WILD ADULT AFRICAN CATFISH *Clarias gariepinus* UNDER FROZEN STORAGE

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Fish is valued for its nutritional qualities as a good source of minerals, vitamins and protein in the amount required for healthy human growth. African catfish, *Clarias gariepinus* is widely consumed in Nigeria but prone to decomposition immediately after harvest, as fish is prone to decomposition, development of rancidity and microbial spoilage, there is a great need to process and preserve harvested fish in order to extend its shelf life for human consumption (Agiopu, 2007). Frozen storage is used to arrest spoilage in the sample fish, both cultured (CWF) and wild (WWF) *Clarias gariepinus* were subjected to frozen storage after removing the slime and blood clots from the skin. Proximate analysis of fish samples were determined before, during and after the frozen storage. Biochemical and mineral contents of fish samples were determined at 14days interval of freezing and the results obtained were subjected to analysis of variance ANOVA at $p<0.05$.

Results

The study showed that cultured *C gariepinus* is similar to wild *C gariepinus* in PV, TBA and food composition values, even better in certain values. As a result, cultured *Clarias gariepinus* is a product with similar quality and high nutritive value as the wild.

Table 1 The proximate composition of *Clarias gariepinus* in fresh state

Parameters	Wild (%)	Cultured (%)
Moisture	5.71 ± 1.41^b	7.10 ± 0.02^a
Ash	9.75 ± 0.18^a	9.47 ± 0.13^b
Crude protein	59.37 ± 0.78^b	68.82 ± 0.09^a
Lipid	17.58 ± 0.42^a	13.78 ± 0.35^b

Table 2 The proximate composition of wild and cultured *Clarias gariepinus* after 14 days of frozen storage

Parameters	Wild (%)	Cultured (%)
Moisture	6.72 ± 1.25^b	8.40 ± 0.70^a
Ash	16.76 ± 0.45^a	9.81 ± 0.22^b
Crude protein	54.19 ± 2.36^b	61.92 ± 0.11^a
Lipid	21.19 ± 0.50^a	18.37 ± 0.52^b

Table 3 The mineral composition of wild and cultured *Clarias gariepinus* in fresh state (mg/100g)

Minerals	Wild	Cultured
Calcium	264.54 ± 3.87^a	201.17 ± 1.09^b
Magnesium	35.47 ± 2.20^a	21.22 ± 2.17^b
Iron	7.43 ± 0.23^b	9.37 ± 1.28^a
Zinc	5.84 ± 0.34^b	7.41 ± 0.87^a
Copper	0.65 ± 0.76^a	0.43 ± 0.03^b

Table 4 The mineral composition of wild and cultured *Clarias gariepinus* after 14 days of frozen storage (mg)

Minerals	Wild	Cultured
Calcium	269.65 ± 14.71^b	289 ± 12.64^a
Magnesium	27.78 ± 2.33^a	19.08 ± 2.53^b
Iron	8.88 ± 0.59^b	10.3 ± 1.46^a
Zinc	5.54 ± 0.48^b	7.55 ± 0.76^a
Copper	0.63 ± 0.01^a	0.47 ± 0.06^b

Table 5 Assessment of biochemical indices of lipids damaged in fresh *C. gariepinus*

Parameters	Wild (%)	Cultured (%)
FFA%	0.13 ± 0.08^a	0.12 ± 0.07^b
PV*(meg/kgfat)	4.22 ± 1.50^a	4.15 ± 1.33^b
TBA*	0.44 ± 0.04^a	0.37 ± 0.04^a
TVBN*(mg)	$7.34.\pm0.42^a$	24.51 ± 0.63^a

Table 6 Assessment of biochemical indices of lipids damaged in *C. gariepinus* after 14 days frozen storage

Parameters	Wild (%)	Cultured (%)
FFA%	0.18 ± 0.08^b	0.22 ± 0.07^a
PV*(meg/kgfat)	4.42 ± 1.50^a	4.31 ± 1.33^b
TBA*	0.15 ± 0.04^b	0.19 ± 0.04^a
TVBN*(mg %)	9.36 ± 0.42^b	27.48 ± 0.63^a

EVALUATION OF INSECT MEAL AND MARINE-MICROALGAE DERIVED INGREDIENTS in ATLANTIC SALMON (*Salmo salar*) PARR UNDERGOING SMOLTIFICATION AND SEAWATER TRANSFER

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After many years of research and development, we are beginning to see greater implementation of insect and microalgae derived feed ingredients in Atlantic salmon diets. Black soldier fly meal (BSFL, *Hermetia illucens*) as a protein source, *Schizochytrium* microalgae as an EPA-DHA source and *Hæmatococcus* microalgae to provide astaxanthin are some of the most promising prospects in this next generation of novel feeds offering sustainable alternatives. This study investigates the effects of dietary inclusion of these novel ingredients in Atlantic salmon parr undergoing smoltification and seawater transfer.

A two-phase feeding trial, performed at Nord university research station, evaluated the effects of combining these novel ingredients in diets for parr and post-smolts. The same four diets, adjusted for nutrient needs between phases, were evaluated: a control diet (CO) with synthetic astaxanthin, a diet with *Hæmatococcus* as astaxanthin source (HA) as well as two other diets (BSFL and BSFL-AO) with *Hæmatococcus* providing astaxanthin and 15% BSFL replaced soy protein concentrate. Furthermore, EPA-DHA was provided by algal oil from Schizochytrium, replacing fish oil in BSFL-AO diet. In the freshwater phase (FW, 80 days), diets were assessed in triplicate tanks with 15-g parr under 24L:0D to 16L:8D light regimes. Smoltification parameters were measured at 5 consecutive sampling points and tissue samples were collected. Seawater challenges (SWC, 48 hours) were performed at the two last samplings. Analysis covered osmolality, ions and smoltification-related gill genes, cortisol and growth hormone. In the seawater phase (SW, 85 days), 140 fish from each FW group were transferred to flow-through tanks. Growth, feed efficiency and morphometric parameters were evaluated during the SW-phase.

Results showed that the inclusion of insect meal and algal oil in the diet of salmon parr and smolts had no significant effect on growth and welfare performance (weight gain, specific growth rate, feed conversion ratio, condition factor, hepatosomatic index). Parr underwent smoltification with no significant differences between the dietary groups, except at the fourth sampling: gill gene expression (HA vs BSFL) and plasma calcium (BSFL versus BSFL-AO). The results of these trials suggest that the inclusion of insect meal, algal oil from *Schizochytrium* and astaxanthin from *Haematococcus* support salmon's physiological adaptation to seawater when compared to a standard commercial diet. This underpins the potential of these novel ingredients to provide sustainable alternative sources of essential nutrients.

Table 1: Growth performance of Atlantic salmon fed diets with novel feed ingredients

	CO	HA	BSFL	BSFL-AO
FW-phase				
WG	447.06 ± 15.97	458.68 ± 18.14	429.15 ± 13.64	442.77 ± 12.75
SGR	2.00 ± 0.034	2.02 ± 0.038	1.96 ± 0.030	1.99 ± 0.028
SW-phase				
WG	372.63 ± 16.40	370.66 ± 10.68	362.33 ± 17.05	370.98 ± 14.37
SGR	1.85 ± 0.04	1.84 ± 0.03	1.82 ± 0.04	1.84 ± 0.04
FCR	0.72 ± 0.02	0.71 ± 0.01	0.71 ± 0.02	0.72 ± 0.01

WG (Weight Gain in %); SGR (Specific Growth Rate in % day^{-1}); FCR (Feed Conversion Ratio)

AQUATECHINN 4.0: THE INTERACTIVE WAY TO LEARN AQUACULTURE

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AQUATECHinn 4.0 represents a cutting-edge training initiative poised to revolutionise the aquaculture sector by harnessing the latest technological and productivity advancements within the framework of Industry 4.0. Designed to meet the evolving demands of the labour market, this comprehensive program features specialised modules covering pivotal areas such as "New Technologies in Fish and Shellfish Aquaculture," "Sustainability Management," and "Animal Health, Biosecurity, Welfare, Nutrition, and Reproduction."

Delivered through an innovative e-learning platform, AQUATECHinn 4.0 integrates state-of-the-art training methodologies, including digital blended learning tools, immersive Virtual Reality simulations, gamified learning experiences, captivating instructional videos, and accessible open educational resources (MOOCs).

They were developed collaboratively by a consortium comprising key European stakeholders from aquaculture industry associations (CNC - Comité National de la Conchyliculture, API - Associazione Piscicoltori Italiani, APA - Associação Portuguesa de Aquacultores, M.A.R.E. Soc. Coop. ar.l., AMA - Associazione Mediterranea Acquacoltori), esteemed universities (Universidade do Algarve, RTEÜ - Recep Tayyip Erdoğan University, University of Bologna – UNIBO, UCH-CEU - Universidad Cardenal Herrera), vocational training centres (E-SCHOOL Educational Group), and renowned research institutions (CCMAR - Centro de Ciências do Mar, FishEthoGroup), this initiative is poised to reshape the landscape of aquaculture education.

Funded by the European Union through the ERASMUS+ Innovation Partnerships Programme, and coordinated by SGS, AQUATECHinn 4.0 aims to bolster Europe's innovation capacity by fostering collaboration and knowledge exchange across higher education, vocational training, and the socioeconomic fabric of the European aquaculture sector, thus driving forward innovation and research in the field.

EFFECTS OF ALGAL DIET SUPPLEMENTATION ON HALIBUT SEMINAL PLASMA ANTIOXIDANT CAPACITY: A CORRELATION STUDY BETWEEN SPERM TRAITS AND EV-CARGO PROTEOME

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Atlantic halibut, *Hypoglossus hypoglossus*, is a marine flatfish farmed in Iceland, UK and Norway. Lack of control over stocks' reproduction, use of wild stocks, inconsistence supply of gametes and larvae, are constraints that need to be addressed to optimise this species' aquaculture. Supplementation of feeds with natural antioxidants might be the approach to improve gamete and offspring quality. Extracellular vesicles (EVs) are cell-derived membrane-surrounded vesicles that carry bioactive molecules and deliver them to recipient cells. These EVs are very important for cell-to-cell communication and are transported in the different body fluids, being specific to each one. EVs and their cargo are considered reliable biomarkers of sperm quality and progeny development. This study aimed to observe the effects of an algal blend supplementation in sperm traits and their correlation with EV-protein cargo in seminal plasma.

Five halibut males from Sogn Aqua Juveniles, Norway, were fed with commercial dry feed during 2022, which was further supplemented with an algal blend in 2023. In the reproductive season, sperm was collected and different quality parameters (i.e., motility, viability, DNA fragmentation, lipid peroxidation and antioxidant status) were assessed. Seminal plasma was obtained and EVs were isolated employing size-exclusion chromatography, where all the fractions containing EVs were collected. EVs were characterised (size and concentration) using the tunable resistive pulse sensing (TRPS) method. EV-proteins were extracted using RIPA buffer followed by freeze-thaw cycles. Tryptic peptides were separated and analysed by LC-MS/MS. Proteins were identified using Fragpipe software at an FDR $< 1\%$.

Significant differences (paired Student's *t*-test $p < 0.05$) were observed in sperm lipid peroxidation, DNA fragmentation, oxidative enzymes (GSR and GPX) between males fed with control and algal diet. There were no differences between treatments in terms of EVs size and concentration. Regarding proteomics, 2146 proteins were identified in EV fractions. A canonical correlation analysis (Pearson; $r>0.9$) between the proteome and the different sperm traits revealed that pathways associated with DNA fragmentation, curvilinear velocity, total motility and oxidative enzymes, like apoptosis, autophagy and cellular response to oxidative stress, were downregulated, while vesicle-trafficking was upregulated, in diet-supplemented fish. These results suggest that algal diet improved the antioxidant capacity of the sperm cells in halibut.

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A QUESTION OF BALANCE – MOVING BEYOND THE BASICS

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Following domestication of shrimp in the 1990's, pioneered by the Oceanic Institute as part of the US Marine Shrimp Farming Program, genetic selection has resulted in major improvements in shrimp productivity. Selection for growth resulted in farmed *Penaeus vannamei* reaching sizes similar to *P. monodon* within the same culture period. Together with development of Specific Pathogen Free (SPF) stocks, this has resulted in an almost complete shift in Asian shrimp production to *P. vannamei*.

In the Americas, successive outbreaks of disease led producers to select survivors from affected ponds as the basis of their breeding programs, the "Specific Pathogen Resistant" (SPR) approach. This resulted in shrimp lines that have better survival in the field than the SPF lines which were produced with no innate resistance but with a slower growth rate.

Because of the antagonistic relationship between growth and resistance, this led to a divergence between lines used in Asia (SPF, selected for faster growth) and lines from the Americas (SPR but with slower growth). Subsequently, this led to differentiation between markets opting for higher resistance or faster growth depending on local farming conditions.

This binary choice would seem to have relatively limited application across an industry where differences in management strategies, environmental conditions and disease threats affect the suitability and success of either the fast growth or resistant line approaches.

Since 2011, SyAqua has adopted a third option, the "balance line", selecting for a combination of growth and resistance traits simultaneously. Simply crossing commercial fast growing and resistant lines typically results in highly variable populations with many slow growing and susceptible offspring. Using a carefully designed "Selection Index" allows us to choose families with the optimal combination of growth and resistance traits. This approach has led to shrimp exhibiting growth patterns similar to those of the best growth lines, while demonstrating heightened resilience to stressors and pathogens over time. Equally important, farmers can achieve better and more consistent returns on investment compared to "pure" lines as seasons, farming conditions and uncontrollable factors fluctuate.

The selection index approach can be adapted to include other economically valuable traits. This allows selection for more traits, addressing specific market needs and ever-changing environmental conditions without losing genetic variability or gains in growth and resistance.

MELATONIN ACTION IN THE REPRODUCTIVE AXIS OF SENEGALESE SOLE: ORIGIN, MECHANISMS AND EFFECTS ON SPERM QUALITY

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Melatonin is a clock-hormone produced at night and responsible for the synchronization of biological rhythms, including reproduction. However, its exact production sites, targets and function in fish are still unclear. This study aimed to ascertain melatonin presence in Senegalese sole (*Solea senegalensis*) seminal plasma, its effects on sperm quality, mechanisms of action in spermatozoa, and its origin. For this, wild and F1 broodstocks were sampled along three reproductive seasons to collect sperm and blood at mid-light (ML) and mid-dark (MD) daytimes. Firstly, using CASA software, the effects of endogenous melatonin on sperm motility and velocity were assessed. In the second year, melatonin was quantified in blood and seminal plasmas through radioimmunoassay (RIA), and the concentration of sex steroids (T and 11KT) and antioxidant status (TAS) were evaluated in seminal plasma with commercial kits. The mechanism of melatonin entrance in the cell was studied using FITC-melatonin and a confocal microscope. Finally, the expression of genes involved in melatonin biosynthesis (*tph1a, tph2, hiomt1, aanat1a, aanat1b,* and *aanat2*) and melatonin receptors (*mel1, mel1c* and *mel2*) was evaluated in the brain, eye and testis, by quantitative real-time PCR.

Blood plasma melatonin was higher at MD, together with sperm motility parameters, likely to be enhanced by the endogenous melatonin found to be present in seminal plasma, with daily oscillations, and contributing to sperm TAS (Fig 1A). Also, it was demonstrated that melatonin easily crosses the plasmatic membrane of spermatozoa, spreading inside the nucleus and mitochondria. Gene expression of receptors *mel1* and *mel2*, and the enzymes *tph1a, aanat1a, aanat2,* and *hiomt1*, was detected in male gonads, indicating testes as a peripherical location for melatonin production (Fig 1B). Throughout the study, wild and F1 males showed distinct patterns, giving new information on the dysregulation of the circadian system in F1 animals.

This study described the melatonin synthesis, pathway and action in the fish reproductive system for the first time, revealing that Senegalese sole testes are an extra-pineal production site of melatonin. Also, the dysregulation in the hypothalamus-pituitary-gonad axis in F1 sole males was highlighted. These findings can enable the aquaculture industry to obtain better sperm quality from F1 males in a non-invasive way (collecting sperm at night) and improve Senegalese sole reproduction success.

This study was funded by FCT through SFRH/BD/148280/2019 and COVID/BD/153473/2023 grants to FF, contract DL 57/2016/CP1361/CT0007 to CCVO, and CCMAR Strategic Project (UIDB/04326/2020, UIDP/04326/2020 and doi.org/10.54499/LA/P/0101/2020); and by "Programa Crescimento Azul (#4)" through BREEDFLAT (PT-INNOVATION-0080) project.

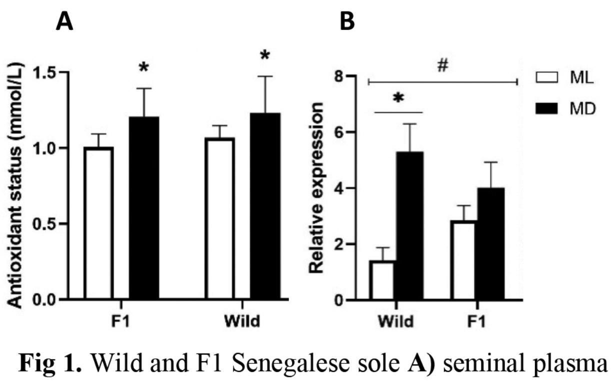

Fig 1. Wild and F1 Senegalese sole **A)** seminal plasma TAS and **B)** *tph1a* expression in testis at mid-light (ML) and mid-dark (MD) daytimes.

MICROBIOME REVEALS THE LINKAGE BETWEEN GUT MICROBIOTA AND COMMON CARP (*Cyprinus carpio*) GROWTH

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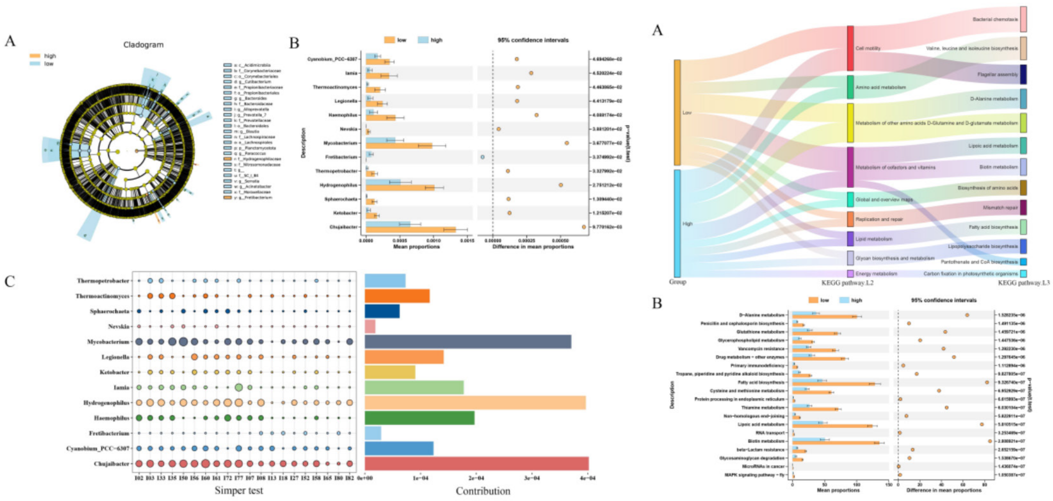
Gut microbiota are increasingly recognized for their crucial role in regulating the health and growth of the host. The mechanism by which the gut microbiome affects the growth rate of common carp, however, remains unclear. In this study, the gut contents of the fast-growing and slow-growing (High and Low) carp were collected for the fish gut microbiome. High throughput 16S rRNA gene sequencing showed that the overall gut microbiota of High group was distinct from that of Low group. For example, the Cetobacterium were highly enriched in the guts of High group (39.9%), vibrio is highly enriched in the gut microbiota of the Low group (20.0%). LEfSe analysis identified 13 different flora between the two groups, mainly located in 17 KEGG pathways, of which nearly 50% were involved in lipid, vitamin, and amino acid metabolism.

Compared with the Low group, the gut microbiota in the High group significantly enriched the pathways involved in Energy metabolism and Biosynthesis of other secondary metabolites. These metabolic pathways enrich some bioactive substances such as diarylheptane and gingerol, which can have a significant impact on the growth rate of carp.This study can not only elucidate the dynamic changes of gut microbiota of carp with different growth rates, but also help us to screen significant flora that affect growth rate and promote the development of microbiome technologies in aquaculture.



ECONOMIC EVALUATION OF FISH MISLABELING IN EUROPE

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Even though the European labeling regulations establish compulsory information, European consumers receive little information about the sea fish and seafood they eat whereby they are not able to discriminate between species. Consequently, there are incentives in the supply chain to cheat when premiums exist for specific species or types of fish. Fish mislabeling results in a series of varied negative consequences as, for example, losses in duties and import taxes for governments as well as economic losses for the consumers that buy an unwanted product at expensive prices.

The aim of this work is to answer the following research question: what is the economic impact of mislabeling in the fish and seafood industry? For it, we have estimated the economic value of mislabeled fish consumed in different European countries. For this estimation, we have employed a framework proposed by Kroetz et al. (2018). According to them, the potential magnitude of mislabeled fish consumed in a country depends on both the mislabeling rates and the consumption of the most important commercial species in that country. Thus:

$$M = R \times C$$

where M is the value of mislabeled product, R is the mislabeling rate ($R < 1$) and C is the consumption (in €) of a specific fish species. To estimate mislabeling rates we have carried out a systematic literature review of 25 papers published between 2008 and 2023 dealing with mislabeling in the fish and seafood industry in different European countries, whereas the consumption of the main commercial species in each country was obtained using the database of households' purchases of fresh fish and seafood seafood products provided by EUMOFA. Due to information limitations, we have only been able to estimate the economic value of mislabeling of two of the main commercial species groups such as gadoids (cod, hake, haddock and other white fish) and scombroids (tunas, mackerels and bonitos) in 9 European countries (see Tables 1 and 2). We can observe that Italy presents the highest mislabeling rates, whereas the lowest rates are from the Netherlands and UK. On the other hand, the largest economic value caused by mislabeling is presented in Spain due to the high fish consumption in this country of gadoids and scombroids species.

Table 1. Mislabeling rates of no-processed fish for consumption by country and species group (%)

Country	All groups	Gadoids	Scombroids
Spain	24.8	30.1	31.9
France	32.8	-	32.8
Italy	46.8	65.1	54.1
Portugal	10.0	-	10.0
UK	2.8	1.2	4.4
Ireland	11.4	19.0	3.8
Denmark	25.3	25.3	-
Sweden	5.5	5.5	-
Netherlands	0.0	0.0	-
All countries	**22.0**	**21.5**	**22.5**

Note: Average values.
Source: Elaborated by the own authors.

Table 2. Economic value of mislabeling by country and species group (th.€)

Country	All groups	Gadoids	Scombroids
Spain	362,722	282,830	79,892
France	9,865	-	9,865
Italy	95,129	88,233	6,896
Portugal	2,068	-	2,068
UK	2,495	1,170	1,325
Ireland	7,813	7,602	211
Denmark	1,747	1,747	-
Sweden	131	131	-
Netherlands	0	0	-
All countries	481,970	381,713	100,257

Note: Calculation based on average values of households' consumption of fresh fish (2013-2023).
Source: Elaborated by the own authors.

Kroetz, K., Donlan, C.J., Cole, C.E., Gephart, J.A., Lee, P. (2018). *Through the Lens of Production and Trade: How Much Mislabeled Seafood Do Consumers Buy?* Resources for the Future, report.

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FORMULATING OF SUSTAINABLE DIETS FOR *Pennaeus vannamei* BY THE INCLUSION OF BY-PRODUCTS AS FISHMEAL SUBSTITUTE IN BIOFLOC TECHNOLOGY

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Introduction: The main limiting factor in shrimp aquaculture sector is the use of fishmeal (FM) in feed formulation, which account for 50-70% of the production cost. In previous studies in *Penaeus vannamei* (*P. vannamei*), relative high FM substitution can be achieved using plant or animal raw ingredients as alternative protein source. The ingredients choice and % substitution limits the growth performance, generally >50% replacement (animal or vegetal) results in lower growth rates. Therefore, the present study aims to evaluate the effect of total or high FM substitution by a combination of animal and plant by-products on productive parameters of shrimp under biofloc technology.

Material and Methods:

The present study consisted of two experimental phases of shrimp growth. **Phase I**, diets with 100% or 92% of by-products inclusion as alternative protein source from animal (A92 and A100), plant (V92 and V100) or a combination of animal and plant origin (AV92 and AV100) were assayed in *P. vannamei* from 2.5 to 8 g in tanks of 1 m^3 (n= 3 replicates/ group). Additionally, a control group with a 15% of FM inclusion were also tested to be compared with the experimental diets (Table 1). All diets were isoproteic and isolipidic, with a total 38% and 10%, respectively. In a **Phase II**, the two experimental groups from Phase 1 that provided the best growth performance were compared with the control from 5 to 14-15 g in tanks of 3.3 m^3. All diets in Phase 2 were isoproteic and isolipidic, with a total 34% and 10%, respectively, according to Ferrando-Juan *et al.* (2022). The growth assays were performed under intensive conditions, with densities of 350 shrimp/ m^3.

Table 1. Feed ingredients (g/kg) used in fishmeal replacement diets for P.vannamei

Feed ingredients (g/kg)	Control	A92	A100	V92	V100	AV92	AV100
Fishmeal	150	75	0	75	0	75	0
Eco wheat	344	405	382	333	324	348	322
Eco soy	210			215	218	163	188
Porcine hemoglobin		100	120			20	37
Meatmeal							
Wheat; Soy hydrolyzed; potato protein	150			210	270	165	180
Fish oil	20	28	36	28	36	28	36
Soy oil	46	32	22	39	32	36	27

Table 2. Growth performance of *P.vannamei* fed different alternative feed ingredients. Letters represent significant differences among diets.

	Feed	Final Weight (g)	SGR (%/day)	FI (g/100g fish day)	FCR
Phase I	**Control**	7.55 ± 0.33	1.46 ± 0.02	3.32 ± 0.1	3.04 ± 0.1^{ab}
	A92	8.29 ± 0.55	1.58 ± 0.09	3.13 ± 0.05	2.67 ± 0.12^{b}
	A100	7.41 ± 0.16	1.45 ± 011	3.36 ± 011	3.07 ± 0.19^{ab}
	V92	7.57 ± 0.41	1.46 ± 0.05	3.35 ± 0.13	3,07± 0.15^{ab}
	V100	7.22 ± 0.34	1.43 ± 0.09	3.46 ± 0.18	3.22 ± 0.2^{ab}
	AV92	8.20 ± 0.32	1.56 ± 0.07	3.19 ± 0.07	2.76 ± 0.15^{b}
	AV100	6.74 ± 0.4	1.31 ± 0.09	3.61 ± 0.16	3.75 ± 0.3 ^{a}
Phase II	**Control**	12.02 ± 0.48	2.41 ± 0.52	2.65 ± 0.44	1.20 ± 0.091
	A92	10.84 ± 0.4	2.25 ± 0.42	2.91 ± 0.36	1.42 ± 0.074
	AV92	12.10 ± 0.4	2.27 ± 0.42	2.52 ± 0.36	1.24 ± 0.074

(Continued on next page)

Results and discussion:

In Phase I, better growth performance was observed in the experimental diet A92 and AV92, with the lowest values of FCR (Table 2). Therefore, it was determined that a 92% of FM substitution with alternative animal and plant/animal by-products was possible without compromising growth performance, with no differences with control group. In Phase II, A92 registered a lower growth but without significant differences. In both phases, the results confirm that a high FM substitution is feasible in *P. vannamei* thanks to a combination of animal and plant by-products as alternative protein source. Previous studies have shown that it is possible to substitute fishmeal with alternative plant protein sources, such as soybean meal, up to 95%, or with animal protein sources, such as poultry by-products, up to 66%. Therefore, the present study provides an alternative and sustainable diet formulation for shrimp production without compromising production.

Acknowledgments:

The study was funded by the Research Project: “Optimizing shrimp feeding and nutrition in biofloc system (BioFlango)” (PID2020-114574RB-C21). S. Ferrando-Juan contract by MICIN Research Personnel Training Grant (PRE2021-098367). Y. Candela and J. Gómez-Aguilera contract was supported by European Union Next Generation-Plan of Conselleria d’innovació, Universitats, Ciència i Societat Digital of Generalitat Valenciana (INVEST/2022/434).

DEVELOPMENT OF LONG AND SHORT-TERM STORAGE PROTOCOLS FOR BIOFLOC CONSERVATION

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Introduction: Systems utilizing Biofloc technology (BFT) exhibit the capacity to rear specific aquatic species, notably shrimp and tilapia, at remarkably high stocking densities. The maturation process of biofloc is characterized by a prolonged duration before achieving full functionality. Initialization of a biofloc system typically involves a low initial density of aquatic organisms, coupled with an input of feed and an external carbon source. Drawbacks associated with this method encompass extended waiting periods, fluctuating water quality, and limited animal densities. Therefore, developing simple Biofloc preservation and reinoculation protocols is an interesting tool in aquaculture production. **Material and Methods:** Microbiological preservation tests were performed on concentrated Bofloc samples to ascertain the most effective preservation techniques. Total cell counts, heterotrophic and nitrifying bacterial growth were evaluated after the samples were subjected to various preservation methodologies.. Experimental groups included a positive control (concentrated and unpreserved Biofloc) and five experimental groups: refrigerated (RF), frozen with 15% glycerol (F-GLI), frozen with 15% glucose (F-GLU), frozen (F), and freeze-dried (LF). Notably, the F-GLI and F-GLU groups exhibited the highest levels of heterotrophic and nitrifying bacteria growth, with the F-GLI group demonstrating superior cell viability among the experimental cohorts (Figure 1).

Subsequently, an experiment was conducted utilizing *Penaeus vannamei* shrimp to assess the efficacy of preserved biofloc samples. Experimental groups encompassed refrigerated in vacuum bags (V), frozen at -80°C with 15% glycerol (F-GLI), and dried (D). Control groups included a negative control (C-), devoid of Biofloc, and a positive control (C+), inoculated with fresh Biofloc. Utilizing 80L tanks with an initial biomass of 80 grams and an inoculum concentration of 1g/L conserved for 5 days, water quality was periodically analyzed via chemical assessment of ammonium and nitrite concentrations

Results and discussion: After 21 days, observations indicated complete transformation of ammonium to nitrite in groups V and F-GLI. While nitrite accumulation was absent in group V, group F-GLI exhibited minimal nitrite accumulation albeit at very low concentrations. Conversely, group D displayed higher nitrite accumulation compared to the negative control, albeit with a more rapid stabilization of ammonium. These findings suggest the feasibility of short-term (one-week refrigeration) and long-term (freezing) preservation of biofloc to expedite the maturation of biofloc tanks accommodating high animal densities.

Acknowledgments: The study was funded by the Research Project: "Optimizing shrimp feeding and nutrition in biofloc system (BioFlango)" (PID2020-114574RB-C21). S. Ferrando-Juan contract by MICIN Research Personnel Training Grant (PRE2021-098367). J. Gómez-Aguilera contract was supported by European Union Next Generation-Plan of Conselleria d'innovació, Universitats, Ciència i Societat Digital of Generalitat Valenciana (INVEST/2022/434).

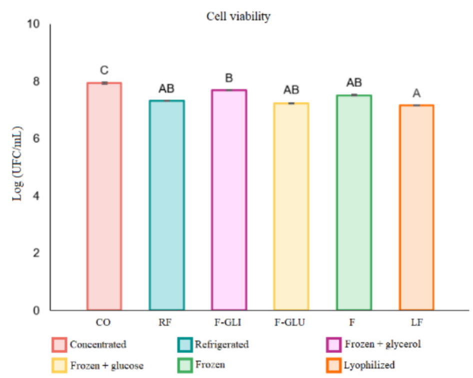

Figure 1. Cell viability after submitting

EXPLORING BLOOD COAGULATION IN EUROPEAN SEABASS *Dicentrarchus Labrax* VIA AUTOMATED ANALYSIS

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Numerous factors (e.g., stress, vitamin K concentration, liver damage) can influence hemostasis in fish. Despite being relatively understudied, the hemostasis of bony fish shows striking parallels to that of other vertebrates, such as mammals, encompassing both intrinsic and extrinsic pathways. This study aimed to measure the prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration (Fib), and antithrombin activity (AT), in plasma samples collected from bony fishes using an automated coagulation analyzer.

We examined K_3EDTA plasma samples obtained from 90 European seabass (weight 154.9 ± 34.1 gr) reared in a recirculation aquaculture system (temperature 23.0 ± 2.5 °C) at the Laboratory of Aquaculture of the University of Bologna, Cesenatico, Italy. Blood samples were collected by venipuncture from the caudal vein, centrifuged at 3,000xg within 2 hours and analyzed within 12 hours from collection using an automated analyzer (Siemens BCS XP + manufacturer's reagents, Siemens Healthineers, Marburg, Germany). Plasma samples were stored at 0 - +4°C until analysis. PT was measured using 50 µl of plasma incubated with Thromborel S (Human thromboplastin containing Calcium). The aPTT was measured by incubating plasma sample (50 µl) with Dade Actin Activated Cephaloplastins reagent to allow the phospholipid and surface activator interaction, followed by the addition of calcium chloride solution as a trigger for the coagulation. For AT analysis, the Innovance Antithrombin kit was used. It consists of adding an excess amount of factor Xa (FXa) to 10 µl of a 1:4 prediluted plasma with a buffer. Subsequently, the factor Xa is complexed and inactivated by the AT present in the sample The remaining, unbound FXa was cleaved into a specific chromogenic substrate and then releasing a dye. Fib was measured following the Clauss method, by adding the Dade Thrombin reagent to the plasma sample (50 µl), prediluted 1:10 with Dade Owren's Veronal buffer. All analyses were conducted at a temperature of +37 °C (default setting) and coagulometric and colorimetric readings were performed at a wavelength of 405 nm. A total of 160 µl of the sample was required for the 4 analyses. All methods used were fully automated and results were available in 9 min.

The analyzer successfully measured aPTT, Fib and AT which results were similar to those found in mammal species (Table 1). In this study, we demonstrated that the intrinsic coagulation pathway, AT, and Fib can be evaluated in the plasma of European Seabass with a commercially available automated coagulation analyzer. PT analysis was unsuccessful probably because this variable is affected by changes in temperature, likely due to the presence of heat-sensitive clotting factors in the extrinsic coagulation pathway as previously reported. The analytical methods used in this study seem promising for prospective research studies concerning hemostasis in bony fish by rapidly processing a high number of samples. An extensive validation of this method is necessary for European seabass, evaluating the repeatability of the results, stability of the samples for analysis and species-specific variations.

Table 1. Results of PT, aPTT, Fib, and AT measurements in European Seabass plasma samples with Siemens BCS XP

	PT (sec)	aPTT (sec)	Fib (g/l)	AT (%)
European Seabass§	-	26 (15.30-66.40)	2.54 (0.98-3.96)	90 (41-174)
Dog; Cat*	6.5-8.9; 9-15	8.0-16.5; 9-20	1.45-3.85; 1.45-3.80	105-166; 98-180

§Data are reported as median (min-max); *In-house reference intervals used at the Clinical pathology service of the Authors' institution.

TOP-DOWN CONTROL OF EUTROPHICATION AND PROVISION OF ECOSYSTEM SERVICES BY THE SOFTSHELL CLAM *Mya arenaria* IN A DANISH COASTAL LAGOON

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Introduction

Ringkøbing Fjord is a coastal lagoon system in western Jutland (Denmark), with a north-south orientation. The fjord is approximately 30 kilometres long and 10-15 kilometres wide. It has an area of almost 300 square kilometres and is on average just under 2 metres deep. The fjord is connected to the North Sea through a sluice and receives freshwater inputs from the Skjern River and smaller streams. Ringkøbing Fjord drains a basin of about 3 500 km^2, where agriculture, which covers 65-70% of the catchment, is the dominant activity.

The Water Framework Directive (WFD – 2000/60/EC) classifies Ringkøbing Fjord as having poor ecological potential due to eutrophication.

In light of this situation, the Ringkøbing-Skjern Municipality brought together relevant stakeholders to promote the development of an ecosystem modelling framework (Fig. 1) for the fjord.

The purpose of this framework was to (i) provide a thorough understanding of the interactions among the catchment, the fjord, and the sluice; (ii) to offer insights into how the stakeholders can work together effectively to achieve the targets set by the WFD; and (iii) to support policy makers.

Approach

Pelagic primary production in the fjord ecosystem is determined by both bottom-up and top-down control—the latter is due to the softshell clam *Mya arenaria*, known locally as sand mussel. This has the potential to become a managed fishery, while at the same time reducing eutrophication symptoms in the fjord and enhancing seagrass recovery.

The well-tested SUCCESS (System for Understanding Carrying Capacity, Ecological, and Social Sustainability) framework (e.g. Ferreira et al., 2023) was applied to Ringkøbing Fjord (Fig. 1). The modelling framework includes (i) a hydrological model to quantify the discharge of water and nutrients into the fjord, including source apportionment; (ii) a sluice model to simulate the exchange between the fjord and the North Sea (Nielsen et al., 2005); (iii) a hydrodynamic model (Delft3D) to simulate the 3D circulation inside the fjord; (iv) a system-scale ecological model (EcoWin) that includes sub-models for both the soft-shell clam *Mya arenaria* and the eelgrass *Zostera marina*.

The key features of the bivalve model are:

- Simulation of key physiological functions;
- Integration of relevant physical and biogeochemical components;
- Environmental feedback of shellfish growth, e.g. removal of phytoplankton and detritus, production of particulate organic waste, excretion of dissolved nitrogen, and oxygen consumption.

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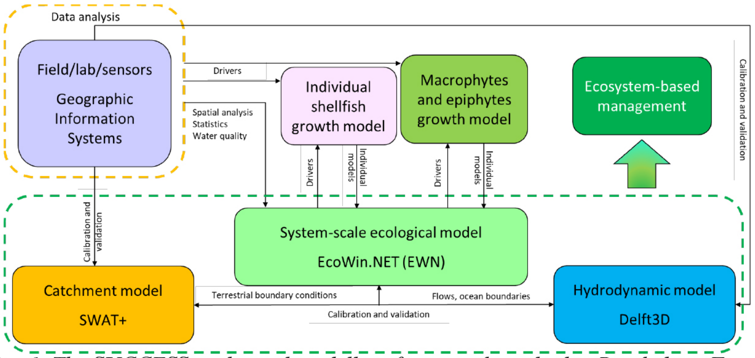

Fig. 1. The SUCCESS ecological modelling framework applied to Ringkøbing Fjord.

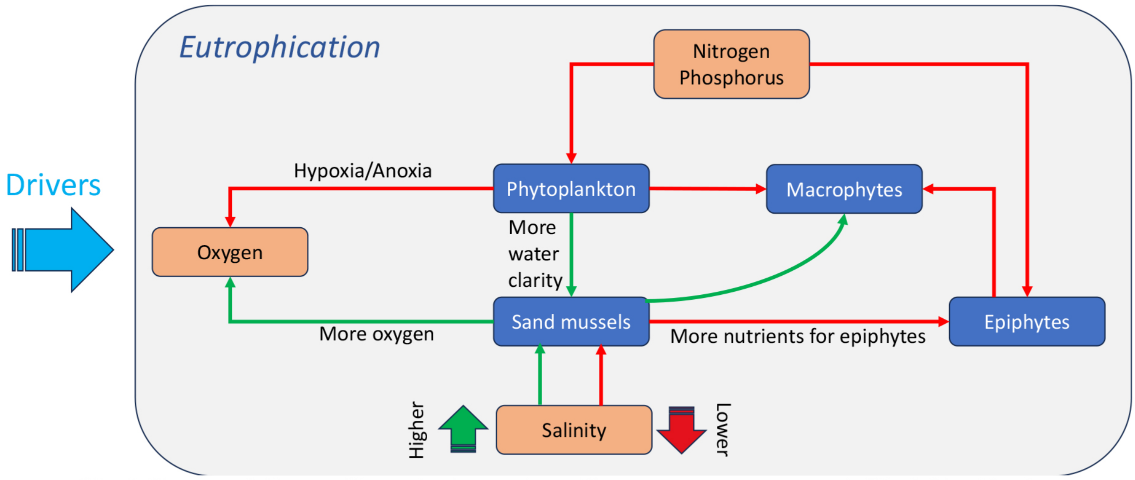

Fig. 2. Conceptual diagram illustrating interactions of key ecosystem components in Ringkøbing Fjord.

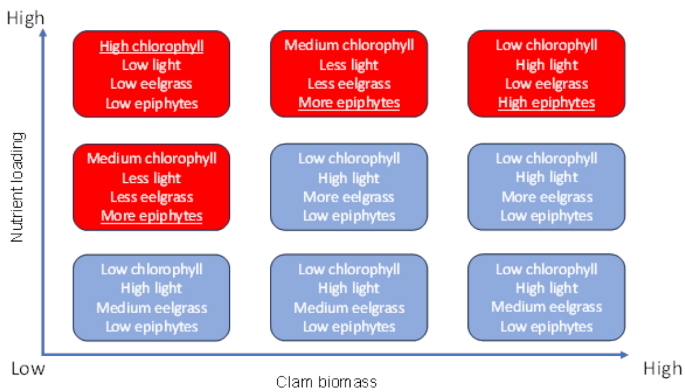

Fig. 3. Risk matrix for eelgrass and epiphytes applied to EcoWin results.

Table 1. Effect of top-down control on a set of indicators for a selection of EcoWin boxes and different scenarios.

Indicator	Scenario	SE area (Box 29)	Central area (Box 45)	Sluice area (Box 47)
Summer chlorophyll a (µg L^{-1})	With *Mya arenaria*	1.3	1.3	1.6
	No top-down control	16.9	13.8	12.7
	Difference (%)	+1191	+955	+709
Winter DIN (mg L^{-1})	With *Mya arenaria*	1.8	1.6	1.6
	No top-down control	1.8	1.6	1.5
	Difference (%)	+1	+1	+1
Macrophyte & epiphyte biomass (kg m^{-2})	With *Mya arenaria*	708	42	373
	No top-down control	705	33	53
	Difference (%)	-1	-22	-86
Epiphyte risk score	With *Mya arenaria*	3.42	1.10	2.70
	No top-down control	3.30	1.08	1.17
	Difference (%)	-4	-1	-57

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Sand mussels are not commercially important in Denmark, but in other markets such as the US (Beal et al., 2002) the soft-shell clam constitutes a valuable managed fishery, with a farmgate price of about 10 USD per pound of meat (National Marine Fisheries Service, 2021), or about 15 € per kg total fresh weight. There is no comparable European market that approaches those valuations.

The modelling framework was run for a period of ten years, and various scenarios were tested, including bottom-up (various changes to agriculture loading) and top-down (with and without soft-shell clams) control. Changes in phytoplankton biomass (chlorophyll as an indicator), dissolved inorganic nitrogen (DIN), and benthic macrophyte biomass were used for comparison of management strategies, and a risk matrix was developed to support management.

Results and discussion

The ecosystem dynamics of Ringkøbing Fjord (Fig. 2) are complex. Under normal discharge conditions and sluice operation, a significant population of soft-shell clams exists in the fjord. In EcoWin, this is simulated through a process of annual seeding (equivalent to recruitment) of about 120 animals (0.5 g each) per m^2 and leads to a simulated annual harvest of about 21,000 tonnes fresh weight, or about 9 animals per m^2, partly due to an annual mortality of 70%, partly because the animals have a two-year growth cycle, so the model simulates crop rotation, and partly because animals below harvestable weight remain in the fjord.

Salinity can fluctuate due to natural events such as high precipitation, and/or management policies such as reduction in water exchange with the North Sea through the sluice. Such events can lead to low salinity conditions, which result in high clam mortality.

If clam biomass is high, top-down control reduces chlorophyll (chl) and increases water clarity, leading to an increase in benthic vegetation.

However, since there is less nutrient drawdown by phytoplankton, excessive nutrients can also result in epiphyte growth on benthic plants. A reduction in nutrient loading coupled with higher clam biomass provides a sweet spot with low chl, eelgrass recovery, and less nuisance epiphytes. This is illustrated in Fig. 3 and was used to interpret EcoWin outputs for the various scenarios tested in this work

Table 1 shows the effect of soft-shell clams on various indicators relevant to the WFD classification of Ringkøbing Fjord. The top-down control of phytoplankton biomass is evident in all areas of the fjord, while no effect can be seen on winter DIN, as would be expected. The response of benthic plants to the presence of soft-shell clams is more pronounced in box 47 (2.2 m water column depth); in the shallow area of box 29 (1.2 m), light penetration to the bottom is less of an issue, and in the deepest box (45), where the water depth is 4.0 m, the underwater light climate is less suitable for benthic vegetation to thrive. This also decreases the epiphyte risk score since the substrate (eelgrass) available to epiphytes is reduced.

Among the various management recommendations instrumental in the recovery of Ringkøbing Fjord is the maintenance of an adequate salinity throughout the year to avoid mass mortality of clams, which result in chlorophyll peaks and addition of organic matter to the system. The potential value of *Mya arenaria* in the fjord can be estimated for provisioning services using a fraction of the US cost, since there is no European market for this bivalve. Even so, considering 10% of the US price, the potential annual revenue is of the order of 30 million euros.

The value of regulatory ecosystem services can be calculated in two ways: the conventional approach would be to determine the N removal associated with the potential clam harvest. However, since there is no clam fishery at the present time, the alternative is to determine the avoided cost of nutrient removal on land associated with chlorophyll reduction through top-down control (Ferreira & Bricker, 2019). The development of such a fishery could potentially have added value through a nutrient credit trading mechanism (e.g. (CDEEP, 2018)) similar to those developed in the United States.

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References

Beal, B.F., 2002. Adding value to live, commercial size soft-shell clams (Mya arenaria L.) in Maine, USA: results from repeated, small-scale, field impoundment trials. Aquaculture, 210, 1–4, 119-135.

Connecticut Department of Energy and Environmental Protection (CDEEP), 2018. Report of the Nitrogen Credit Advisory Board for Calendar Year 2018 To the Joint Standing Environment Committee of the General Assembly Concerning the Nitrogen Credit Exchange Program. CT DEEP, 79 Elm Street, Hartford CT. https://portal.ct.gov/-/media/DEEP/water/municipal_wastewater/NitrogenReport2018Final.pdf

Ferreira, J.G., Bricker, S.B., 2019. Assessment of Nutrient Trading Services from Bivalve Farming. In: Goods and Services of Marine Bivalves. Aad C. Smaal, Joao G. Ferreira, Jon Grant, Jens K. Petersen, Øivind Strand, Editors. p. 551-584.

Ferreira, J.G., Bernard-Jannin, L., Cubillo, A., Lencart-Silva, J., Diedericks, G.P.J., Moore, H., Service, M., Nunes, J.P., 2023. From soil to sea: an ecological modelling framework for sustainable aquaculture. Aquaculture 557, 1-14.

National Marine Fisheries Service, 2021. Fisheries of the United States, 2019. U.S. Department of Commerce, NOAA Current Fishery Statistics No. 2019. 167 pp.

Nielsen, M.H., Rasmussen, B., Gertz, F., 2005. A simple model for water level and stratification in Ringkøbing Fjord, a shallow, artificial estuary. Estuarine, Coastal and Shelf Science 63, 235-248.

CARRYING CAPACITY ASSESSMENT FOR SUSTAINABLE OYSTER AQUACULTURE IN DEEP BAY, HONG KONG

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Deep Bay is a shallow estuary covering an area of around 115 km^2 located to the north of the New Territories of Hong Kong and to the south of the city of Shenzen, China. Its waters are home to a major aquaculture industry for the Hong Kong oyster, *Magallana hongkongensis*, cultivated on rafts.

The significant increase of oyster rafts in recent years has affected the coastal environment, highlighting the need to strengthen the management of oyster farming activities in the area. In this context, a comprehensive assessment of the ecological dynamics of Deep Bay was carried out with the objective of quantifying the bay's environmental carrying capacity and determine the optimal level of production in the different aquaculture zones. An integrated soil to sea modelling framework was developed and applied to Deep Bay (Fig. 1).

The framework combines the simulation of (i) land-based loads with the Soil and Water Assessment Tool (SWAT) catchment model; (ii) hydrodynamics of the Deep Bay using the Regional Ocean Modeling System (ROMS) model; (iii) and the *Magallana hongkongensis* individual growth model. All three components are included within the well-tested EcoWin ecological model (e.g. Ferreira et al, 2008; Bricker et al. 2018, and references therein).

The EcoWin model domain applied to Deep Bay was divided into 17 horizontal boxes and 2 vertical layers totalling 34 individual computation units, using a multicriteria approach based on administrative boundaries, physics, and water quality. In parallel, detailed mapping of the 10,000 rafts, with a total capacity of around 500 million oysters, was carried out to parameterise spatial distribution and stocking density of oyster farming (Fig. 2).

The EcoWin model was calibrated and validated against field measurements and is able to reproduce the observed ranges and seasonal patterns of nutrients and particulate matter concentrations. The implementation of an individual growth model specific to *Magallana hongkongensis* and the ability of the EcoWin model to simulate management scenarios such as different stocking densities, seeding and harvest sizes and periods, and mortality, provide an integrated framework for supporting local stakeholders and policy makers with respect to management options for oyster aquaculture in Deep Bay. We present estimates for the carrying capacity of Deep Bay for oyster culture, the effect of top-down control on eutrophication in the bay, and discuss options for optimisation of bivalve aquaculture, taking into account the suitability of different areas, the harmonisation of different water uses, and the role of different actors in the bay ecosystem, including conservation interests.

References

Bricker, S.B., Ferreira, J.G., Zhu, C., Rose, J. M., Galimany, E., Wikfors, G., Saurel, C., Miller, R. L., Wands, J., Trowbridge, P., Grizzle, R., Wellman, K., Rheault, R., Steinberg, J., Jacob, A., Davenport, E. D., Ayvazian, S., Chintala, M., Tedesco, M. A., 2018. Role of Shellfish Aquaculture in the Reduction of Eutrophication in an Urban Estuary. Environ. Sci. Technol. 52:173–183. https://doi.org/10.1021/acs.est.7b03970

Ferreira, J.G., Hawkins, A.J.S., Monteiro, P., Moore, H., Service, M., Pascoe, P.L., Ramos, L., Sequeira, A., 2008. Integrated Assessment of Ecosystem-Scale Carrying Capacity in Shellfish Growing Areas. Aquaculture, 275, 138-151.

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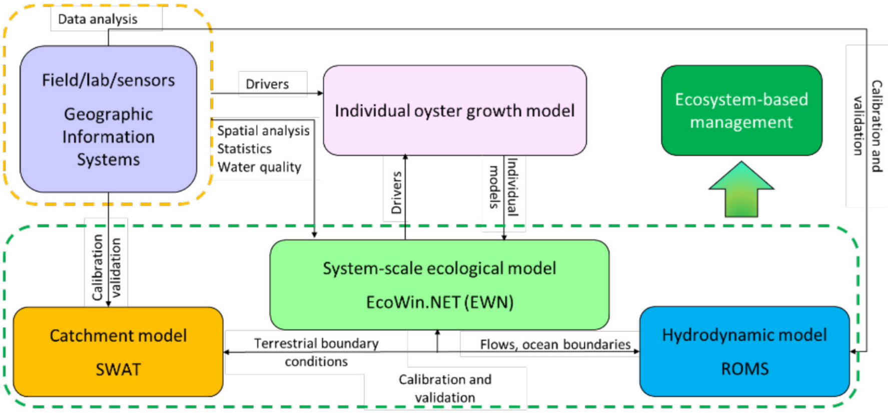

Fig. 1. Ecological modelling framework applied to Deep Bay.

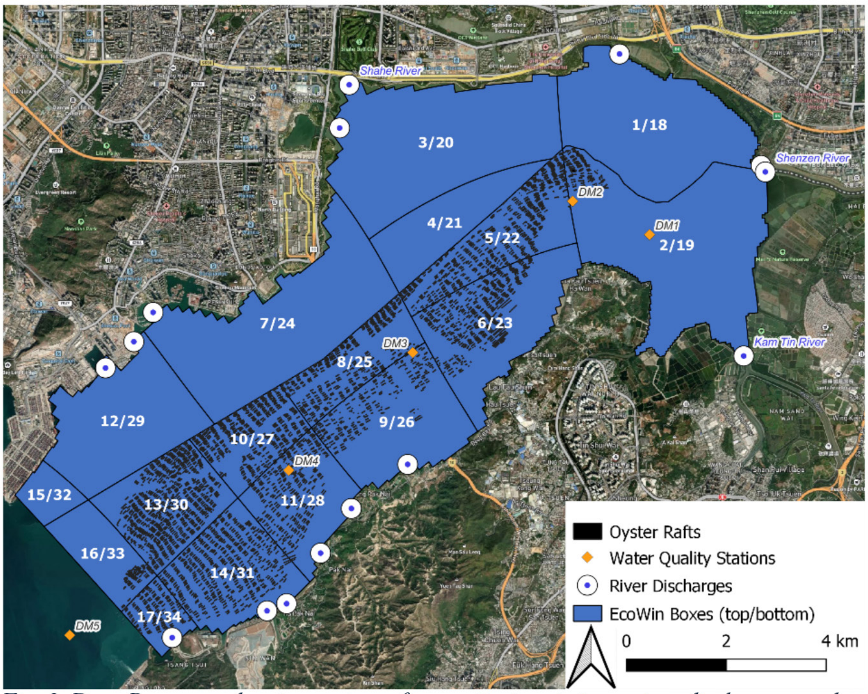

Fig. 2. Deep Bay area, showing oyster rafts, monitoring stations, river discharges, and EcoWin boxes.

MODELLING NEAR- AND FAR-FIELD EFFECTS OF AQUACULTURE: APPLICATION OF THE FINS MODEL TO PARTICLE DEPOSITION, DISSOLVED NUTRIENTS, AND PATHOGEN CONNECTIVITY

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Introduction

Sustainable aquaculture requires tools to address the key environmental issues associated with animal husbandry. Because water is the medium, the fate of waste substances is more difficult to determine than in agriculture, and engineering solutions for mitigating the impact of waste are more challenging.

In open water aquaculture, organic particle emissions are tightly coupled with the bottom, although water movement in a 3D environment results in deposition plumes that transport and deform the farm footprint due to horizontal transport and dispersion during the sinking process.

Regulations are normally set on the basis of (i) loading rates to the sediment (1 gC m^{-2} d^{-1} is often used); and (ii) thresholds of selected indicators such as sulphide, redox potential, and particulate organic carbon (POC) in the sediment below the farm. While the sediment indicators are measurable quantities, loading rates are measurable only by means of sediment traps, which often give ambiguous results in relatively shallow water (tens of metres).

In order to quantify the relationship between particulate emissions from a farm (waste feed for fed aquaculture, faeces, pseudofaeces for bivalves) and sediment quality, it is necessary to measure and model emissions, model the trajectory to the sea (or lake/reservoir) bottom, and use the calculated loads to feed diagenetic models that can predict sediment indicators—these can then be compared with measured values, closing the loop between emission and impact.

Far-field effects of aquaculture are often neglected; these include eutrophication due to the emission of dissolved nutrients (typically up to 5X more nitrogen than in particulate waste), and potential pathogen contamination, which may occur following a disease outbreak on a farm and will impact neighbouring farms depending on connectivity and other risk factors.

The Farming In Natural Systems (FINS) framework was developed to address the various issues described above, including the production and ecological pillars (e.g. Inglis et al, 2000;McKindsey et al, 2006) of carrying capacity, with the far-field ecological component including both eutrophication and disease.

Models that quantify near- and far-field impacts of aquaculture are available, but typically their use is beyond the scope of farmers and managers, which limits their application in any screening process. This is particularly the case in data-poor areas, which is often where expansion pressure is highest in the aquaculture sector.

FINS was designed to integrate complex models into a simple platform, allowing practitioners and policy makers to review potential siting and stocking, evaluate risk, and analyse development options in a multi-stakeholder environment.

In that context, this work aims to:

1. Present the application of FINS to the cage emission-organic load-diagenesis chain and compare the results of the model with measured outputs;
2. Examine the risk profile of a set of farms within an ecosystem, with respect to connectivity and pathogen outbreaks;
3. Illustrate how cutting-edge computational techniques, in particular using the Graphics Processing Unit (GPU) and AI, can be used to optimise model outputs and contribute to farm siting.

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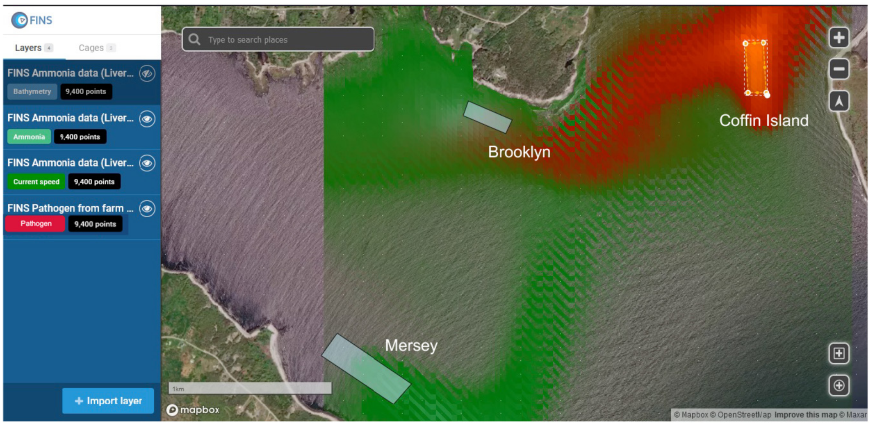

Fig. 1. WebFINS (with GPU) for farmers and managers showing three salmon farms (one existing and two potential developments), satellite imagery, ammonia dispersal and pathogen emission from the Coffin Island farm (eastern Canada).

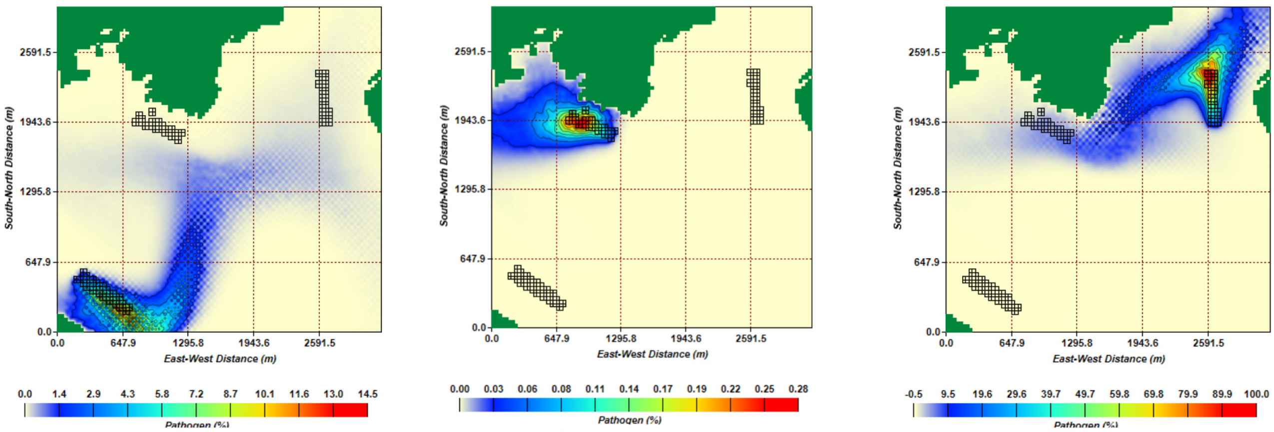

Fig. 1. Outputs for pathogen dispersal from the ORGANIX model based on data exported from FINS: emissions from three farms are shown: farm 1 (Mersey, left), farm 2 (Brooklyn, centre), and farm 3 (Coffin Island, right). Farm 3 has the highest connectivity.

Table 1. Relative connectivity of different farms in Liverpool Bay (%).

Effect	on farm 1	on farm 2	on farm 3
of farm 1	-	10.232	24.090
of farm 2	0.128	-	0.0321
of farm 3	0.081	100	-

Approach

The standard approach used in FINS for both near-field and far-field simulations was to (i) use inputs from more detailed models such as FVCOM (Chen et al, 2002), FARM (e.g. Cubillo et al, 2016; Cubillo et al, 2021), and ABC (Ferreira et al, 2021); (ii) simplify these inputs in terms of temporal and spatial resolution while maintaining acceptable accuracy; and (iii) develop a map-based platform to allow managers and industry to interact with the models, optimising runtimes and providing a rich user experience.

At present, FINS is coupled offline with the ORGANIX model (Cubillo et al, 2016) which simulates emission and dispersion of particulates for near-field effects, and ammonia, oxygen demand, chlorophyll uptake (bivalves), and pathogen dispersal.

The FINS framework has been applied to a number of ecosystems in the Canadian province of Nova Scotia, including Liverpool Bay, Whitehead Bay, Lobster Bay, and Port Mouton. 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)

The addition of the disease component to the model required the development of an AI algorithm to identify individual farms (such as the three shown in **Error! Reference source not found.**), implementation of pathogen decay (through turbulence and natural mortality), and automated farm-by-farm modelling of pathogen emission and dispersal to calculate a connectivity matrix for a bay, estuary, or lake.

The calculations required to plot the FINS output surfaces on a map are computationally intensive, but a significant optimisation can be obtained if they are performed in parallel. Consequently, the computations are executed by the GPU, resulting in a considerable increase in calculation speed allowing for real-time display and quasi-instant updates when maps are panned or zoomed.

Results and Discussion

The results presented in this paper focus on the pathogen component of FINS and on the link between particulate emissions from cages and diagenetic processes.

Fig. 1 shows conceptual pathogen emissions from each of three farms in Liverpool Bay, Nova Scotia, over a fourteen-day cycle. Because pathogen outbreaks are notifiable for any major disease, the maximum period for a pathogen simulation in FINS is 30 days. The results show that the farms have different connectivity with each other, not only in terms of trajectory but also in terms of magnitude.

For the hydrodynamic conditions considered, farm 3 has the most significant effect on the others—this is expressed in Table 1 as a percentage, based on the highest overall value at a target farm using a normalised pathogen emission.

The table, which shows relative risk, shows that the maximum risk from farm 1 is on farm 3, but only about a quarter of the risk of farm 3 on farm 2. The effect of farm 1 on farm 2 is about 10% of the maximum risk, and farm 2 has a very low risk on the other farms (less than 1%) under these water circulation conditions. The data can be interpreted as follows: siting of farm 2 (Brooklyn) at the proposed location poses little hazard in terms of disease to other farms considered, but a disease outbreak in farm 3 (Coffin Island) would have potentially serious consequences on the new farm.

Although not shown due to space constraints, we also present results of the cage-to-sediment chain with respect to loading of organic particles and its effects on sediment key performance indicators. The representation of these results is shown in the web version of the FINS application.

Acknowledgements

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References

Chen, C. H. Liu, R. C. Beardsley, 2002. An unstructured, finite-volume, three-dimensional, primitive equation ocean model: application to coastal ocean and estuaries. Journal of Atmospheric and Oceanic Technology,20, 159-186.

Cubillo, A.M., J.G. Ferreira, S.M.C. Robinson, C.M. Pearce, R.A. Corner, J. Johansen, 2016. Role of deposit feeders in integrated multi-trophic aquaculture - a model analysis. Aquaculture, 453, 54-66.

Cubillo, A., Ferreira, J.G., Lencart-Silva, J., Taylor, N.G.H., Kennerley, A., Guilder, J., Kay, S., Kamermans, P., 2021. Direct effects of climate change on productivity of European aquaculture. Aquaculture International 29(1–2) DOI:10.1007/s10499-021-00694-6

Ferreira, J.G., Taylor, N.G.H., Cubillo, A., Lencart-Silva, J., Pastres, R., Bergh, Ø., Guilder, J., 2021. An integrated model for aquaculture production, pathogen interaction, and environmental effects. Aquaculture 536, 1-16, doi.org/10.1016/j.aquaculture.2021.736438

Inglis, G.J., Hayden, B.J., Ross, A.H., 2000. An overview of factors affecting the carrying capacity of coastal embayments for mussel culture. NIWA Client Report CHC00/69, Christchurch, New Zealand.

McKindsey, C. W., Thetmeyer, H., Landry, T., Silvert, W., 2006. Review of recent carrying capacity models for bivalve culture and recommendations for research and management. Aquaculture, 261(2):451-462.

IDENTIFICATION OF *Tenacibaculum maritimum* EXTRACELLULAR PRODUCTS: *IN VITRO* AND *IN VIVO* PROTEOMICS APPROACH

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Among the numerous bacteria that can affect fish species, *Tenacibaculum maritimum* has consistently been a cause for concern due to re-emergent outbreaks in aquaculture sites. Recent evidence suggests that the extracellular products (ECPs) secreted by *T. maritimum* are among these bacteria's main virulence mechanisms. However, despite their importance to pathogenesis development, the protein content of *T. maritimum*'s ECPs remains largely unexplored, both *in vitro* and *in vivo*. Therefore, *in vitro* and *in vivo* experiments were developed using the same *T. maritimum* virulent strain, comparing the proteins produced in a more controlled environment and during the host-pathogen interactions.

In vitro experiments were developed to disclose the identity of some of the proteins secreted by *T. maritimum*. These experiments allowed the collection of cell and cell-free supernatants at 6, 12, 24, 36, and 48 h to obtain SDS-PAGE protein profiles. Selected protein bands were excised from the gels and analyzed by NanoLC-MS/MS. In addition, an *in vivo* trial using a proteomics analysis of the skin mucus of European sea bass (*Dicentrarchus labrax*), previously bath-challenged with the same *T. maritimum*, was used to identify which proteins are being secreted during infection. Groups of sea bass were divided into bath-challenged (infected) or mock-challenged fish. Skin mucus samples were collected (n=12) at 24 h post-challenge, pooled to obtain 4 pools per treatment (each containing mucus from 3 fish), and subjected to proteomics (NanoLC-MS/MS) to search for the presence of *T. maritimum* proteins.

In the *in vitro* experiments, several proteins were identified, including sialidase, metalloprotease, collagenase, outer membrane proteins (like TonB-dependent receptors), lipoproteins, and type IX secretion system (T9SS)-related proteins. A C-terminal (CTD) signal suggests these proteins are being secreted. Most identified proteins are predicted virulent factors for *T. maritimum* and display fundamental roles in the pathogenicity of other phylogenetically close bacteria. This may indicate that these proteins may present an essential role during tenacibaculosis infection. The identification of the *in vivo*-produced proteins in the skin mucus of bath-infected European sea bass (cumulative mortality 30%) is currently under analysis and could bring valuable knowledge into the complex host-pathogen interactions between *T. maritimum* and fish at the mucosal level.

Work funded by the European Union's Horizon Europe research and innovation program (GA No. 101084651 - project IGNITION).

DIETARY SUPPLEMENTATION WITH CHROMIUM-METHIONINE COMPLEX ENHANCES GROWTH PERFORMANCE OF AFRICAN CATFISH

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Feed is one of the highest cost factors in fish farming, and it has become evident that the utilization of diets for aquatic species must be as efficient as possible to reduce environmental pollution. Decades-long research has demonstrated that supplementation of aqua feeds with different nutrients, vitamins or minerals can be beneficial for fish health, growth and overall feed efficiency, especially for diets low in fishmeal. Chromium is an essential mineral for humans and certain animals, although an essentiality could not be demonstrated in fish based on the definition of an essential trace element. However, dietary supplementation with Cr in fish diets has resulted in enhanced growth performance as well as improved immune response and stress sensitivity in numerous fish species. Trivalent chromium (Cr^{+3}) can support the metabolism of carbohydrates, lipids and proteins by elevating the activity of digestive enzymes and potentiating the action of insulin. Since knowledge about efficiency of organic trace mineral supplementation in different species of fish is lacking, effects of chromium-methionine (Cr-Met) complex in African catfish nutrition were evaluated. Four commercially based diets with increasing Cr-Met complex supplementation level (0, 0.2, 0.4 and 0.6 mg Cr kg-^{1}) in the form of Availa®Cr 1000were fed to African catfish (*Clarias gariepinus* B., 1822) in quadruplicate groups at Fraunhofer IMTE in Büsum, Germany, for 84 days. Growth performance parameters (final body weight, feed conversion ratio, specific growth rate, daily feed intake, protein efficiency ratio, protein retention efficiency), health parameters (mortality, hepatosomatic index, spleen somatic index, hematocrit) and mineral retention efficiency were assessed at the end of the feeding trial. Evaluation of health parameters revealed that the applied levels of Availa-Cr 1000 inclusions are not toxic to African catfish. Moreover, specific growth rate (SGR) was significantly increased in fish fed diets with 0.2 mg Cr-Met kg^{-1} and 0.4 mg Cr-Met kg^{-1} supplementation in comparison with Control (Fig.1). Based on a second-degree polynomial regression analysis, supplementation with 0.33 mg Cr-Met kg^{-1} was optimal in commercially-based diets for African catfish.

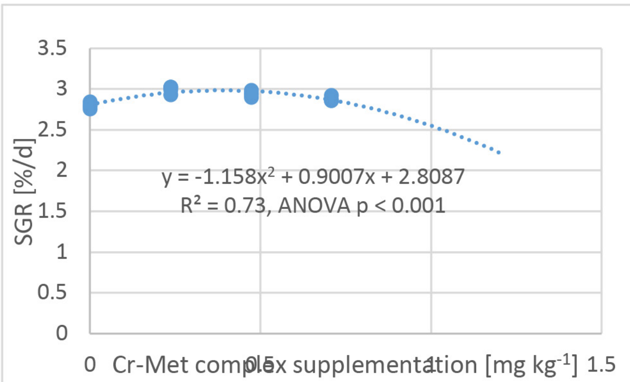

Figure 1: Effect of Cr-Met supplementation on Specific Growth Rate (SGR) of African Catfish

REPLACEMENT OF INORGANIC MINERALS WITH METAL-AMINO ACID COMPLEXES REDUCES MINERAL LOSSES IN RAINBOW TROUT

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Traditional flow-through systems reuse 0% of the water, whereas the most advanced RAS technologies reuse 95-99% of the water through a series of water treatment steps, making them environmentally more attractive. By regulating the culture conditions and shifting production to land, RAS can be built practically everywhere, regardless of local conditions, and can alleviate lack of available space and competition for access to water, all of which are critical in a world of limited natural resources. While being important for the fish, trace mineral (TM) levels in the water may have a negative impact on the natural environment and potentially on the fish. To reduce the environmental impact of aquatic animal feeds on (open) water quality, several countries have regulated the maximum level of mineral supplementation. The European Union, for example, set a whole feed Zn limit of 180 mg/kg for salmonids and 150 mg/kg for other fish species. As a consequence, aquafeed manufacturers need to develop methods to be more efficient with TM supplementation.

Our study investigated two factors that can affect the TM availability/uptake by the fish and the water/effluent level of TM: composition of the TM premix (inorganic vs TM-AA complexes) and the water refreshment level (RAS vs. flow through system), resulting in four different treatments. With this purpose, two diets were formulated to be equal in their nutrient composition with the exception of the type and level of TM used. One of the diets was supplemented with inorganic forms of TM (TM-I; sulphates of Fe, Zn, Cu, Mn and Se) and the other with TM-AA complexes (TM-AA; Fe-, Zn-, Cu-, Mn- and Se-AA complexes). In the TM-AA diet, TMs were supplemented at half the level of the inorganic. The two diets were fed for 8 weeks to ±78 g initial body weight rainbow trout reared in two different systems differing in water refreshment rate, high (HWR; 1.5 times the system volume) and low water refreshment (LWR; ±311 L / kg feed). At the end of the feeding period, the effect of the diet, rearing system and their interaction were evaluated in terms of growth performance, fish composition, TM retention and losses, plasma and hepatic TM content, and activity of hepatic enzymes playing a key role in the antioxidant defence (e.g. SOD, GPx, CAT). Rainbow trout grew better (SGR %/d) in the LWR (1.98) system compared with the HWR (1.92), regardless of the diet fed. Replacement of inorganic TM with TM-AA complexes by the half has significantly improved digestibility of P, Zn, Mn and Se. Moreover, and regardless of the system type, the supplementation with TM-AA complexes reduced significantly Fe, Cu, Mn and Se total losses while maintaining TM body retentions similar. The analysis of hepatic TM content revealed a reduction on Fe and Se contents with TM-AA vs TM-I and in the Se content of trout raised at HWR vs LWR system. However, neither the diet nor the system type affected the activity of enzymes playing a key role on antioxidant defense system. Plasma analysis showed higher levels of Mn and lower levels of Zn in trout fed TM-AA vs TM-I diet, regardless of the system type. In summary, our findings prove that replacement of inorganic with TM-AA complexes can offer an interesting strategy to reduce TM losses into the environment, while respecting EU legislation for upper limits of TM and still maintaning fish performance and health.

CHROMIUM-METHIONINE COMPLEX IMPROVES PERFORMANCE AND RETENTION OF PROTEIN AND PHOSPHORUS IN ATLANTIC SALMON

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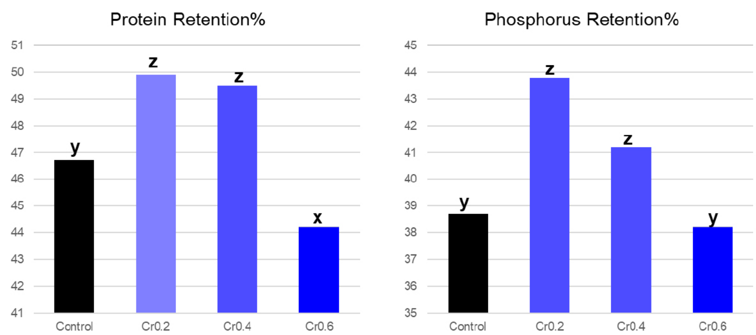
Complexes of trivalent chromium (Cr3^{+}) with organic acids and amino acids have been shown to play a key role in carbohydrate, protein and lipid metabolism. Although not completely understood, improved utilization and regulation of carbohydrates (glucose) by Cr^{3+} associates with increased insulin sensitivity, activation of intracellular insulin signaling, and glucose transporter translocation into the cell membrane. Dietary supplementation with Cr methionine (Cr-Met) complex proved to be consistently effective in improving feed utilization in crustaceans and fish, regardless of their trophic level (from carnivorous to omnivorous species), optimum temperature (warm- to cold-water species) or salinity tolerance (seawater to freshwater).

The efficacy of graded dietary levels of Cr-Met complex, in the form of Availa®Cr 1000, was recently evaluated in terms of performance and whole-body nutrient retention in Atlantic salmon (*Salmo salar*). The trial was comprised of four dietary treatments: a control diet (Control) without Cr-Met complex supplementation; and three other diets, based on the Control formulation, but supplemented with Cr-Met complex at increasing doses (0.2, 0.4 and 0.6 mg.kg^{-1}). Quadruplicate groups of 25 Atlantic salmon, with a mean initial body weight of 57.1 ± 3.2 g were fed one of the four experimental diets during 95 days. At the end of the trial, the final body weight ranged between 239.6 and 253.6 g. Specific growth rate varied between 1.51 and 1.57 %/day. When compared to Control, supplementation with Cr-Met complex at 0.2 and 0.4 mg.kg^{-1} resulted in significant improvements for weight gain and feed utilization (FCR and PER). Moreover, supplementation with Cr-Met complex at only 0.2 mg.kg^{-1} significantly increased protein (by 6.8%) and phosphorus (by 13.2%) retention (% nutrient intake). The positive effect of Cr-Met complex shown in performance and nutrient retention seems to be associated with a better utilization of glucose as an energy source, which allows for protein to be spared for growth purposes. Supplementation with Cr-Met complex has significantly increased the activity of glucokinase and glycogen content in liver of salmon, which is in agreement with the effect shown by Cr-Met complex in the glucose metabolism of other fish and shrimp species. These results indicate that supplementation with Cr-Met complex at doses as low as 0.2 mg.kg $^{-1}$ can be an efficient strategy to improve performance, while reducing excretion of nitrogen and phosphorus into to the environment, contributing therefore, to the sustainability of Atlantic salmon production.



COMPARATIVE ANALYSIS OF ECOSYSTEM- AND FARM-SCALE MODELS IN BIVALVE AQUACULTURE: IMPLICATIONS OF SPATIAL AND TEMPORAL RESOLUTION

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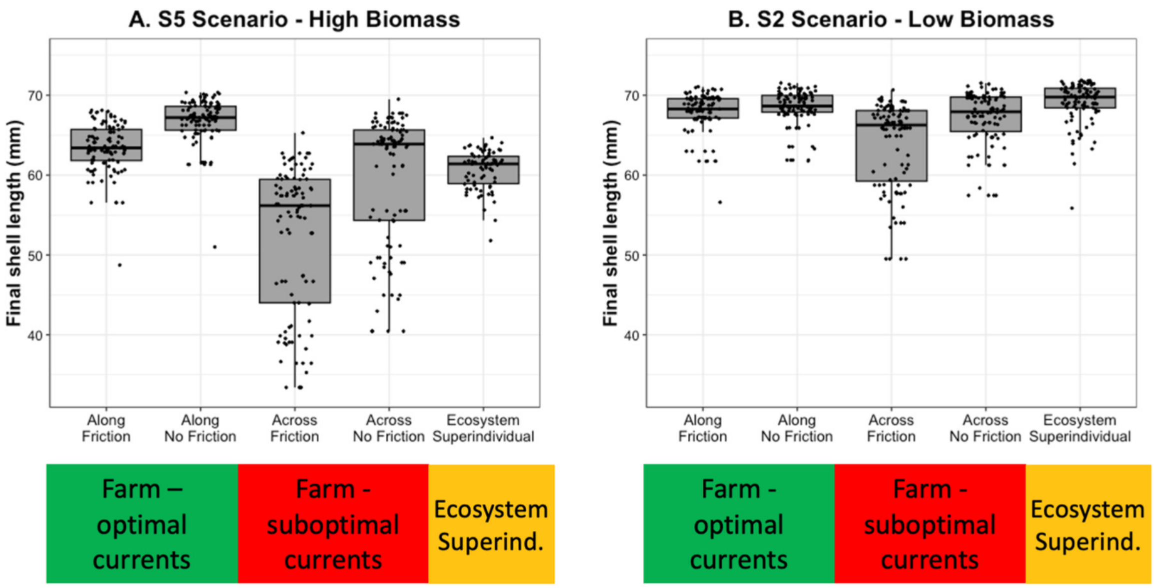
Mathematical models play a crucial role in bivalve aquaculture research, facilitating predictions and optimization of coastal aquaculture. These models can vary in the complexity of simulated processes and can be applied at different spatial and temporal scales and resolutions, which have implications for the potential research question to be addressed and for the computational power required to run simulations. This study examined the performance of ecosystem and farm-scale models in simulating bivalve growth and farm productivity, focusing on the effects of spatial and temporal resolution under a range of environmental conditions in Hardangerfjord, Norway. The study relies on two previously published models: an ecosystem model, NORWECOM.E2E, which has been recently coupled to a Dynamic Energy Budget (DEB) to simulate the bioenergetics of mussels, and a farm model that simulates the effect of the suspended canopy on local hydrodynamics to predict mussel growth using DEB theory. A series of numerical experiments revealed that the characterization of water currents in relation to the orientation of the farm is challenging and can lead to uncertainty in farm-scale models. Further, the discrepancies between modelling approaches were strongly dependent on the stocked biomass, with low biomass scenarios minimizing these discrepancies. Based on the results, the NORWECOM.E2E-DEB ecosystem model can be used to explore site selection and aquaculture environment interactions without impacting the estimations of farm production predicted by the farm-scale model to a critical level. The study underscores the potential of combining both modelling approaches for holistic coastal and farm management, providing valuable insights for future aquaculture development.



ADVANCING KNOWLEDGE ON FACTORS AFFECTING SOCIETALLY-ENDORSED SUSTAINABLE SALMON AQUACULTURE

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Despite its role as a key socio-economic driver, salmon farming continues to be a controversial topic with public opposition and conflicts challenging the sustainability of the sector. From a societal perspective, the seemingly intractable challenge centres around gaining acceptance of aquaculture as a complementary valued activity along with other more established uses of the coastal and marine environment. As a result, a deeper understanding of public perceptions and multi-scalar factors influencing opinions becomes critical for developing sustainable and socially acceptable aquaculture. In this presentation, we discuss the findings from a seven-year research project (www.coastalfutures.ca) focused on gaining a better understanding of societal needs for the sector in a manner that potentially contributes to global opportunities of aquaculture to be realized. Organized around five sub-modules, we focus our interdisciplinary expertise on acquiring knowledge on perceptions of social licence and how these may be influenced by occupational health and safety, aquaculture-community dynamics, socio-ecological finfish carrying capacity and the role of communities in marine spatial planning. Our path-breaking approach to aquaculture development – which requires both scientific excellence and social responsibility – highlight a range of factors and linkages that need to be integrated if companies, regulators, communities and other stakeholders are to better understand how societally-endorsed sustainable aquaculture may be achieved.

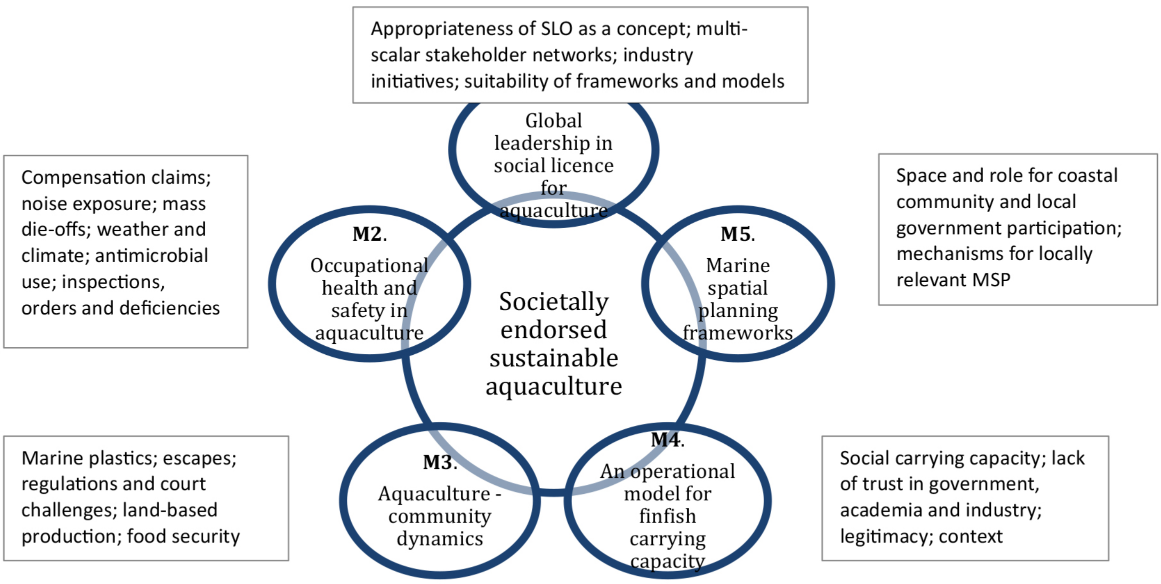

Figure 1: A societally-endorsed sustainable aquaculture research framework

INTERNAL FISH ESCAPEES COMPROMISE BIOSECURITY IN RECIRCULATION SYSTEMS: LESSONS LEARNED FROM NORWAY

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Recirculation Aquaculture Systems (RAS) are dependent on a mature and robust biofilm within the bioreactor to efficiently convert harmful ammonium excreted by the fish. Furthermore, a biofilm matured for several years contributes strongly to a stable and diverse microbiota within the system, including fish tanks, which is beneficial for the operational stability of the water treatment as well as for fish welfare.

The strategy of sustaining the biofilm in a RAS for several years is in theoretical conflict with the "all inn, all out" biosecurity principles due to a suspected potential of the biofilm to function as a reservoir for fish pathogens. The Norwegian regulations (NS-9416) define how a land-based aquaculture installation should be designed to avoid fish escaping out of the facility and in to the wild. Hence, the main installations to avoid escapees are strategically placed sieves towards the facilities boundaries as well as in the fish tanks. Both set of sieves have light openings in relation to the minimum fish size defined by the production plan. Internal escapees which have breached the primary barrier in the fish tanks, have shown to be residing in pipes, drum-filters, bioreactors, CO_2-degassers and oxygen-cones within a RAS department.

Pure Salmon Technology (PSTech) has the past year high-lighted the potential of internal escapees in RAS to breach the biosecurity principle of "all inn, all out". In a field study of two Infectious pancreatic necrosis virus (IPNV) infected RAS-departments in an Atlantic salmon *Salmo salar* Norwegian hatchery, we demonstrated that internal escapees were sub-clinically infected by IPNV. These individuals would serve as a reservoir for the virus and thus pose a risk for the new batch of fish introduced into the department.

Based on the presented case study and similar experiences by other smolt producers in Norway, we will document why internal escapees within RAS must be removed before any biosecurity measures are taken towards the biofilm in the bioreactor. "Humane" euthanasia solutions (using approved sedative at high dose) procedures will be presented to help eliminate individuals acting as potential pathogenic hosts in the system without compromising the biofilter efficiency and performance.

EFFULENT NUTRIENT ANALYSIS OF AEROBIC AND ANAEROBIC SLUDGE MINERALIZATION FROM CLEAR-WATER AND HYBRID BRACKISH-WATER RAS

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Water reuse is a key feature of inland, brackish-water, recirculating aquaculture systems (RAS) and mineralization of thickened waste may play a role in reducing water discharge. In clear-water systems solids filtration is robust; hybrid systems in comparison, operate with less filtration. By allowing the collected sludge to remain in contact with an external water body, the process of mineralization or decomposition of solids, can release nutrients and minerals important for plant production in brackish-water aquaponics. It is unclear how waste from the two system types might differ and what effects aerobic versus anaerobic environments may have on mineralization of the material. This trial compared aerobic and anaerobic mineralization of marine shrimp sludge from two system types over a four-week period to determine changes in concentrations of dissolved nitrogenous compounds, phosphate and mineral content over time.

Sludge was collected from separate clear-water (CW) and hybrid (HY) shrimp RAS settling chambers. A 28-day trial examined each water type in aerobic (AE) and anaerobic (AN) conditions. The two water types and two oxygen levels created four treatments: CW-AE, CW-AN, HY-AE, HY-AN. Each treatment was randomly assigned to four replicate, 18-L containers. The water in each container was analyzed weekly for total ammoniacal nitrogen (TAN), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), phosphate (PO_4) and alkalinity (ALK). Additional water samples were collected weekly and filtered, then sent to an independent lab for elemental analysis via inductively coupled plasma mass spectrometry (ICP-MS).

Concentrations of ALK, TAN and PO_4 tended to be higher overall and NO_3-N tended to be lower overall in anaerobic treatments. Because the denitrification process reduces NO_3 and produces alkalinity, results indicated denitrification occurred in anaerobic treatments. Plants generally prefer uptake of NO_3 over ammonia and use less energy to do so; however, both compounds can be assimilated into plant tissues. The CW-AE treatment showed a gradual increase in PO_4 concentration that may continue past the duration of this project. However, anaerobic treatments generated far more PO_4 than aerobic treatments in the four weeks of this trial. The difference (initial – final) in elemental concentrations showed an increase of dissolved Ca and decrease of Mn and Fe concentrations in aerobic systems. There was an overall decrease in all treatments of Na, Mg, Ca, K and Sr concentrations, and an overall increase in all treatments of S, Li, Ni, Si, P and Br concentrations. A two-week mineralization time in anaerobic treatments yielded the highest TAN, NO_3 and PO_4 concentrations, suggesting additional mineralization time is unwarranted for these nutrients. However, it is likely that other plant essential nutrients could be optimally mineralized at different time intervals, or could become more bioavailable over time. Results indicate that system type may not be very impactful with regard to waste mineralization; however, aerobic versus anaerobic environments, and perhaps time, can make large impacts on what compounds are mineralized into the water column and at what concentrations. This information may be used by aquaponics producers to select for particular compounds best suited to the nutritional needs of their plant species being cultivated.

ENRICHED INGREDIENTS AND POTENTIAL BIOACTIVE EXTRACTS FROM SOLID STATE FERMENTATION OF FRUIT AND VEGETABLE DISCARDS TO IMPROVE DISEASE RESISTENCE IN EUROPEA SEA BASS (*Dicentrarchus labrax*)

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A significant amount of fruits and vegetables are wasted each year, causing negative impacts on both the economy and the environment. Utilizing them as animal feed would help reduce feeding costs and, simultaneously, would align with efforts to prevent resource wastage and improve the economic efficiency of processing plants. The present research aimed to optimize a solid-state fermentation (SSF) process to produce nutritionally and functionally enriched potential aquaculture feed. SSF led to an increase of protein and fats, improving essential amino acid profile and fatty acid concentration in the fermented ingredients. Extraction led to a bioactive extract product with in vitro antioxidant and antimicrobial activity against bacteria from *Vibrio, Tenacibaculum, Bacillus and Staphylococcus* genera (figure 1). *In vivo* trials using fermented products and extracts resulted in no differences in the productivity parameters compared to a control diet in sea bass, however, the inclusion of 0.5% of fermented extract in the diets led to a reduction in mortality when sea bass was exposed to the pathogen *Tenacibaculum maritimum* (table 1).

SSF succeeded in obtaining a stabilized material enriched in protein, fat and digestible proteins that can be used in 5% inclusion of aquaculture diets without affecting productive performance. In addition, fermented products could be extracted to obtain a functional ingredient with positive effect in the immune system of sea bass.

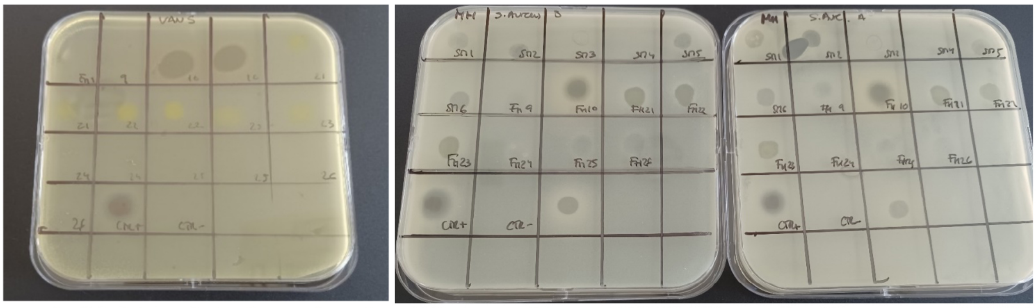

Figure 1. Inhibition halo formed against *Vibrio angillarum* CECT 522 and *Bacillus cereus* CECT 131 in agar diffusion assay.

Group	A	B	C	Control
Mortality (%)	36.67±5.77 [a]	133.33±5.77 [b]	0.00±0.00 [c]	133.33±5.77 [b]

Table 1. Mortality rates for each bacterial load. The values are expressed as the mean of replicates ± standard deviation. Entries indicate significant differences *P value*<0.005. A: Control + 5.0% Inclusion of fermented fruit and vegetables (FFV), B: Control + 5.0% Residual pellet and C: Control + 0.5% Fermented extract.

EFFECT OF VACCINATION AND SELECTIVE BREEDING AGAINST INFECTIOUS SALMON ANAEMIA IN ATLANTIC SALMON (*Salmo salar*) IN TWO DIFFERENT CHALLENGE MODELS

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Infectious salmon anemia (ISA) caused by the infectious salmon anemia virus (ISAV) leads to disease outbreaks that entails considerable economic and welfare complications for Atlantic salmon farming.

This study examined the impact of vaccination and selective breeding on mortality and load of virus by assessing the susceptibility to ISA of two genetics groups (high and low resistant against ISAV) of *S. salar* vaccinated with a commercial ISAV vaccine. Groups injected with a vaccine without the ISAV component, were included as a negative control. The fish were challenged by intraperitoneal (IP) injection or cohabitation. The evaluation of protection was assessed based on differences in specific accumulated mortality and viral loads between vaccinated and control groups and the different genetic groups.

The groups vaccinated with the ISAV component resulted in reduced mortality compared to the groups vaccinated without the ISAV component in both infection models, from above 72% mortality in groups vaccinated without the ISAV component to below 16% mortality in groups vaccinated with the ISAV component. There was a significant difference between the genetic groups vaccinated without the ISA component from the IP challenge (p-value = 0.001), and an indication but not a significant contrast between groups from the cohabitation challenge (p-value = 0.085). Based on the result comparisons of mortality in the different groups, additional analysis was performed to assess the viral load between groups using qPCR. There were significantly lower viral loads in the high-resistant genetic group compared to the low-resistant genetic group, both vaccinated with the ISAV component, from the cohabitation challenge (p-value = 0.020).

The use of an ISAV component in vaccines for Atlantic salmon reduced mortality after challenge. The results also indicate that selective breeding for increased ISAV resistance leads to reduced mortality, and significantly reduced viral loads. Vaccination, as well as selective breeding leads to reduced mortality and viral loads, which could reduce viral shedding to the surroundings.

GLYOXAL ACID-FREE (GAF®) AS AN ALTERNATIVE HISTOLOGICAL FIXATIVE TO FORMALDEHYDE IN FISH SPECIES

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For over a century, the gold standard for fixation of histological samples is characterized by a solution of formaldehyde in water, also known as 10% formalin. Although this fixative has notable merits in preserving the morphology of cells and tissues, environmental authorities are increasingly concerned about the objective toxicity of formalin. As a volatile reagent, formalin displays allergenic, neurotoxic, and cancerogenic properties. In recent years, alternative fixatives have been considered as substitutes to formalin with the aim to reduce the described negative effects. In particular, fixatives utilizing a glyoxal solution deprived of acids, have garnered attention due to their non-volatile nature and lack of carcinogenic hazard. Although glyoxal acid-free (GAF®) has been tested on tissues from human and terrestrial animals, there is a lack of knowledge regarding its efficacy in fish species.

The aim of this study was to test the performance of GAF® fixative in comparison with those of two distinct fixatives: 10% formalin and 4% paraformaldehyde. Two different formulations of glyoxal acid-free (GAF®) have been considered: the first, previously tested on samples from terrestrial animals, while the second was specifically formulated for samples from fish species. For the trial, we selected zebrafish (*Danio rerio*) to assess fixation in freshwater fishes, and European sea bass (*Dicentrarchus labrax*) as marine species. Various target organs such as liver, muscle, gills, and gonads were sampled in adults of both species; moreover, in zebrafish, whole animals as well as heads and trunks were collected. The samples were maintained in the respective fixatives for 48 hours. The preservation of morphology was evaluated on histological sections by using haematoxylin-eosin as well as Masson's trichrome staining. Furthermore, the maintenance of relative nucleic acid integrity was evaluated on histological sections by using in situ hybridization.

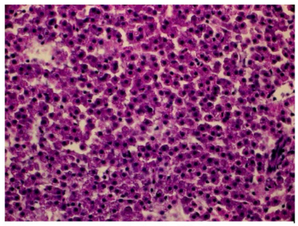

Figure 1 GAF fish formulation, zebrafish liver.

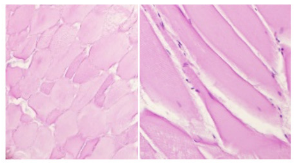

Figure 2 GAF fish formulation, zebrafish (left) and European sea bass (right) muscle.

The formulation of GAF® initially tested for terrestrial species exhibited suboptimal results in both species. However, upon adjusting the GAF® formulation for fish species, we observed improved results (see Figure 1 and 2), which more closely resembled those obtained with 10% formalin and 4% paraformaldehyde. Interestingly, Masson's trichrome staining did not reveal any notable differences among the different fixatives. In GAF®-fixed specimens, in situ hybridization results matched those observed with 10% formalin and the signal was clearer and more pronounced.

In conclusion, GAF® fixative, especially the formulation set up for fish species, could serve as a valuable fixative alternative to formaldehyde for both freshwater and marine fish species.

VITAMIN K REQUIREMENT FOR EUROPEAN SEA BASS *Dicentrarchus labrax*

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Vitamin K is a lipid-soluble vitamin that acts as a co-factor of the enzyme glutamyl-carboxylase (GGCX). Several proteins involved in blood coagulation (clotting factors) and bone mineralisation depend on the action of GGCX to transform a glutamic residue to Y-carboxy glutamic to become active. Vitamin K deficiency might lead to longer coagulation time and changes in bone mineralisation. To our knowledge, there are not studies carried out in European Sea bass (*Dicentrarchus labrax)*.

A dose response trial with increasing levels of supplemented vitamin K in the feed (0, 4, 7, 10, 17 and 29 mg vitamin K/kg feed) was carried out in triplicate tanks in sea water at 22C with European sea bass from 35 to 150g. At the end of the trial, 5 fish/tank were sampled for blood analysis (activated partial thromboplastin time, aPTT; fibrinogen concentration; haematocrit value; HT), x-ray, mineral content of the spine, vitamin K content in liver and vitamin K dependent proteins gene expression in bone and liver. Specific growth rate (SGR), feed intake (FI), feed conversion rate (FCR) were determined at the end of the trial.

There was no correlation between increasing vitamin K in the diet and SGR, (Figure 1), feed intake or FCR. For blood variables, increasing dietary vitamin K content did not change aPTT results of fish). Fibrinogen content was higher in diets containing 4 mg or more of vitamin K/kg compared to the non-supplemented diet (Figure 2).

Based on the results the optimal vitamin K supplementation for European sea bass seems to be at the low range of dietary supplementation.

Figure 1. SGR (%) of fish with increasing vitamin K concentration in feed (mg/kg)

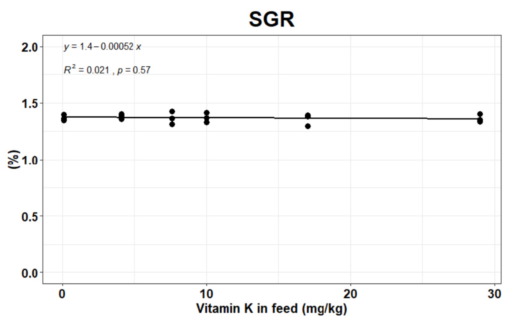
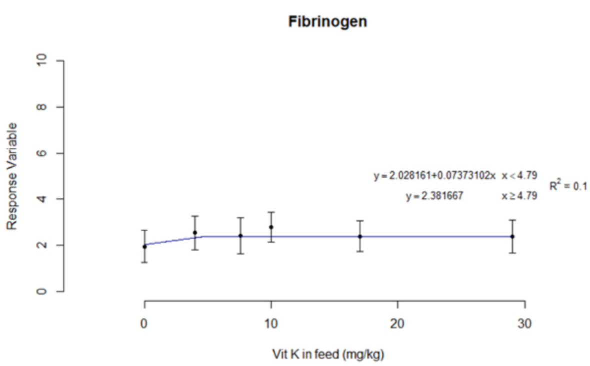


Figure 2. Fibrinogen concentration in blood (g/L) of fish with increasing vitamin K concentration in feed (mg/kg)



QUANTIFYING ENVIRONMENTAL IMPACT OF AQUACULTURE THROUGH CERTIFICATION-FACILITATED IN-SITU FARM DATA COLLECTION

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The Aquaculture Stewardship Council (ASC) is one of the leading certification schemes for farmed seafood, certifying a wide range of species including marine and freshwater fish, molluscs, crustaceans and seaweed. Currently, 58 unique species are in production by ASC certified farms across 52 countries operating a variety of production systems. One of the distinguishing features of ASC's Farm standards is the measurable nature of a variety of requirements herein that each producer needs to conform to, to become or remain certified.

As part of their ASC certification, producers need to record and submit production data, key environmental and social data to ASC, associated with these requirements. The purpose of the data is related to assurance, transparency, traceability, standard improvements, impact assessment and research. The wide variety in production systems used, the diversity in species produced and global distribution of ASC certified producers offers the unique opportunity to assemble an extensive database on data associated with the environmental impact of aquaculture operations. Due to this variety that ASC deals with and the learnings from the past 10 years, we understand the challenges and inherent risks which enhanced our ability to develop a uniform, systematic data collection procedure and improve data use capabilities.

Here we present the developed procedure, and outline 1) what in-situ variables are collected related to the state and drivers of impacts on water quality, benthic environment, wildlife and climate, 2) how farm in-situ data submissions are technically facilitated by ASC using online tools, 3) how by this extensive and standardized data collection, ASC is enabled to quantify region, production system and species-specific impacts of aquaculture, and 4) where we have identified key limitations and risks in the application of these data.

VGLL3 DOES NOT EXPLAIN FAMILY DISCORDANCE IN MALE MATURATION FOLLOWING DIFFERENT SMOLT PRODUCTION REGIMES IN ATLANTIC SALMON

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Atlantic salmon farming is moving towards extended periods of land-based production to minimise the time at sea, but this increases the risk of unwanted male sexual maturation. As the timing of puberty is driven by genetics (e.g. *vgll3*) and the environment, we used a domesticated strain of *vgll3* heterozygous broodstock to produce three all-male half-sibling families (A-C) using three different production regimes to assess the timing of first puberty. Firstly, "large smolts" were produced in a flow-through system on 13°C freshwater and constant light from first feeding (day 0) up to 1kg (day 354). Genetics explained a significant ($p<0.05$) amount of the variation, with 93, 71, and 34% of the expected early (EE), intermediate (EL), and late (LL) maturing *vgll3* genotypes being pubertal on day 354, respectively (Figure 1A). Secondly, "post-smolts" were produced by switching half the large smolts to 13°C seawater at 420g (day 272) and growing them in tanks to 0.95kg (day 354). This led to a significant 14% reduction in the total incidence of puberty by day 354 compared to the large smolts (Figure 1B). However, the regulation of pubertal timing by *vgll3* did not interact with salinity. Thirdly, traditional "under-yearling" smolts were produced using periods of natural or manipulated temperature and photoperiod, followed by transfer to a sea-cage at 150g in December (day 290) where they stayed for one year until harvest at 4.8kg (day 648). None of these fish were mature at sea transfer, but 9% were at harvest, with 22, 7, and 2% of the early, intermediate, and late *vgll3* genotypes being mature, respectively. When comparing the regimes, family effects outside of *vgll3* on the prevalence of sexual maturation were significant for the large-smolt regime, but not in the under-yearling regime (Figure 1C). In conclusion, i) selecting for the late *vgll3* allele is an effective method to delay puberty over a range of production regimes, ii) family effects outside of *vgll3* were not consistent across regimes, and iii) using seawater reduced the incidence of precocious puberty during land-based production.

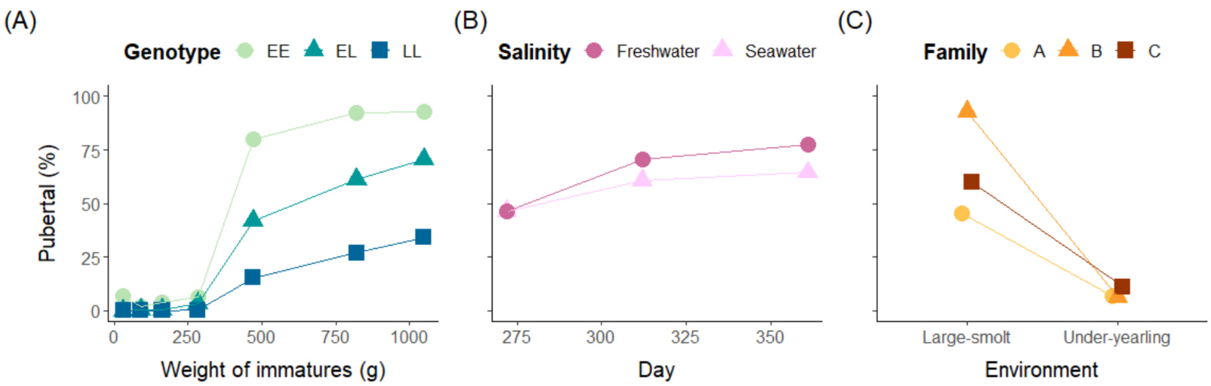

Figure 1. The incidence of puberty relating to (A) vgll3 genotype in large smolts, (B) salinity, and (C) family in different environments. Data is the mean for each group.

TEMPERATURE AND PHOTOPERIOD REGULATE POST-SMOLT MATURATION IN ATLANTIC SALMON

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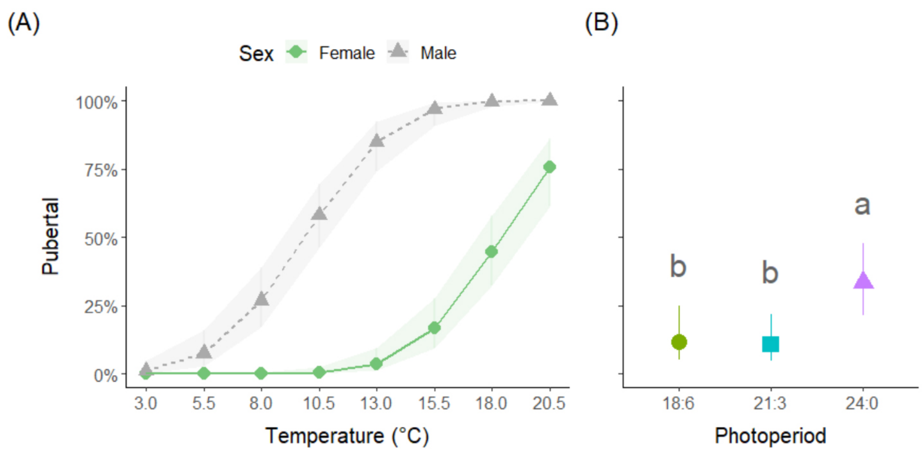
Atlantic salmon are increasing being reared on-land to larger sizes, but this is associated with an increased risk of unwanted sexual maturation. This is especially true of post-smolt maturation, a phenotype not typically observed in wild salmon. The environment plays a significant role in regulating the timing of maturation in salmon, especially temperature and photoperiod. Here, we present data from two experiments exploring the use of constant temperatures and photoperiods, methods often used in land-based facilities. In the first, we reared 200g post-smolts in seawater at one of eight temperatures ranging from 3 to 20.5°C (in increments of 2.5°C) on constant light for 140 days. The occurrence of pubertal fish was lower in females compared to males, but both increased with rearing temperature (Figure 1A). Female puberty was evident at 13.0°C and above, while male puberty was evident from 5.5°C and above. At the end of the trial, there was 75 and 100% female and male puberty, respectively, at 20.5°C. This was not associated with growth rate, as the quickest growth was found in those reared at 10.5°C. In the second experiment, 120g salmon in freshwater were exposed to a square wave (long-short-long) photoperiod to trigger smoltification (seawater adaptation). After the period of short daylength, salmon were exposed to 16°C and one of three "long" photoperiods; constant light (24:0 light/dark), 21:3, or 18:6. The idea being to see if a short scotophase could be used to impede maturation. Indicators of puberty were assessed for six weeks. At the end of the trial, the total prevalence of male puberty was 33, 10, and 12% in the 24:0, 21:3 and 18:6 treatments, respectively (Figure 1B), whereas no females showed any signs of sexual maturation. Again, the prevalence of puberty was not associated with growth rate in the different photoperiods. In conclusion, both temperature and photoperiod have a regulatory effect on the prevalence of post-smolt maturation. As such, salmon producers are advised to use lower rearing temperatures (e.g. ≤8°C) and avoid the use of constant light when rearing fish over 100g.

Figure 1. (A) The incidence of puberty in female and male Atlantic salmon after 140 days on one of eight temperatures. (B) The incidence of male puberty in Atlantic salmon after six weeks on one of three photoperiods.



THE ENVIRONMENTAL AND GENETIC REGULATION OF CATARACTS IN ATLANTIC SALMON

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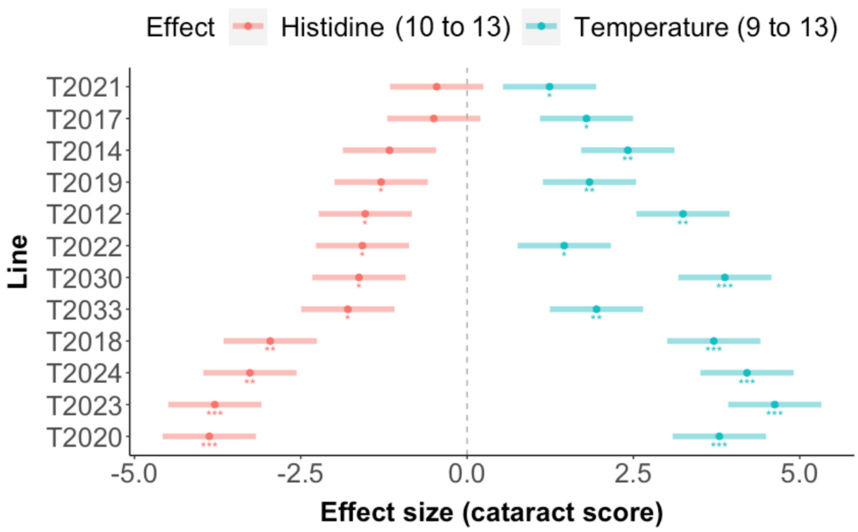
Farmed Atlantic salmon can suffer from ocular cataracts, a condition that leads to clouding of the lens which reduces vision and feed intake. Cataracts have a multifactorial etiology in salmon, and have especially been linked to higher temperatures, the dietary level of the essential amino acid histidine, and genetics (family). Here, we report results from two experiments that have used various combinations of genetic backgrounds, temperature, and dietary histidine following smoltification, a developmental stage when salmon are particularly prone to develop cataracts. In the first experiment, 12 clonal lines based on a domesticated strain were used, in combination with two seawater temperatures (9 and 13°C), and two levels of dietary histidine in seawater (10 and 13 g/kg). The dietary histidine levels were chosen based on the requirement for maximal growth (8 g/kg) and the requirement to minimize cataracts (13 g/kg). In general, high temperature and low dietary histidine elevated the risk of cataract development. However, these effects interacted with family, as some lines were more resistant to temperature and/or dietary histidine level (Figure 1). In the second experiment, we have assessed seawater temperature (9, 12, 15°C), photoperiod prior to sea transfer (continuous light versus a winter signal), body size (50g versus 110g) at the start of the winter signal, and genetics (landlocked versus anadromous strains) on the development of cataracts. The inclusion of photoperiod and size was to assess whether the smoltification regime influenced cataract development in seawater. The analysis of the second experiment is ongoing, but early results show cataracts are positively associated with temperature, but the effect size is family dependent. As in, some families are more prone to develop cataracts, irrespective of the environment. The landlocked strain had relatively low level of cataract development, whereas the farmed and wild anadromous strains had both higher and lower values than the landlocked based on family. Photoperiod prior to sea transfer had a minimal effect on cataract development.

Figure 1. The effect size of additional histidine (13 compared to 10 g/kg) and increased temperature (13 compared to 9°C) on cataract development in 12 families (Line) of salmon.



IRON-FUNCTIONALIZED CALCIUM CARBONATE NANOPARTICLES: ENHANCING LETTUCE GROWTH IN AQUAPONICS

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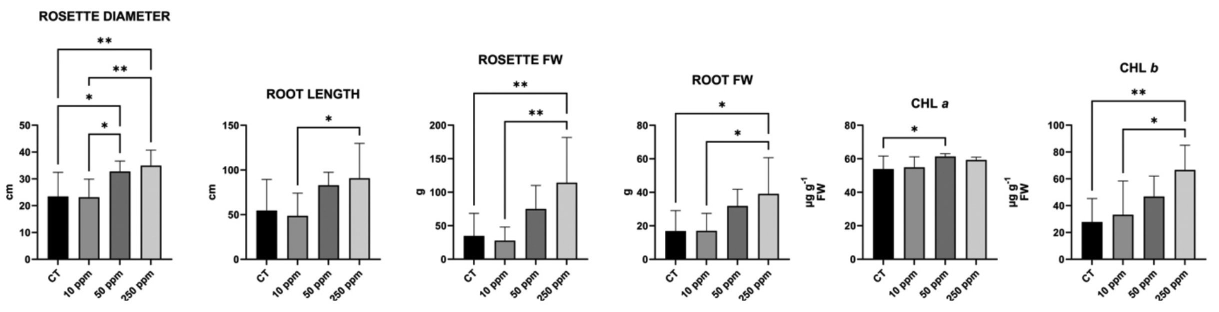
Aquaponics offers a much more sustainable alternative to traditional farming methods by combining aquaculture and hydroponic plant cultivation. In aquaponics, a recirculating water process complements the symbiotic relationship: plants absorb nutrients from fish waste, while beneficial bacteria convert ammonia into nitrates. This closed-loop system ensures water continually cycles through the environment, fostering a harmonious ecosystem where plants flourish, fish thrive, and bacteria play a vital role in nutrient cycling. This approach enhances resource efficiency and minimizes environmental impact compared to conventional agriculture. It addresses challenges such as climate change and soil degradation, providing a sustainable solution for food production. However, aquaponics still faces obstacles like micronutrient depletion, particularly iron deficiency, which can affect plant growth. Nanotechnology provides a means to surmount this challenge. The study suggests the nanoparticles (NPs) as a remedy for the lack of iron and highlights the sustainability of these NPs, given that their synthesis method occurs through an enzyme (anhydrase synthase) that use atmospheric CO_2 as substrate.

From March to July 2023, the aquaponic system employed in this study integrated the breeding of *Oreochromis niloticus* L. with the cultivation of *Lactuca sativa* L. cv. Foglia di Quercia Verde. The primary focus was to examine the effects of iron NPs, administered as a leaf spray at three concentrations: 10, 50, and 250 ppm. Various parameters were evaluated to assess the impact of the iron NPs on plant growth and physiology (Figure 1). These included measurements of rosette diameter, root length, fresh weights of rosettes and roots, soluble solids, photosynthetic pigments (chlorophyll *a*, *b*, total, and carotenoids), phenols, and flavonoids content.

Notably, the 250 ppm treatment emerged as the most effective, showing significant improvements in various parameters compared to the other concentrations. The most notable enhancements were observed in biomass production, encompassing both size and weight, as well as the synthesis of photosynthetic pigments (CHL *a* and *b*). This bears immense significance, particularly from a commercial standpoint. Hence, this study may offer fresh perspectives on the potential utilization of nanofertilizers in aquaponics, opening avenues for further exploration and application in agricultural practices.

Figure 1. Morphological parameters and photosynthetic pigments content.



AUTOMATIC VISUAL SEGMENTATION AND TRACKING OF RAINBOW TROUT *Oncorhynchus mykiss* AND ATLANTIC SALMON *Salmo salar* IN RAS ENVIRONMENT

Hilla Fred*, Mogens Agerbo Krogh, Paw Petersen, Tapio Kiuru, Laura Ruotsalainen, Matti Pastell

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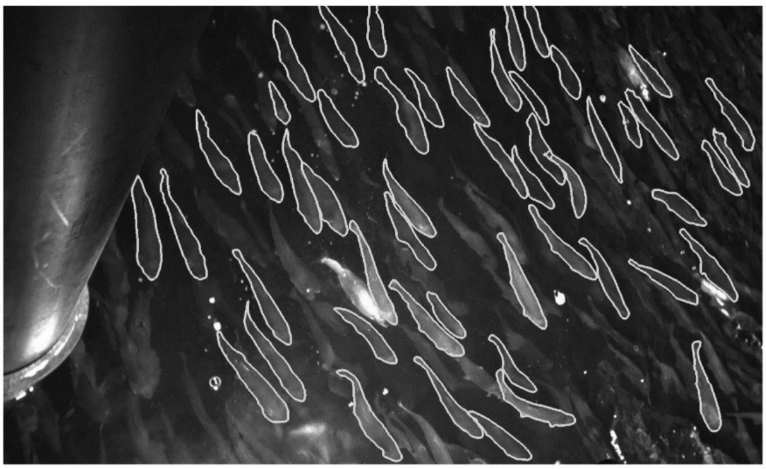
Computer vision based methods in fish farming are under active development, as automatic tracking of the animals using cameras would allow for cost-efficient monitoring and precise collection of data on fish behaviour. Methods that do not rely on costly manual annotation work are crucial for them to be applicable in real-world systems. We tried to solve this problem by leveraging the recent advances in instance segmentation foundation models, namely the state-of-the art benchmark performance reaching Segment Anything model (SAM). In instance segmentation, individual objects are identified in the image, resulting in segmentation masks that cover the pixels belonging to the corresponding objects.

To develop and test our methods on real-life data, video data was recorded on two commercial RAS farms, one farm cultivating Rainbow trout and one focusing on Atlantic salmon, using four RGB cameras positioned over the fish tanks. For the dataset, 2654 images were extracted at different times over several days' timespan to ensure a varied dataset, and the fish individuals were manually annotated in the images using bounding boxes.

We studied how we could automatically segment fish from the images without any annotations using SAM and compared the performance to using hand-annotated prompts. We find that with carefully chosen parameters the automatic segmentation can find a considerable number of good quality individual fish masks, while typically also producing more bad quality masks than the hand-annotated input. To tackle this, we propose a classifier-based post-processing method for filtering out bad quality masks using properties of the mask contours. Using the method together with a Kalman filter based tracking-by-detection algorithm, we can track the visible fish individuals to get estimates on their swimming speed and direction. We aim to show the connection of the swimming behaviour to fish health, feeding and tank environmental conditions, combining known production data to several months of video observation data.

Figure 1: Instance segmentation masks visualized on RAS farmed Rainbow trout



IMPACT OF PARTICLE LOAD ON WATER QUALITY AND MICROBIOTA IN SALMON RECIRCULATING AQUACULTURE SYSTEMS

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Recirculating aquaculture systems (RAS) are increasingly used in salmon smolt production as they enable flexible production cycles with reduced freshwater use and time in net-pens at sea. Maintaining good water quality is essential in RAS, and the microbiota is key in this. It is important to maintain a stable community of nitrifying bacteria in the biofilter, and the water microbiota can affect fish health. Small particles typically accumulate in RAS, and this can negatively impact the microbiota by facilitating growth of heterotrophic bacteria. This can be detrimental for biofilter functioning, water quality, and fish health.

In the MikroRAS study, funded by the Norwegian Seafood Research Fund, we conducted an experimental study with six identical pilot-scale RAS stocked with Atlantic salmon. Three systems were operated at high and three at low particle loads (Figure 1). We followed the systems for four months through the freshwater and brackish phases and conducted a simulated transport followed by further sampling over three months in salt-water flow through systems (simulating open sea-cages). We have utilized amplicon and shotgun sequencing to investigate the microbiota of the RAS environment (water, biofilter) and salmon (gut, gill, and skin). We relate the microbiota data to water quality metrics and particle size distribution as well as fish health and performance.

Our results show that particle load had limited impact on biofilter functioning and water quality, although we observed trends towards more favourable conditions in the low particle load RAS. The RAS operated at a low particle load had lower levels of ammonia and nitrite, likely due to nitrifying microorganisms in the biofilter facing less competition from heterotrophic bacteria. Initially, in the freshwater phase, the RAS microbiota was surprisingly unperturbed by the difference in particle load. However, after transition to brackish water, the newly established saline-tolerant microbiotas diverged strongly between the RAS with high and low particle loads. We observed minimal mortality and the fish remained healthy throughout the study. However, the salmon in RAS with low particle load trended towards increased feed intake and growth, resulting in heavier fish with a higher condition factor at the end of the RAS phase. The study ultimately aims to help optimise operational parameters in RAS, thereby improving fish welfare, cost-effectiveness, and sustainability of salmon production in RAS.

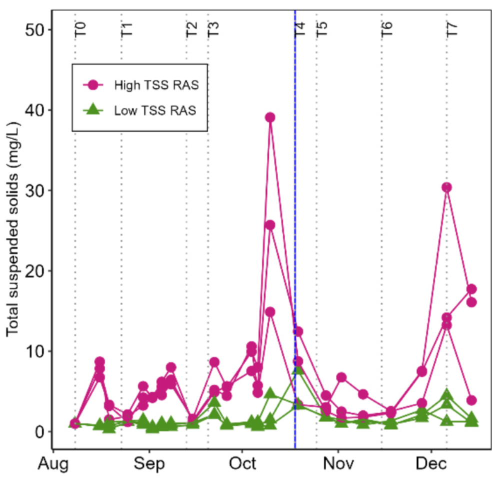

Figure 1: The amount of total suspended solids in each RAS. Microbiota sampling timepoints are shown as vertical lines. Particle load was most increased at the end of the freshwater (T4) and brackish (T7) phases.

NUCLEOTIDES IN AQUACULTURE: INSIGHTS FROM EMBRYONIC DEVELOPMENT, LARVAL GROWTH, AND MACROPHAGE ACTIVATION STATUS IN ZEBRAFISH

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Aquaculture plays a crucial role in meeting the world's growing demand for seafood. However, challenges such as disease and environmental stressors pose significant threats to the sector's productivity. As a potential strategy to improve the health and resilience of farmed fish, the use of natural supplements such as nucleotides has gained attention. Nucleotides are essential components of DNA and RNA and are involved in various physiological processes, including immune function. The aimed of this study was to investigate the effects of nucleotides on zebrafish (*Danio rerio*) early-stage development and immune system activation.

Zebrafish embryos were water-exposed to 5 different nucleotides premix concentrations (0, 5, 10, 20 and 40 mg L^{-1}), from 24 to 120 hour post fertilization (hpf). Hatching and survival rate were daily monitored. Body length (BL), swim bladder development and yolk sac size were measured at 120 hpf, and locomotion behaviour analysed. The transgenic zebrafish line *Tg(mpeg1:EGFP)* was used for *in vivo* evaluation of macrophages activation status (fig. 1A). To this purpose confocal analysis was used for counting macrophages total number (M1, pro-inflammatory; M2, pro-healing), and their relative percentage.

Results suggest that nucleotides positively affected zebrafish embryo development, leading to improved growth parameters. Fish exposed nucleotides, showed a dose dependent effect for BL (fig. 1B) and swim bladder area. Yolk sac size was lower in all the treated groups compared to the control. The percentage of M2 macrophages was higher in fish exposed to 10 mg L^{-1} of nucleotides, in comparison to control, 5 mg L^{-1} and 40 mg L^{-1} (fig. 1C). This result suggests a potential role in strengthening the fish defence mechanisms. Finally, fish exposed to 10 mg L^{-1} of nucleotides showed the best locomotor performance, while those exposed to 40 mg L^{-1} showed the worst (fig. 1D). Concluding, the use of nucleotides might enhance fish growth performances, health, and resilience against biological and environmental stressors. Further research is needed to confirm their effect when nucleotides are feed included.

A FUNCTIONAL FEED SOLUTION TO COMPENSATE FISH MEAL REDUCTION AND ASSOCIATED NUTRITIONAL STRESS IN SEABREAM *Sparus aurata*

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Aquaculture production has undergone remarkable growth during the past few decades, and it will continue to rise in the coming years to meet higher demand for safe and healthy seafood products.

For economic, environmental and societal reasons, a lower usage of finite marine-harvested resources has been a major trend in the aquafeed industry. Grain and oilseed by-products are promising sources of protein and energy for aquaculture feeds. However, high dietary inclusion levels of plant proteins have often been associated to detrimental effects on growth.

In the scope of this sustainable approach, Mixscience (France) developed a specific Phytogenics based solution that helps to reduce the negative impact of challenging feeds.

The aim of this study is to examine the impact of this new solution on the zootechnical performance, nutrient digestibility, intestinal mucosal health and stress resilience in gilthead seabream (*Sparus aurata*) challenged by a nutritional stress and some additional environmental stress.

The Seabream trial includes 3 treatments.

- A positive control **(PC)** = optimal diet for the targeted fish
- A negative control **(NC)** with lower levels of digestible protein and digestible energy due to fish meal replacement by challenging ingredients derived from defatted soybean meal and sunflower meal, which tend to reduce digestibility and negatively affect intestinal integrity.
- An additional diet, based on the NC formula, supplemented with the tested product **(PHY)** at a dose of 200 g/tonne of feed

The growth assay includes two distinct experimental phases. During the first 62 days, the fish are raised under normal breeding conditions to allow optimal zootechnical performance.

Subsequently, the fish are subjected to stressful rearing conditions for 12 days, which involve a combination of a high density stress for 10 days and 2 additional days under oxygen reduction. Apparent nutrient digestibility and stress response are measured alongside the performance trial.

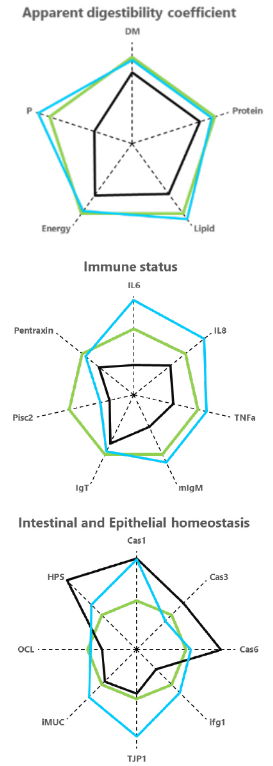
The results clearly indicate that in seabream, a challenging food (NC) induces negative metabolic reactions: reduction of overall immune status, reduction of antioxidant defenses, degradation of intestinal homeostasis with particular deterioration of intestinal morphology **(Figure1).** This outcomes in lower feed efficiency, and performance of fish **(Table 1).** The negative impact of a challenging feed can be compensated by specific phytogenics that activate key metabolic activities to save energy and maintain overall resistance that limits dysbiosis and intestinal disorders under stressful conditions and helps maintain a healthy optimal feeding efficiency and an overall fitness of the host.

Table 1. Growth performance after **74 days** of feeding

	PC	NC	*PHY*
Survival, %	99.4	100.0	99.4
Initial Body Weight, g	41.3	41.4	41.3
Final Body Weight, g	106.8c	95.0a	102.3b
Weight gain, g/fish	65.6c	53.6a	61.0b
Feed Conversion Rate	1.13a	1.36b	1.16a
Feed Intake, %BW/day	1.53a	1.62b	1.49a
Protein Efficiency Ratio	2.01b	1.67a	1.96b

Values are means ± standard deviation (n=4).
Values within a row with different superscripts, denote a statistical difference (P<0.05).

Figure 1. Results of the relative overall activity of PHY (in blue) on immune response, intestinal and epithelial homeostasis and apparent nutrient digestibility compared to the negative control (NC in black) and the positive control (PC in green)



nEW FEED SOLUTION TO ALLOW A FISH MEAL REDUCTION IN AQUAFEEDS - EXAMPLE WITH SEABREAM *Sparus aurata*

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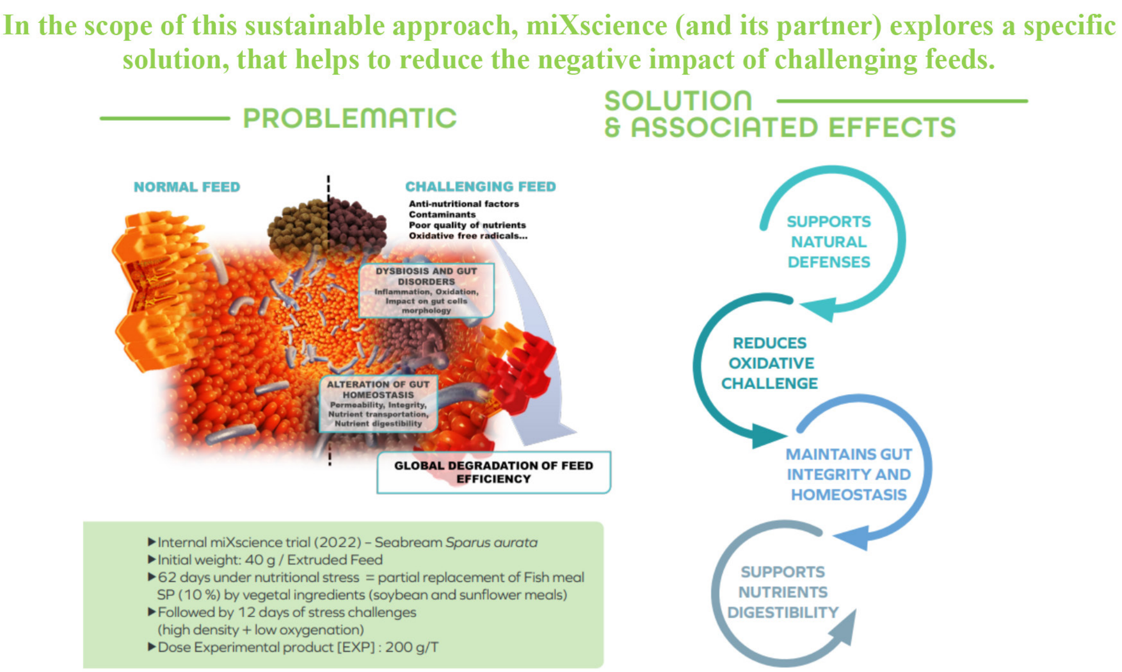
For economic, environmental and societal reasons, a lower usage of finite marine-harvested resources has been a major trend in the aquafeed industry. Grain and oilseed by-products are promising sources of protein and energy for aquaculture feeds.

However, high dietary inclusion levels of plant proteins can be challenging since they are often been associated to detrimental effects on growth, feed efficiency, intestinal dysbiosis and immune response, threatening the profitability of major aquaculture species.

In the scope of this sustainable approach, miXscience (and its partner) explores a specific solution, that helps to reduce the negative impact of challenging feeds.

The present study confirms that dietary supplementation with this new feed solution is an effective strategy to maintain the zootechnical performance of gilthead sea bream under conditions of dietary and environmental stress.



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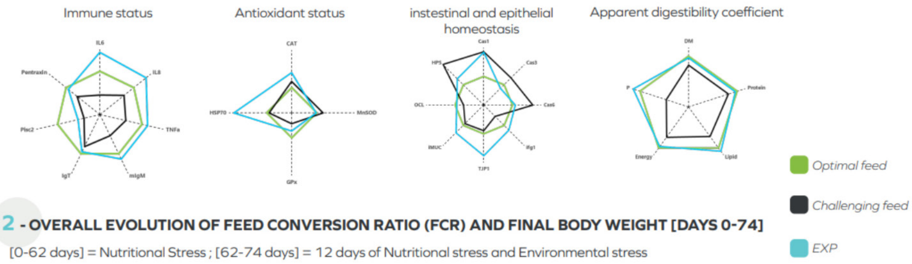
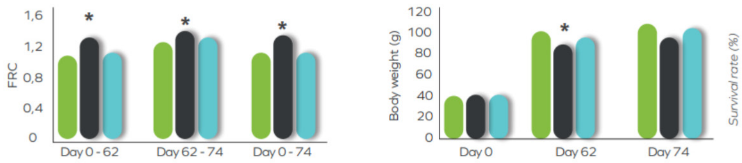
KEY RESULTS

1 - RELATIVE STRESS RESPONSE OF SEABREAM UNDER NUTRITIONAL CHALLENGE



2 - OVERALL EVOLUTION OF FEED CONVERSION RATIO (FCR) AND FINAL BODY WEIGHT [DAYS 0-74]

[0-62 days] = Nutritional Stress ; [62-74 days] = 12 days of Nutritional stress and Environmental stress



IN CHALLENGING CONDITIONS, THE PRODUCT :

1 - Activates key metabolic activities to save energy and maintain an overall resistance that limits dysbiosis and gut disorders under stressful conditions

2 - Helps to maintain an optimal feed efficiency and global host's fitness

A GUT HEALTH ENHANCER TO IMPROVE RESISTANCE OF AQUATIC FARMED SPECIES AGAINST PATHOGENIC PRESSURE

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Aquaculture industry is highly challenged by diseases outbreaks, introduction and spread of specific new pathogens, having significant impacts on the economics of the farms and on animal performance.

In recent years, chemicals like antibiotics have been chosen as first solutions, but their massive use is now a high public health concern.

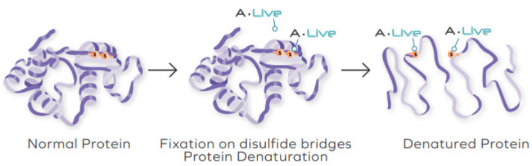
We concluded that this feed solution provides an efficient control against a large variety of pathogens and could be considered as an universal and sustainable way of reducing the use of antibiotic in aquaculture systems.

In the scope of its sustainable approach, miXscience (France) developed a specific solution called A-Live, based on plant products, with a wide spectra of actions, that helps to control the pathogenic pressure in all aquaculture farming systems.

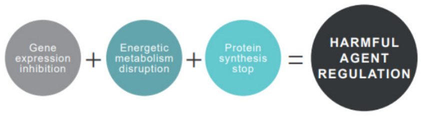
MODES OF ACTIONS

The ubiquitous efficacy of this product is linked to its universal mode of action.

It acts on denaturation of proteins, the main component of microbial pathogens…



… that induces the following cascade effect:



KEY RESULTS FROM LAB TO FIELD

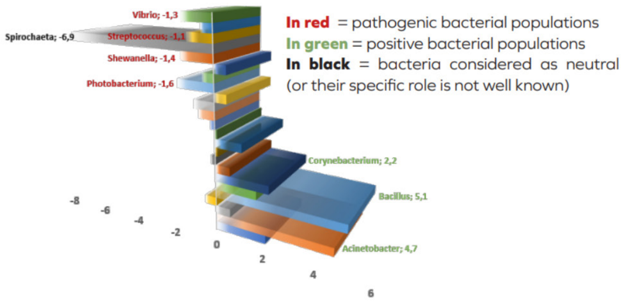
1 - COMPARATIVE INHIBITION OF MICROORGANISMS VISIBLE GROWTH

Internal miXscience trials (2016) based on MIC (ppm)

	A·Live	Competitor A	Competitor B	Competitor C
Aeromonas salmonica	+++	+++	++	-
Edwardsiella tarda	++	-	+	-
Photobacterium damsela	+++	+	-	-
Streptococcus iniae	++	+	-	-
Tenacibaculum maritimum	+++	+++	+++	-
Tenacibaculum discolor	+++	+++	++	-
Vibrio harveyi	+++	-	-	-
Vibrio alginolyticus	++	-	-	-
Vibrio anguillarum	+++	+	-	-
Yersinia ruckeri	+++	-	+	-

2 - EXAMPLE OF THE RELATIVE % OF VARIATION OF THE GUT MICROBIOME OBSERVED IN SEABREAM AFTER 90 DAYS

A-Live vs Control at the end of the trial
External miXscience trials (2019) run in Greece * p<0.05



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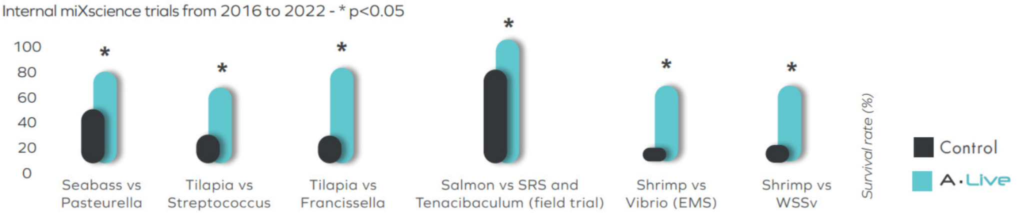
3 - COMPARISON OF SURVIVAL (%) WITH DIFFERENT AQUATIC SPECIES AND THEIR ASSOCIATED PATHOGENS

Internal miXscience trials from 2016 to 2022 - * $p<0.05$



AT LAB AND FIELD SCALES, THE PRODUCT:

1 - Regulates a wide range of harmful agents

2 - Positively modulates the gut microflora by increasing its biodiversity and abundance and by reducing the negative populations

3 - Contributes to improve global resistance of different aquatic species under challenging conditions

STABILITY OF CYNATRATOSIDE-C: AN ANTIPARASITIC COMPOUND AGAINST *Ichthyophthirius multifiliis*

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Cynatratoside-C (CyC), a C-21 steroidal saponin isolated from *Cynanchum atratum*, is a promising candidate for treating freshwater fish infected with the ciliated parasite *Ichthyophthirius multifiliis*. To promote the drug development process of CyC, the present study investigated the stability of CyC under heating, sterile water, and aquaculture water treatments. An accelerated stability test demonstrated that the thermal degradation kinetics followed a first-order reaction with a predicted half-life ($t_{0.5}$) of 3472 days (9.5 years) at a temperature of 25°C. According to the Arrhenius model, the activation energy Ea of CyC was calculated as 91.7 kJ/mol. When CyC was stored in sterile water for 3 years, the mortality rates of theronts and nonencysted tomonts were decreased significantly in the low CyC concentration treatment group, and the minimum concentration that inhibited the production of encysted tomonts increased from 0.06 mg/L to 0.125 mg/L. When CyC was stored in sterile water for 6 years, it showed a nearly complete loss of antiparasitic efficacy, indicating that water may promote the degradation of CyC. When CyC was stored in aquaculture water, the antiparasitic efficacy of CyC against theronts and tomont was decreased significantly at 2 days post-storage, indicating that microorganisms in aquaculture water could absorb and degrade CyC. The present study provides important information concerning the stability of CyC and the drug approval process.

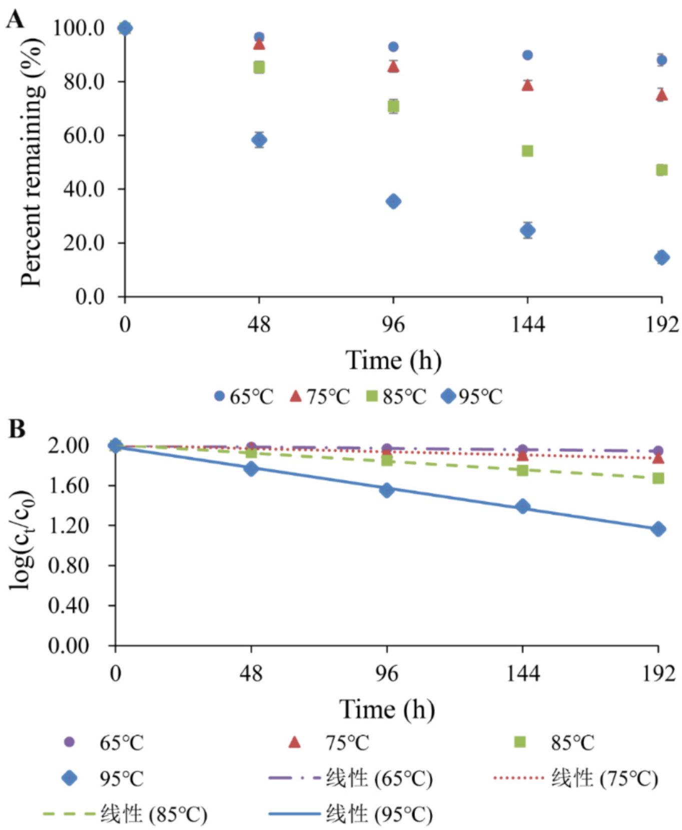

Figure 1. Content changes and degradation kinetics of CyC at temperatures from 65°C to 95°C. (A) The value of each point was calculated as the peak area of CyC at time *t*/peak area of CyC at the initial point × 100%. (B) First-order degradation regression plots of CyC at different temperatures.

SUPPLEMENTING A LOW-FISH MEAL DIET WITH FISH MEAL SOLUBLE FRACTION IMPROVES GROWTH PERFORMANCE AND DIGESTION IN YELLOWTAIL *Seriola quinqueradiata*

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Fish meal (FM) is a key protein source in fish feed. Diets for yellowtail *Seriola quinqueradiata*, a carnivorous fish, typically contain high levels of FM. Reducing the FM content can result in low feeding amounts and hindered growth. This study aimed to investigate the function of FM by utilizing a soy protein concentrate (SPC)-based diet supplemented with a water-soluble fraction of FM (FMS).

Four experimental diets were formulated: an FM-based diet (FM diet), SPC diet, SPC diet supplemented with the water-soluble fraction of Chilian jack mackerel meal (S+JM diet), and SPC diet supplemented with the water-soluble fraction of Peruvian anchovy meal (S+A diet). Yellowtail (mean body weight = 40.0 g) were divided into 12 tanks (15 fish per tank in triplicate). The fish were fed the experimental diets once daily for eight weeks, and growth performance was assessed. At eight weeks, the hypothalamus and pyloric ceca were collected from three fish pre-feeding and three fish post-feeding (3 h) per tank. The gene expression of orexigenic hormones (neuropeptide Y [NPY] and agouti-related protein 1 [AgRP1]) in the hypothalamus, as well as digestive hormones (cholecystokinin [CCK] 1 and 2) and digestive enzymes (trypsin and lipase) in the pyloric ceca, were quantified using RT-qPCR.

Growth performance (Fig. 1): Differences in body weight were observed among the groups at eight weeks in the following order: S+JM group > S+A group > SPC group > FM group. Gene expression in the hypothalamus: A significant increase in *npy* and *agrp1* expression was observed in the S+JM and S+A groups from pre- to post-feeding. Gene expression in the pyloric ceca: A significant increase in *cck1* and *lipase* levels was observed in the S+JM and S+A groups from pre- to post-feeding.

FMS stimulates physiological responses, leading to improved growth in yellowtail. Identifying the effective substances in FMS could facilitate the development of improved low-FM diets for carnivorous fishes.

This work was supported by a Grant-in-Aid for JSPS Fellows (21J23499 and JSPS KAKENHI JP21H02286) and a JST Grant (JPMJPF2117).

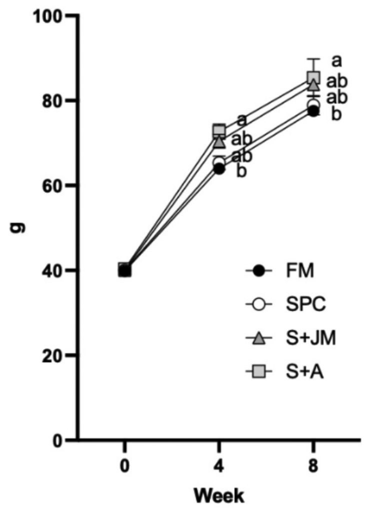

Fig.1 Changes in average body weight (mean ± standard error, $n = 3$). Different letters indicate significant differences in two-way ANOVA followed by Tukey's multiple comparison tests ($p < 0.05$).

AQUAPONIC PRODUCTION OF ASIAN SEABASS *Lates calcarifer* AND CHERRY TOMATO *Solanum lycopersicum* IN FLOATING VERSUS MEDIA-BASED SYSTEMS

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According to the European Parliamentary Research Service (EPRS), aquaponics is one of the ten technologies that could change our lives. Since aquaponics uses a recirculating system, the amount of water used is significantly reduced compared to traditional methods of agriculture and aquaculture, and wastewater (containing nutrients) is not discharged into the environment but utilized for growing plants in the system. However, aquaponics can be profitable only if the species grown in the system have high commercial value. There is continuing research to test several species for growing in specific types of aquaponic systems. In previous experiments, we successfully produced Nile tilapia (*Oreochromis niloticus*) together with several plant species.

The experiment aimed to determine the most suitable type of aquaponic system for producing Asian seabass (*Lates calcarifer*) and cherry tomato (*Solanum lycopersicum*), which are considered more profitable compared to other species commonly grown in aquaponic systems. Two types of aquaponic systems were compared: floating and media-based (hydrostones) systems, both made of fiberglass tanks and equipped with sedimentation and biofiltration tanks. The dimensions of the fish tanks were 90 x 60 x 50 cm, and the plant tanks were 90 x 60 x 30 cm. Four replicate tanks were used for each type of aquaponic system.

Asian seabass, with an initial body weight of 0.5 g, were fed a commercial feed at 5% of body weight, later reduced to 3% of body weight. Growth and survival rates of Asian seabass were found to be similar in both aquaponic systems, with an average body weight of 271 g and an average survival rate of 82% after 12 months.

Cherry tomatoes were grown in two batches over 12 months. In the first batch, each plant tank contained one tomato plant, while in the second batch, each plant tank contained two tomato plants. The number of cherry tomato fruits was higher in the media-based system (306 fruits per plant) compared to the floating system (174 fruits per plant).

While temperature, dissolved oxygen, and pH did not differ significantly between the two systems, ammonia levels were higher in the floating system, indicating less efficient nitrification and production of nitrates to support tomato fruit production.

In conclusion, the study suggests that the media-based aquaponic system is more suitable for the production of cherry tomato fruits, alongside Asian seabass. This system demonstrated higher fruit yields per plant compared to the floating system, likely due to better nutrient availability facilitated by efficient nitrification processes.

CHARACTERIZATION OF IMMUNE PARAMETERS IN ACELLULAR AND CELLULAR FRACTION IN MALE AND FEMALE OF ATLANTIC BLUE CRAB (*Callinectes sapidus*)

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Despite their economic significance, brachyurans are relatively understudied among invertebrate groups compared to other decapods, with numerous fields remaining to be explored, including the immune response. Therefore, the aim of this study was to characterize several immune-related enzymes and activities of the Atlantic blue crab (*Callinectes sapidus*) in cell-free haemolymph (CFH) and in the haemocyte lysate supernatant (HLS), considering the gender factor and the suitability of measuring each activity in CFH or HLS. Data were expressed by millilitres of haemolymph or milligrams of protein to identify which of these units would be more robust and informative. For this, haemolymph from crabs of each gender was collected, and centrifuged to obtain CFH samples, whilst the cells were sonicated to get the HLS. Our results revealed that the protein concentration was higher in CFH compared to HLS. Considering milligrams of protein as units, phenoloxidase and esterase activities were lower in CFH regarding to HLS in both genders. Similarly, the lysozyme and phosphatase activities were lower in CFH compared to HLS but only in females. The values of lysozyme activity were higher in HLS of females compared to males. Studying millilitre of haemolymph as units, lysozyme activity exhibited higher activity in CFH compared to HLS in both genders, whilst phenoloxidase and esterase activities were higher in HLS, showing variations only in males. Interestingly, alkaline phosphatase activity was undetected in CFH male. Any variation was observed between the activities measured in both genders. Protease and antiprotease activities were higher and lower in CFH compared to HLS values, respectively, whilst any variation was observed between genders. Bactericidal activity against *V. harveyi*, *V. alginolyticus* and *V. anguillarum,* exhibited stronger values in HLS than in CFH, independently of gender, as well as against *P. damselae* in females. Considering gender factor, the bactericidal activity against *V. alginolyticus*, *V. anguillarum* and *P. damselae* was higher in HLS of females than in males. However, the bactericidal activity against *V. alginolyticus* and *P. damselae* was higher in CFH of males compared to females. Our results could contribute to gender selection in future brachyurans studies considering the variability between males and females due to their distinct biology. Additionally, we provide data regarding the baseline values of several immune-related activities, establishing the most appropriate units from the biological point of view.

IMPACT OF DIFFERENT STOCKING DENSITIES ON GROWTH PERFORMANCE AND WELFARE OF *Penaeus vannamei* IN RAS

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Introduction and methodology

Despite the widespread farming of *Penaeus vannamei*, there remains a lack of recognition and assessment of welfare standards and chronic stress markers (David et al., 2020; Wuertz et al., 2023). This study adopts a holistic approach to elucidate the effects of different stocking densities on *P. vannamei.*

In a RAS three different stocking densities were tested in replicate: low stocking density (1 kg/m^2), medium (2 kg/m^2) and high (4 kg/m^2). A stress phase of 21 days is followed by a recovery phase of 21 days at a low density (0.8 kg/m^2). After every phase growth, hemolymph parameters and morphological status in 4 grades (0 = no abnormalities and 3 = severely impacted) were evaluated. Further an ethogram of stress-related behaviours including cannibalism, swimming abnormalities, loss of balance, body cramps and strong escape behaviour was created.

Results and Conclusions

High stocking densities have a negative impact on survival and growth performances (Tab. 1). However, recovery and partial compensatory growth are observed in the second phase.

Furthermore, physiological parameters in hemolymph (glucose and total proteins) do not exhibit significant variations related to the treatments in both phases (Tab. 1).

Notably, antennae and uropods sustain significant damage under higher density conditions (Fig. 2), likely due to cannibalism and abrasions caused by limited space. Rehabilitation occurs within three weeks, with no discernible differences among treatments.

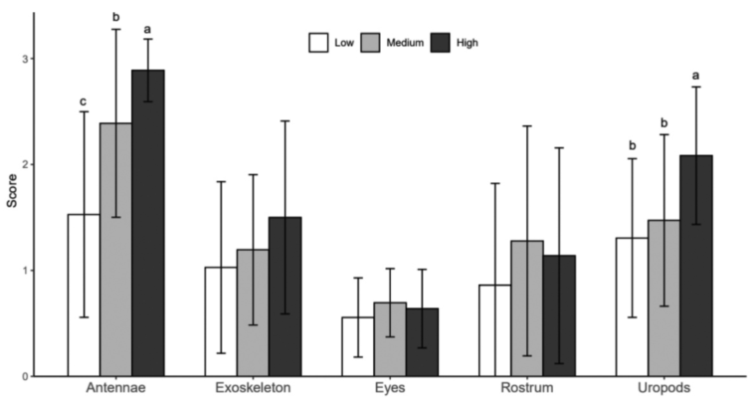
In the mixed model analysis (p-value < 0.001), treatments influence the frequency of stress-related behaviour occurrences, while time does not. On the contrary, the recovery phase does not show differences among treatments and trends over time. This investigation evidences the effect of stocking density on *P.vannamei* and its capacity to recover promptly when the stressor is removed. The results can support the optimisation of farm practice in aquaculture.

Table 2. Survival, growth performance and hemolymph parameters after 21 days under three stocking densities and after 21 days of recovery. Mean ± SD.

	Stress phase			Recovery phase		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**
Survival (%)	97.95^a±3.21	87.61^b±4.10	64.19^c±4.15	97.04±3.39	97.65±3.57	95.91±2.76
Initial weight (g)	14.87±0.41	14.89±0.22	14.45±0.32	18.18^a±0.79	16.58^b±0.47	15.83^b±0.63
Final weight (g)	18.18^a±0.79	16.58^b±0.47	15.83^b±0.63	20.95^a±1.03	19.23^b±0.69	19.21^{ab}±0.68
RWG (%)	0.22^a±0.03	0.11^b±0.04	0.09^b±0.04	0.15^b±0.02	0.16^b±0.03	0.21^a±0.01
DWG (g.day^{-1})	0.16^a±0.02	0.08^b±0.03	0.06^b±0.03	0.13±0.02	0.13±0.02	0.16±0.01
SGR (%)	0.95^a±0.10	0.51^b±0.18	0.40^b±0.17	0.67^b±0.09	0.71^{ab}±0.13	0.92^a±0.04
Glucose (mg/dL)	49.83±8.67	46.33±10.12	45.33±15.94	53.33±8.58	50.67±11.03	54.22±9.13
T. Proteins (g/dL)	15.02±1.67	13.33±1.67	13.26±2.79	15.96±1.52	15.33±2.43	15.93±1.53

RGW = Relative weight gain; DWG = Daily weight gain; SGR = Specific growth rate

Figure 1. Morphological status of *L.vannamei* after 21 days at different stocking density in RAS. N=36, mean ± SD.



References

1. David, P.N.F., Manipol, N.E.P.; Madamba, J.A.B.; Mariano, R.A (2020). Good Aquaculture Practices Adoption and Certification of Shrimp Aquaculture Farms in Bulacan, Philippines: Status, Issues and Prospects. J Glob Bus Trade 2020, 15, 11–26.
2. Weiss, M., Rebelein, A., & Slater, M. J. (2020). Lupin kernel meal as fishmeal replacement in formulated feeds for the Whiteleg Shrimp (Litopenaeus vannamei). *Aquaculture Nutrition*, *26*(3), 752-762. https://doi.org/10.1111/anu.13034.
3. Wuertz, S., Bierbach, D., & Bögner, M. (2023). Welfare of Decapod Crustaceans with Special Emphasis on Stress Physiology. *Aquaculture Research*, *2023*.

GREEN MACROALGAE *ULVA* EXTRACTS MODULATE RAINBOW TROUT IMMUNE RESPONSES AND GROWTH PERFORMANCE *IN VIVO*

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Introduction

A group of potentially interesting immunomodulatory feed additives are marine sulphated polysaccharides (MSP) isolated from cell wall matrices of marine macroalgae (Mohan et al., 2019). MSP are highly complex sulphated polysaccharides, named ulvans when extracted from green macroalgae. Previously, the effects of different extracts from green macroalgae (*Ulva* sp.) and red macroalgae (*Solieria* sp.) both rich in MSP, were studied for direct antibacterial properties and induction of innate immune activity *in vitro*. Inhibition of bacterial growth, induction of reactive oxygen species (ROS), nitric oxide (NO) and immune gene expression were studied in primary head kidney leukocytes as read-out parameters in rainbow trout and Nile tilapia (Petit et al., 2024). The earlier *in vitro* studies led to a *in vivo* study in which the effects of green algae (*Ulva* sp.) derived MSP-rich extracts on fish health and growth performance were explored.

Materials and methods

Rainbow trout (*Oncorhynchus mykiss*) were reared at 14.6–16.2 °C, with a 12-12h light-dark cycle. The fish were fed a trout specific research diet twice per day. Prior to the start of the experiment, fish were starved for 24 hours, caught from a common batch and randomly distributed over three different dietary treatments: Control, 3 g kg-1 of crude processed *Ulva sp*. concentrate (UC) and 3 g kg-1 of crude processed and heat-treated *Ulva sp*. concentrate (2 hours at 80 °C) (UC-T). Fish were randomly distributed in four tanks per dietary treatment at a stocking density of 30 fish per tank. The duration of the feeding trial was 42 days.

On day 0, fish were batch weighed and 10 fish per dietary treatment were sampled for proximal analysis and 5 fish per dietary treatment for baseline gene expression of pro-inflammatory cytokines: tumour necrosis factor (TNF**α**), interleukin-1 (IL1b), interleukin-12 (IL12p40) and type II interferon (IFN**γ**); the anti-inflammatory cytokine interleukin-10 (IL10), and two tight junction genes; occludin and marvelD2 (Tricellulin). On day 42, fish were batch weighed and 5 fish/tank were sampled for proximal analysis and 2 fish/tank sampled for gene expression of cytokines and tight junctions.

On day 48, 5 fish per dietary treatment were intra-peritoneally injected with 200 **µl** PBS or 200 **µl** of the viral mimic synthetic double-stranded RNA (Poly (I:C), P1530 Sigma-Aldrich) at 5 **µg**/gram fish, under mild sedation. At 24h post-injection, fish were euthanized, and posterior intestines were collected and stored in RNAlater at -20°C until RNA isolation for gene expression analysis of cytokines, tight junction and antiviral associated genes (*viperin* and *pkr*).

Total RNA from posterior intestinal tissue were stored at □80°C. Prior to cDNA synthesis, total RNA was treated with DNase I, Amplification Grade (Invitrogen), and cDNA was synthesized using random primers (300 ng) and Superscript III First-Strand Synthesis for RT-PCR (Invitrogen). 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 (Thermo Scientific) in a Rotor-Gene Q (Qiagen), and fluorescence data were analysed using Rotor-Gene Analysis software version 1.7. The relative expression ratio (R) of each sample was calculated according to the Pfaffl method based on the take-off deviation of sample versus each of the PBS controls and normalized relative to *elongation factor 1□* (*elf1□*) as reference gene.

A one-way ANOVA was used to test for significance the effects of dietary supplementation with MSP-rich extracts from green (*Ulva sp*) algae on growth performance and apparent digestibility. When significant effects were found, results were checked with a Tukey HSD test. Gene expression data presented as fold-changes were transformed with natural logarithm. 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 one-way ANOVA, followed by a least significant difference (LSD) post hoc. All statistical analysis was performed in IBM SPSS statistical data editor version 26.

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Results

Rainbow trout fed with diets with MSP-rich extracts (UC and UC-T) showed no significant differences in terms of growth performance and apparent digestibility when compared to the control group. On day 42, significant differences in baseline gene expression coding for cytokines (TNF□, IL*1b*, IL*10*, IL*12p40*, IFN□) and tight junction proteins (*occludin* and *marvelD2*) were observed in both UC- and UC-T-enriched diets when compared to the control group but differed in the extent of their effect. Moreover, the immune challenge with poly (I:C) triggered a modulation of the gene expression of cytokines in diets with MSP-rich extracts (UC and UC-T). The expression of antiviral related genes *viperin* and *pkr* was significantly up-regulated in control group and groups fed with MSP-rich extracts (UC AND UC-T). MSP thermal processing during extraction had some effect on the parameters for immune modulation, but not on growth performance.

Conclusion

Overall, in this *in vivo* study, both, maintenance of growth performance and modulation of the immune responses suggest that MSP-rich extracts derived from green algae (*Ulva sp*.) can modulate physiological responses of rainbow trout.

EFFECTS OF BUTYRATE AND TAURINE SUPPLEMENTATION ON GROWTH PERFORMANCE AND GUT MICROBIOTA COMPOSITION IN GILTHEAD SEABREAM (*Sparus aurata*) FED FISHMEAL FREE DIETS

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Feed additives are emerging as a suitable strategy to mitigate the potential negative effects of low fishmeal (FM) diets on fish growth and gut health. Dietary butyrate and taurine supplementation can reverse, at least partially, some of these drawback effects and alter the gut microbiota composition. However, their effects are underexplored in gilthead seabream (*Sparus aurata*) and other farmed fish. Thus, this study aimed to evaluate the potential benefits of butyrate and taurine dietary supplementation on fish performance and gut microbiota composition of gilthead seabream juveniles after 10 weeks of feeding and after crowding stressor exposure. To this aim, juveniles (~11 g) were fed with an FM-based diet (CTRL) or FM-free diet without supplementation (NOFM), supplemented with 0.1% butyrate alone (BUT), or with 0.1% butyrate plus 0.5% taurine (BUTTAU). After the nutritional trial, fish were exposed to a crowing stress for 10 days with a two-fold increase in rearing density. After the nutritional trial and after the stressor event, DNA from mucosal adherent bacteria from the anterior intestine was collected for the amplification and sequencing of 16 rRNA V1-V3 regions, using the Oxford Nanopore MinION. The best growth performance was achieved in fish fed the BUTTAU diet compared with CTRL, NOFM and BUT fish groups. In agreement with this, Partial Least Square-Discriminant Analysis (PLS-DA) of gut microbiota clearly separated NOFM fish, clustering together CTRL, BUT and BUTTAU fish with a high level of confidence (pR2Y=0.002; pQ2=0.002), explaining the two first components more than 96% of the total variance. Such separation was driven by 222 discriminant taxa (VIPs $\geq$ 1) that comprised 9 abundant genera (at least 1% in one of the groups). Seven out of nine, including *Photobacterium* and *Shewanella*, were over-represented in NOFM fish and decreased in BUT and BUTTAU fish. The other two abundant discriminant taxa (*Enterobacter, Escherichia*) decreased with the FM replacement and the BUT supplementation but increased again in BUTTAU fish. The key role of *Enterobacter* was depicted also by Bayesian network clustering, emerging as a pivotal parent node. On the other hand, it is of relevance that most of the gut microbiota changes driven by the matrix diet (CTRL vs NOFM) persisted after the stressor challenge, but the reshaping role of supplemented diets was transient, and it mostly disappeared after 10 days of crowing exposure. Altogether, the results reflect the important driving role of the environment upon gut microbiota, reinforcing the dynamic nutrition, environment, and microbiota interactions in farmed fish.

This work was supported by the TNA programme (PID:26681) within H2020 AQUAEXCEL3.0 project (871108) to I. García-Gallego for accessing to IATS-CSIC facilities, H2020 Marie Sk□odowska-Curie ITN Programme (EATFISH; 956697), and by the Portuguese Foundation for Science and Technology (Ministry of Science and Higher Education, Portugal) through UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020 to CCMAR and contract DL57/2016/CP1361/CT0033 to CA. This abstract reflects the views only of the authors, and the European Union cannot be held responsible for any use which may be made of the information it contains.

ANTIVIRAL ACTIVITY OF PLANT EXTRACTS AGAINST REDSPOTTED GROUPER NERVOUS NECROSIS VIRUS

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In aquaculture, viruses are one of the major concern related to infectious diseases, since they cause high economic losses. Therefore, the search for new antiviral compounds to prevent or mitigate these losses is of great importance. In this work, we evaluated the antiviral activity of four plant extracts: *Vitis vinifera, Rosmarinus officinalis, Glycyrrhiza glabra* and *Punica Granatum*, to search for new antiviral compounds, since the use of plant extracts as antimicrobials is known to have certain benefits, for example, they do not contaminate the aquatic environment and generally do not produce viral resistance. Therefore, we evaluated the antiviral activity of these plant extracts against red spotted grouper nervous necrosis virus (RGNNV). This virus is one of the most important viruses in aquaculture, as it affects a large number of wild and farmed fish species and is distributed globally.

This work was carried out using the E-11 cell line (clone of SNN-1 (striped snakehead whole fry cells)), since it is a cell line highly susceptible to RGNNV. As mentioned above, *Vitis vinifera, Rosmarinus officinalis, Glycyrrhiza glabra* and *Punica Granatum* plant extracts were chosen because of their potential antiviral capacity. For that, the working concentrations of the extracts were determined by a cytotoxicity assay. Then, the evaluation of the antiviral activity was performed as follows. First, the antiviral capacity of the plant extracts was evaluated by pretreating the cell monolayer with the extracts 24 hours prior to infection. Second, the virucidal capacity of the extracts was evaluated by pretreating the virus for 2 hours before infection. Finally, the antiviral activity of the extracts was evaluated by treating the cells during the course of infection, 2 hours after infection. The viral load was analyzed by RT-qPCR by means of the expression of RGNNV polymerase (RdRP). The results obtained showed the high antiviral activity of the plant extracts, some of them reducing up to 90% the expression of RdRP. These results demonstrated the potential of these extracts as antivirals against RGNNV. The final aim of this work will be to include these extracts in animal feed to reduce or mitigate the effect of this virus in aquaculture.

Acknowledgements: This study was funded by ThinkInAzul programme, GVA-THINKINAZUL/2021/020, supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.1) and by Generalitat Valenciana.

PARTICIPATION AND INFLUENCE OF WOMEN IN SEAFOOD SYSTEMS: INSIGHTS USING THE FISHERY AND AQUACULTURE PERFORMANCE INDICATORS

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Women play diverse roles within the global seafood system, and organizations globally have called for improved understanding of their contribution and influence to support gender-explicit policy. This paper uses a unique dataset of 150 fishery and 57 aquaculture case studies to compare gender participation and influence in global seafood systems and examine the relationship between gender participation and socioeconomic and environmental sustainability outcomes. Nearshore fisheries and aquaculture, such as bivalves, crabs, and seaweeds, have facilitated greater participation of women in the seafood sector; however, influence of women in decision-making is not equitable across countries. Wages and access to community services was also low in seafood sectors where women participation was high, suggesting inequities exist.

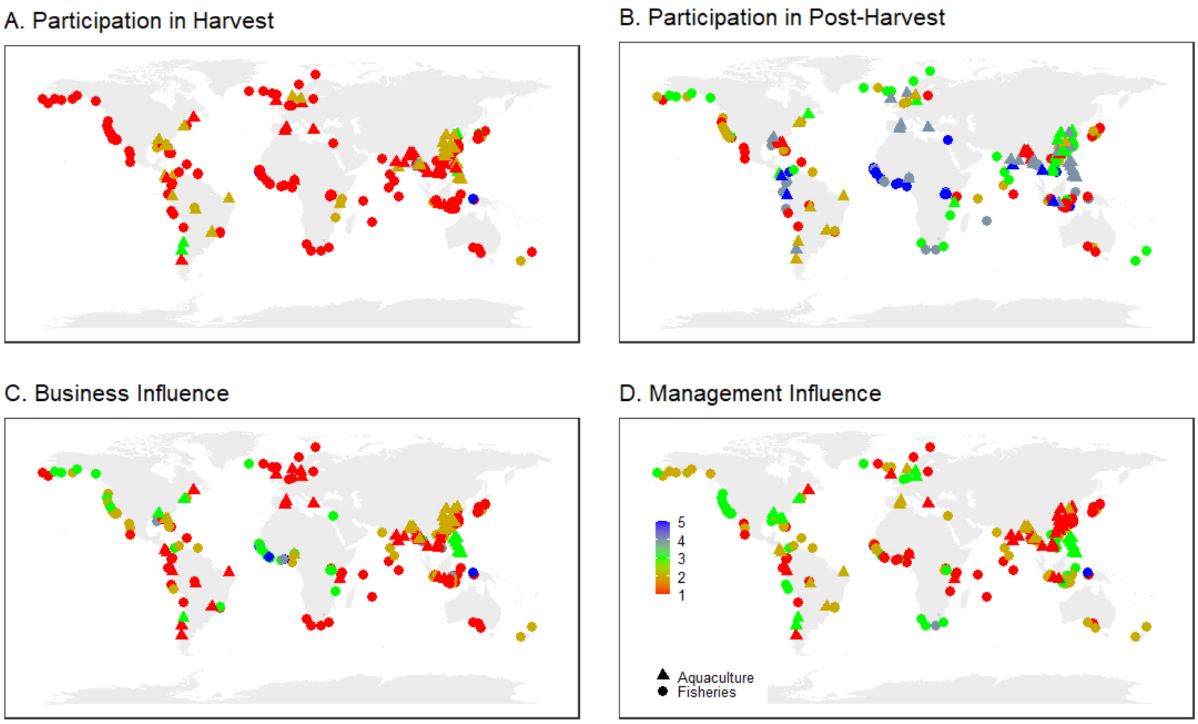

Figure 1. Gender participation and influence in fishing and aquaculture industries. All panels use a 1-5 scale, where 1=dominated by men, 3=balance between men and women, and 5=dominated by women.

UNDERSTANDING THE CONTRIBUTION OF INDIVIDUAL VARIATION IN STRESS-RESPONSE TO INCREASCE WELFARE IN AQUACULTURE: A CASE STUDY IN A DIURNAL AND NOCTURNAL SPECIES

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Background: Overcoming stressful situations is a crucial part of individuals' life and such capacity is defined as coping styles, i.e. a set of behavioural and physiological traits, constant over time and stable in different/various contexts [1]. Within-population, individuals react differently to their environment showing high variation in their coping styles, which reflects their success in adeptly responding to the stressor events, especially in a dynamically changing environment [2]. However, in farming conditions, individuals are constantly under new pressures that they have not been selected for. Since recent studies have found that individual variation of coping style in stress response are underpinned by genetic and environmental factors [3], one of the future challenges for the aquaculture is predicting the most appropriate genotype for rearing, i.e. selective breeding. Although an increasing number of studies reported the presence of coping styles in the most important farmed fish species [4], the methodology and the behavioural parameters used to measure individual variability of coping stress responses are species-specific. Furthermore, farmers commonly breed fish as diurnal for practical reasons, therefore not considering the behavioural and physiological needs of nocturnal species. Nocturnal species might not be adapted to the new conditions, which causes negative consequences for their welfare and economic loss for the producer, given the onset of stress-related problems, i.e. reduced growth, increase inter-individual competition and higher mortality rate.

Aim: By use of a set of behavioural paradigms, the aim of the present study was to characterize behavioural traits in stress-response in two commercial interest species with different daily activity pattern: the nocturnal black bullhead catfish *Ameiurus melas* and the diurnal largemouth bass *Micropterus salmoides*. We assessed individual variation of coping style using Operational Welfare Indicators (OWIs), which provide an objective assessment of the welfare state that could be extensively applied for other species [5], and fish behaviours were observed in different contexts during their peak of daily activity (middle of the day for the diurnal *M. salmoides*; middle of the night for the nocturnal *A. melas*) and across different contexts.

Methods: In a previous experiment (unpublished data), we recorded (duration 1-month) the two species *A. melas* and *M. salmoides* to assess their locomotor activity over days. Results confirmed that *A. melas* is a nocturnal species showing an activity peak during the dark phase, while *M. salmoides* showed an opposite pattern as a diurnal species. According to this, behavioral assessment was performed respectively during the daily peak of activity for each species. Each subject underwent three standard procedures used for studying behavioral response to novelty in fish: open-field test, a scototaxis test, and a laterality test in which pattern of movement was observed in a circular arena. The full test was repeated after 14 days to assess whether the observed behaviours were stable across time. Since the nocturnal catfish are commonly breed as a diurnal species in the farms, we decided to perform an additional test during the middle of the day to simulate the common breeding conditions. For each test, we used the Ethovision XT software (Noldus, the Netherlands) to track fish from the video recording, which allowed to automatically determine several parameters: i) distance covered (cm), ii) number of inspections towards an open area, iii) latency to move (s), iv) freezing state (s). In the laterality test, we also manually scored the time spent by each subject in turning clockwise and anticlockwise, as an index of stereotyped response to a stressful situation.

After 14 days from the behavioral assessment, we measured basal plasma cortisol and glucose levels (REF) aiming whether these physiological parameters could explain individual variability in coping styles in stress response.

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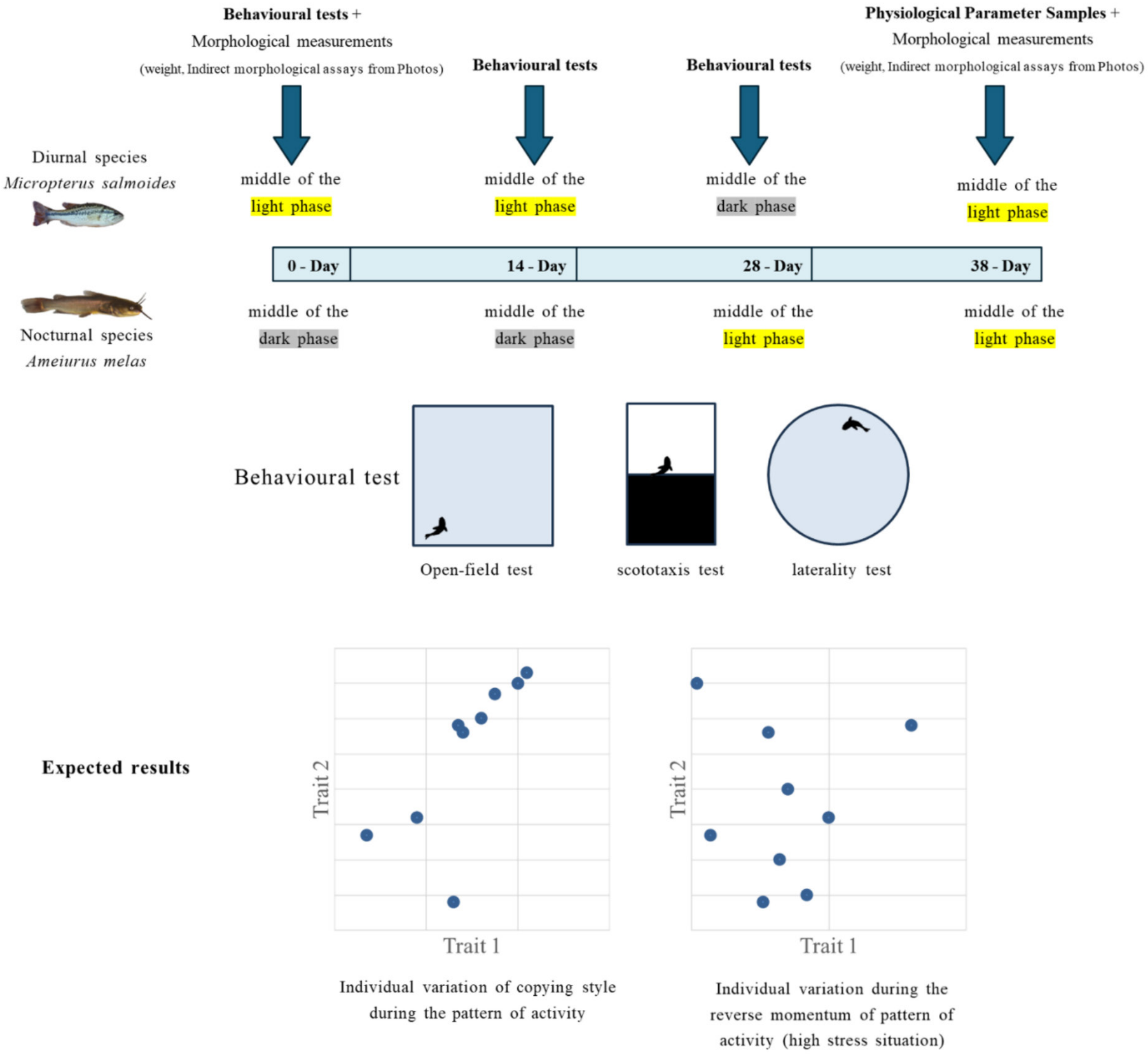

Results: The behavioural analysis is undergoing, and the analysis of video recordings will be completed by the end of the month. We expect to see both species manifesting repeatable and constant coping style in stress-responses during the peak of activity. However, we expect that testing fish during their resting response would disrupt their behavioural phenotypes, as would be confirmed by the lack of correlation among physiological parameters and individual differences in coping style. These results would highlight the importance of refining the breeding condition of fish in captivity according to their activity patterns.

Bibliography

1. Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., De Jong, I. C., Ruis, M. A. W., Blokhuis, H. J. (1999). Coping styles in animals: current status in behavior and stress-physiology. Neuroscience & Biobehavioral Reviews, 23(7), 925-935.
2. Beever, E. A., Hall, L. E., Varner, J., Loosen, A. E., Dunham, J. B., Gahl, M. K., Smith, F. A., Lawler, J. J. (2017). Behavioral flexibility as a mechanism for coping with climate change. Frontiers in Ecology and the Environment, 15(6), 299-308.
3. Coppens, C. M., de Boer, S. F., & Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: towards underlying mechanisms. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1560), 4021-4028.
4. Castanheira, M. F., Conceição, L. E., Millot, S., Rey, S., Bégout, M. L., DamsgAard, B., Kristiansen T., Höglund, E., Øverli, Ø., Martins, C. I. (2017). Coping styles in farmed fish: consequences for aquaculture. Reviews in Aquaculture, 9(1), 23-41.
5. Martins, C. I., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M. T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J. C, Carter, T., Planellas, S. R., Kristiansen, T. (2012). Behavioural indicators of welfare in farmed fish. Fish Physiology and Biochemistry, 38, 17-41.

DIGESTIBILITY OF FUNGAL PROTEIN FROM NON-FOOD BIO RESOURCES AS ALTERNATIVE PROTEIN IN DIETS FOR RAINBOW TROUT *Oncorhynchus mykiss*

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Background: The growing aquaculture industry has an increasing demand for novel protein sources for aqua-feed that are produced in a sustainable way.

Aim: To determine the apparent digestibiltiy coefficients (ADCs) of four novel fungal feed ingredients in Rainbow trout, namely; *Paecilomyces variotii* (PEK), *Aspergillus oryzae* (AO), *Rhizopus oligosporus* (RO) and *Rhizopus delemar* (RD).

Materials and Methods: All fungi were grown on various industrial side streams such as beet vinasse, thin stillage and whole stillage. The test diets were based on the multicellular fungi mentioned above and titanium oxide was added as the inert marker. Diets were produced by extrusion technology. The dietary treatments consisted of a control diet and test diets with 30:70 test ingredient: control. The experimental period lasted for 40 days. 20 fish were kept in each tank with 3 replicates per dietary treatment. One-way ANOVA was performed to compare the means of the groups with each other. $p < 0.05$ was considered significant.

Results: The DM digestibility of PEK was significantly higher than AO, RD and RO, all with similar digestibility. The crude protein ADC for PEK was 91.6% which is significantly higher than the other fungi sources. The ADC of AO and RO were 77.7% and 75.7%, respectively, which was significantly higher than that of RD which was 68%. The crude fat digestibility of the ingredients were 83%, 98.7%, 49.7% and 87.3% for AO, PEK, RD and RO, respectively. AO, PEK and RO have similar crude fat ADC compared to each other but RD has significantly lower crude fat ADC compared with the other ingredients tested.

Discussion: PEK seems to have high ADC for all macronutrients tested. A life-cycle analysis will further help in determining its suitability as an alternate, sustainable protein source for rainbow trout feed. Also, further growth performance trials are required to assess their potential as ingredients in aqua feed.

References:

Cho, E. Y. a. S., S.L. (1979). Apparent Digestibility Measurement in Foodstuff for Rainbow Trout. *World Symposium on Fin Fish Nutrition and Fish Feed Technology*, 239-247.

Langeland, M., Vidakovic, A., Vielma, J., Lindberg, J. E., Kiessling, A., & Lundh, T. (2016). Digestibility of microbial and mussel meal for Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*). *Aquaculture Nutrition*, *22*(2), 485-495.

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POSITIVE EFFECT OF MARINE SYMBIOTICS SUPPLEMENTATION ON FISH INTESTINAL MICROBIOTA DYSBIOSIS INDUCED BY ANTIBIOTIC TREATMENT

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Aquaculture industry is continuously interested in a deeper understanding of the impacts of farm practices on the nutrition and health of targeted species with the aim to improve and adjust its sustainable development. It is well known that fish intestinal microbiota has strong interactions with numerous physiological processes like immune responses, homeostasis and metabolism among others. Intestinal microbiota composition fluctuates depending on various parameters, including species, diet, environmental conditions and the exposure to chemical components like antibiotics, which can lead to rapid emergence of resistant bacteria threating global human health.

A 130-days feeding trials was performed in open circulating aquaculture system on healthy *Sparus aurata* (8.3g initial body weigth, n=48 fish/50L tank, 4 replicates, water temperature of 20°C±2) receiving a 90-days feed supplementation of a marine probiotics consortium constituted of four marine *Bacillus* strains encapsulated in algae (marine symbiotics) to evaluate, first of all, the positive modulation on the intestinal microbiota in normal condition. Supplementation was then stopped and a 10-days antibiotic treatment with oxytetracycline (OTC, 90 mg of active principle/kg) was administered before a new period of 30-days of supplementation with marine symbiotics. A control group fed with a conventional food instead of probiotics underwent the same antibiotic treatment. Probiotics presence in intestinal tract (qPCR with specific probiotics strains primers) and evolution of microbiote composition (metabarcoding) before and after the OTC treatment and at the end of the 30-days supplementation period was analyzed. The well known dysbiosis induced by OTC treatment in healthy gut microbial community will be presented (on going analysis). Dietary inclusion impact of marine symbiotics after OTC treatment will be shown on the intestinal microbiota recovery. We already demonstrated that the colonisation of intestinal microbiota by marine symbiotics was associated with the emergence of other beneficial bacterial. This study characterized the dysbiosis induced by an antibiotic treatment on fish gut microbiota and how marine symbiotics could enhanced and improved the restoration of the intestinal flora in an innovative way to limit negative impact on fish health and economic loss. A similar study on *Salmon salar* from commercial farm are on going to confirm results of the present study.

VIRAL DISEASES SCREENING FOR THE BROODSTOCK COLLECTION OF EUROPEAN EEL (*Anguilla anguilla*) FROM NERETVA DELTA, CROATIA

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Viral infections, often neglected, have been suggested to play an important role in the decline of the panmictic population of the European eel (*Anguilla anguilla*). Despite the importance of knowledge about pathogenic eel viruses, little is known about their spread in the wild population of this species and only a few pathogenic viruses have been described so far. This knowledge is necessary for the eel broodstock selection from natural populations. Neretva Delta, Croatia, presents an ideal place for possible broodstock selection, in which commercial eel fishing is a tradition that has continued to this day, despite the decline in catches. The aim of this study was to investigate the presence of two most often reported causes of viral diseases in the *A. anguilla*, anguillid herpesvirus 1 (AngHV-1) and eel virus European X (EVEX).

Asymptomatic eels were collected from the commercial fisherman catch during winter and spring 2021. Three different organs: liver, spleen and kidney were collected and stored in 70% ethanol. A total of 32 samples were used for detection of AngHV-1 by PCR. After chopping individual organs with sterile scissors their total DNAs were extracted using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma), following the manufacturer`s instructions with the overnight lysis step at 55°C. Subsequent nested PCR reactions for amplification of partial DNA polimerase gene of AngHV-1 were performed using EmmeraldAmpMaxHS PCR Master Mix with DNA volumes, primers and cycling conditions. Presence of PCR products from both reactions (509 bp and 312 bp) was checked in 1.7% agarose gels.

To examine the presence of RNA virus EVEX, parts of the L and M genes were amplified using RT-PCR. Isolation of total RNA was performed using TRI reagent (Invitrogen). RT-PCR reactions were performed in one step using the Transcriptor One-Step RT-PCR Kit (Roche) according to the manufacturer's instructions. The presence of amplified products was checked on a 1.5% agarose gel by gel electrophoresis.

A total of 14 out of 32 fish were positive on AngHV-1 (Fig. 1), while all samples were negative on EVEX (Figure 1.).

These results highlight the importance of broodstock health assessment and biosecurity measures, including quarantine, for eel hatcheries in order to assure higher survival and better quality of larvae.

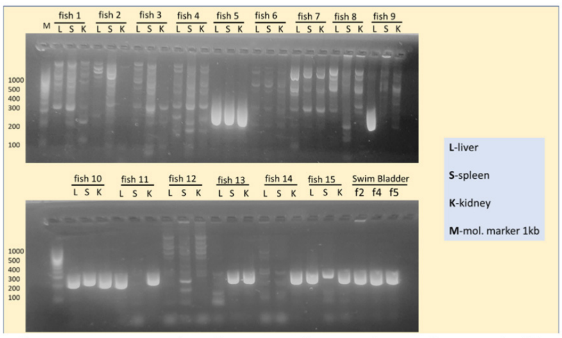

Figure 1. Gel electrophoresis of partially amplified DNA polimerase gene of AngHV-1 (312 bp) in nested PCR reactions from the different eel organs sampled in Winter 2021.

SUSTAINABLE NUTRITION FOR EEL LARVAE: A TRIAL OF NEW DIETARY FORMULATIONS

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A significant challenge in the advancement of European eel aquaculture has been the establishment of larval cultures for the first-feed stages and the nurturing of leptocephalus larvae. Despite considerable efforts, research on identifying suitable feeds tailored to the specific nutritional requirements of European eel larvae remains in its infancy (Benini., 2023; Butts., 2016). The primary focus of current research in eel larviculture around the development of an appropriate initial feeding regimen for the European eel. While advancements in stomach content analysis have provided valuable insights, the natural diet of eel larvae remains incompletely understood (Ayala et al., 2018; Miller et al., 2019; Riemann et al., 2010; Tomoda, 2018). However, the sustainability of traditional feeding practices poses significant challenges, prompting the exploration of alternative dietary formulations to address environmental concerns and optimize growth performance. This study is based on feeding trials to test the effectiveness of new sustainable diets for eel larvae, aimed at promoting both economic viability and sustainability in aquaculture operations.

The trial employed a controlled experimental design, where eel larvae were subjected to various dietary formulations comprising novel ingredients sourced from sustainable and environmentally friendly sources. Parameters such as growth performance, survival rates, feed conversion efficiency, and biochemical composition will be monitored and analyzed throughout the trial period.

Three prototype diets were developed and subjected to pioneering triplicate feeding experiments to assess their impact on the survival and growth of first-feeding eel larvae (figure 1). Diet 1 was based on the regimen used for Japanese eel larvae, while the subsequent diets represented modifications aimed at enhancing nutrient availability and sustainability. Diet 2 incorporated hen's egg yolk and lupins rich in long-chain fatty acids to partially replace the original protein component, while diet 3 consisted of milk protein and the core protein elements of diet 1 (table 1). The results of this study are still preliminary due to ongoing trials. However, the incorporation of new 3D-printed tanks helped mitigate some environmental challenges. This trial will provide valuable insights for future research efforts to refine diets and feeding protocols for European eel larval cultures.

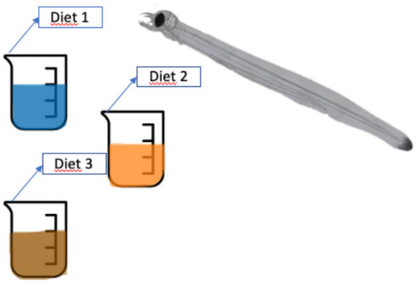

Figure 1: experimental setup of the study

Table2. Formulation and proximate composition of experimental diets (%)

	DIET 1	DIET 2	DIET3
Frozen shark egg	81.9	-	-
Rotifers paste	12.3	-	18.2
Hen egg yolk	-	27.7	16.1
Skimmed milk powder	-	35.9	16.1
Lupin	5.3	26.2	15.2
Casein	-	-	26.5
Fish oil	-	9.4	6.0
Vitamin mix	0.5	0.8	2.0

CRYOPRESERVING EUROPEAN EEL (*Anguilla anguilla*) SPERM: A STEP TOWARDS A SUSTAINABLE CRYOBANK

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The European eel (*Anguilla Anguilla*) is listed as 'critically endangered' on the IUCN Red List of Threatened Species. One of the strategies used to address this drastic decline is the expansion of aquaculture systems.

When artificially breeding this species, it has become increasingly common to use wild females and farmed males. This is because the wild population is declining and the density is decreasing in the various basins, and they differentiate sexually by developing the female sex and adapting to the adverse conditions. In this regard, several research groups have experimented with cryopreservation of the seed in recent years for both Japonica (Tanaka et al.2002) and European eel (Müller et al. 2004; Asturiano et al. 2004). Cryopreservation is an increasingly common practice in fish (Suquet et al.2020; Kopeika et al.2007; Harvey et al. 2003). Cryopreservation of spermatozoa is a crucial method for conserving endangered species and maintaining biodiversity ex situ. This technique allows for the preservation of genetic material and the potential to restore original populations following environmental recovery. It is used in conservation programs, genetic improvement, and selective breeding of fish. The study aimed to develop a technique for preserving *Anguilla anguilla* sperm to establish a national sperm bank.

For this experiment, four wild males (mean weight: 128,63 gr ± 33,89 and average length: 143,10 gr ± 4,31) captured in November 2023 were induced following standard protocols by weekly injections with 1 IU/g BW hCG and started spermiation after a 12-week treatment.

The fish were previously anesthetized by using MS222 (Sigma-Aldrich).

For each male, three ejaculates were collected on different 3 dates and treated as follows: semen samples were diluted in Tanaka's extender solution, ratio 1:8:1 semen/extender/methanol (Tanaka et al.2002) and working on refrigerate condition (4-6°C) The samples were packaged in French straws of 0,25 ml and frozen using nitrogen vapor by maintaining the straws at about 3 cm from the surface of the liquid nitrogen for 3 minutes.

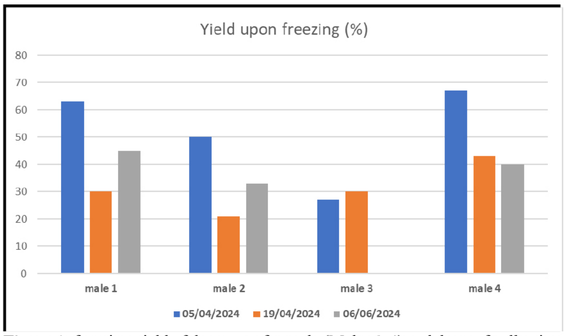

Figure 1: freezing yield of the semen for male (Males 1-4) and dates of collection and processing.

The frozen straws were conserved in liquid nitrogen. To evaluate the effect of cryopreservation on sperm quality, each semen was analyzed for the determination of motility, before and after thawing, using both microscope and image analysis CASA systems (IVOS II – Hamilton Thorne).

The motility average value of fresh semen, before cryopreservation was 53±10,97% while, after thawing was 22,45 ±7,95%, (representing a yield upon thawing of 43,5±15,1%), with an activation time post-tawing of 35.5±3.8 seconds. The total number of straws obtained was 240 with a mean sperms' concentration of 2.600 x 10^6 /ml.

The graph in Figure 1 shows the freezing yield of the eel semen for male (males 1-4) and date of collection and processing. In general, the freezing yield of the ejaculates collected the first day (05/04/24) showed a higher freezing yield compared to those of the other two dates, despite the same processing conditions and similar motilities of the fresh semen among ejaculates and dates. The reproductive period of the eels and the timing of the hormonal induction and semen collection might probably influence the quality of the semen.

This preliminary study allowed to set up a cryopreservation protocol for the semen of European eel. The yield upon thawing revealed very satisfying, despite the motility average values of the fresh semen, which resulted quite low. Further study will be focused on the application of cryopreserved sperm on eggs to perform fertilization trials, to confirm the sperm quality after thawing and the effectiveness freezing protocol for the sperm cryobank implementation.

EFFECT OF ENVIRONMENTAL VARIATION ON THE SWIMMING PERFORMANCE OF EUROPEAN SEABASS

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Introduction

The well-being of farmed fish significantly affects aquaculture productivity and profitability. Changes of environmental factors such as water temperature (T) and dissolved oxygen (DO) can directly impact fish energy demands and swimming performance, thus affecting overall health and growth. Technological advancements like cameras and environmental sensors offer promising opportunities for real-time fish welfare monitoring. This study examines the impact of water temperature on European seabass swimming performance in cages during the summer of 2023 when a heat wave occurred in Greece, compared to the relevant period in 2022, focusing on swimming speed as the behavioral variable.

Materials and methods

A group (N = 22,500 individuals) of E. seabass fish was reared during the two studied periods 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). Rearing was performed in circular polyester cages (40 m diameter, 9 m depth). A submerged network camera (Fyssalis V3.1) capturing at 10 fps was used for monitoring and video recording during daylight hours constantly for a period of two years, i.e. 2022 and 2023. The camera was positioned at 4 m depth using a gyroscopic gimbal stabilizer to ensure its orientation remained directed 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. Bioceanor real time data logger (Aquabox) with T, DO, pH sensors (AquaTroll 500) were also deployed in the cage at 1m depth continuously collecting data. All data were transferred using multiple protocols (MQTT, HTTP) to the Aquareal platform. The mean weight of the fish ranged from 170 gr in March 2022 to 500 gr in September 2023. We tested for differences in the speed between years and between different temperatures.

Results

An increase in the environmental parameters was observed in summer 2023 and temperature increased from $T_{2022} = 24.80 \pm 0.57$ °C to $T_{2023} = 25.38 \pm 0.63$ °C (fig.1). Similar trend appeared in the fish speed as the average speed increased 0.08 b/sec ($s_{2022} = 0.38\pm0.17$ bd/sec and $s_{2023} = 0.46\pm0.09$ bd/sec). Figure 1 shows how the speed and the temperature (averaged values) varied within a day for both years. The fish showed the highest activity in the morning in both, 2022 and 2023. However the duration of the morning excitation lasted longer (apx. 2 hours longer) in 2023 than in 2022. In addition, there is a second excitation peak that appeared in 2023 at around 16:00 in the afternoon, and this peak coincides with the maximum peak the temperature had (25.71 *°C*).

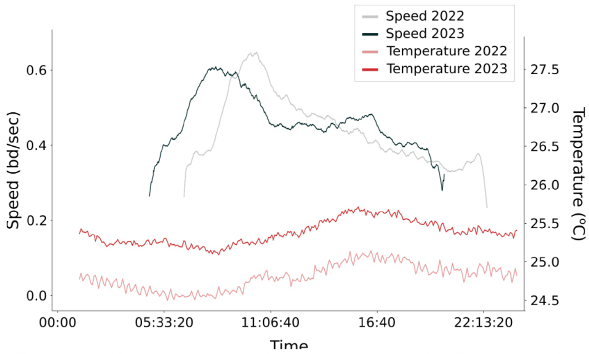

Fig. 1. Time series of the speed (black) and the temperature (red) for the years 2022 (light color) and 2023 (dark color).

(Continued on next page)

Conclusion

From the preliminary results showed here, we can confirm that during the 2023 heatwave there was a change in the activity profile of the fish. Overall, the fish seem to show higher activity as they show higher average speed but also higher duration of increased activity. Previous studies have highlighted the varying energy demands of fish across different temperature ranges, underscoring the intricate relationship between environmental conditions and metabolic processes. Given the multifaceted implications of environmental conditions on fish welfare, continued research in this domain is essential.

Acknowledgement

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References

Barreto, M.O., Planellas, S.R., Yang, Y., Phillps, C., Descovich, K. Emerging indicators of fish welfare in aquaculture. Reviews in aquaculture 14, 343-361. https://doi.org/10.1111/raq.12601

Georgopoulou, D.G, Vouidaskis, C. and Papandroulakis, N. (2024) Swimming behavior as a potential metric to detect satiation levels of European seabass in marine cages. Front. Mar. Sci. 11:1350385. doi: 10.3389/fmars.2024.1350385

WATERBORNE CORTISOL: TOWARDS A KEY FISH-BASED OPERATIONAL WELFARE INDICATOR IN FISH FARMING?

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In most vertebrates (humans included), the steroid hormone cortisol is released to the blood in situations of stress and can be used as a marker for stress/immediate welfare status. In fish, small amounts of cortisol diffuse passively to the surrounding water, mostly through the gills. Waterborne cortisol (WC) is often used as stress indicator in fish research, under very controlled conditions. It has potential to become an ideal welfare indicator for fish in fish-farming facilities, as it is non-invasive and directly informative about the fish stress/welfare status. The main current disadvantage as a fish welfare indicator relates to the fact that WC analysis is time-consuming and involves sending water samples to a laboratory, making it a laboratory-based welfare indicator – LABWI, impractical to use as a routine indicator in fish facilities. However, ongoing technological efforts towards the development of online cortisol sensors could change the situation and turn WC into a good candidate for a fish-based operational welfare indicator (OWI), able to provide real time information on the physiological status of captive fish.

Even if new technology succeeds in providing tools for the continuous monitoring of WC, another problem to solve relates to the poor knowledge available about the extent of release and about the chemical fate of WC in different types of fish facilities/fish farms. First, the passive release of cortisol to the water not only depends on the concentration of cortisol in the blood of the fish, but also on the permeability of the gill to cortisol, which could be affected by different factors (species, size, biorhythms, health status, etc.). Second, there is a need for validation work to better understand how different factors in the fish production systems affect cortisol degradation/permanence in the water upon release, including water quality (temperature, salinity, organic matter, particles, microbial load, etc.) and facility components, design and operation protocols, among others. Detailed information about normal WC levels, normal variation, extent of change upon challenges and routine operations, etc., for different facility types is also lacking, and would be critical to evaluate the potential of WC as welfare indicator in fish farming. This presentation will review current knowledge about advantages and drawbacks of WC and its potential use as an operational welfare indicator for farmed fish.

COMPARISON OF THE GUT MICROBIOME IN WILD AND FARMED ATLANTIC SALMON SMOLTS

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The gut microbiome plays a critical role in different aspects of fish biology; through direct involvement in the digestive process or its active role in immune response and barrier function with major consequences for the fish's health and welfare. Farmed salmon is known to be less resilient compared to the wild salmon. In particular, smolts experience extreme stress when transferred from land-based aquaculture facilities to sea cages where the conditions are much less controlled and where they are exposed to an array of pathogens. Consequently, seawater transfer is one of the major culprits for economic loss and impaired animal welfare in the salmon industry. The microbiota is known to change as the fish moves to marine environments. Comparing the microbiome of wild and farmed smolts will improve our understanding of an important yet understudied aspect of the adaptation of salmon smolts to marine conditions and this critical transitional stage. Therefore, we sampled the posterior intestine of Atlantic salmon one week after sea transfer from a land-based flowthrough aquaculture system to sea cages, as well as wild smolts of comparable size from both fresh- (river) and brackish water (fjord) environments. Total genomic DNA was extracted for metataxonomic, DNA amplicon libraries were generated targeting the V3-V4 regions (341F/R805) of the 16S rRNA gene, and the sequencing was performed on the Illumina platform. Analysis of the sequences was performed following standards pipelines for metataxonomic analysis. Clean sequences chimeric detection and deletion, followed by Amplicon Sequence Variant (ASV) assignment was completed using the DADA2 plugin. Taxonomy was assigned with a 99% similarity level using the q2-feature-classifier plugin with the SILVA138 database. Alpha (Shannon) and beta (Bray Curtis) diversity indices were calculated for each sample using the vegan R package. A strong inter-individual variability was observed in the results, even between fish from the same environment. No significant changes in alpha diversity were overserved with high variability and overlap between conditions. Although beta diversity showed also high dispersion between samples, most gut microbiomes were dominated by the *Proteobacteria* phylum, except for several individuals where *Firmicutes* or *Spirochaetota* were more abundant. At the genus level, the intestinal microbiotas in the fish from rivers and sea cages were dominated by *Stenotrophomonas sp.* And *Delftia* sp., although this pattern was weaker in fish from the fjord. Farmed salmon had a significantly lower abundance of *Carnobacterium*, *Mycoplasma*, and *Deefgea* compared to wild ones, while the relative abundance of the *Aliivibrio* genus was increased. A small proportion of the ASV (~2%) was shared between conditions suggesting the existence of a "core" microbiome shared between wild and captive Fish. The present results represent the first step in our study to be complemented with metagenomic data to define a healthy and resilient Atlantic salmon microbiome. Results from this study could help to shed light on the differential role of rearing environments on the gut microbiota, this will contribute to improving the performance and welfare of farmed salmon.

Acknowledgments: Funding: BlueRemediomics-Grant No.101082304, EU Horizon Europe Programme. Farmed fish were sampled from Lerøy´s land and sea facilities in Norway.

DRYING-OUT FISH PONDS AS A KEY AGRO-ECOLOGICAL PRACTICE TO PROMOTE ECOSYSTEM SERVICES?

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Fish ponds are, in the same time, ecosystems used for extensive fish production, which can contain significant biodiversity with rare, endemic or endangered species, and that can also play a part in climate mitigation. Indeed, fishponds, like aquatic environments as a whole, play a major role in the global carbon cycle. Anthropogenic in origin, their functioning is mainly influenced by management practices. One such practice is drying-out, which consists to drain the pond for an entire production season. This practice aims to rejuvenate the ecosystem and mineralize the organic matter that has accumulated during the production years. However, there is a knowledge gap about its role in terms of ecosystem services (fish production, maintenance of biodiversity and carbon storage).

To fil this knowledge gap, we analysed data (fish yields, abundance and diversity of primary producers, carbon storage and emissions) collected on numerous fish ponds in the Dombes region, France, since 2007. Management information, in particular the distance to the last dry-out, was collected from managers.

Our results show that the dry out year allowed a recolonization of aquatic plants during the first year with water, with levels of diversity and cover at their highest while the phytoplankton concentration is at its minimum. They are decreasing during the following wet years, in parallel with an increase in algae. The best balance between the two primary producers is reached in the second year, where we can also observe the best fish yields. These high levels of primary producers' abundance in the early years of the cycle enable good carbon storage through sedimentation. The dry year is marked by high emissions of greenhouse gases (CO_2 and CH_4), tending to make ponds sources of carbon over the entire production cycle.

By encouraging the development and recolonization of the environment by macrophytes, and also by enabling an optimum unstable state of presence of the two primary producers, drying out appears to be a major agro-ecological practice, in extensive pond fish farming, structuring the functioning of the ecosystem and the services rendered. Nevertheless, drying-out a fishpond represents a major disturbance, marked by significant carbon emissions. These opposing effects will undoubtedly call for a rethinking of the hierarchy of desired ecosystem services.

UPGRADED FEATURES IN THE IBSEM-MODEL PROVIDE NEW INSIGHTS INTO POPULATION-DEMOGRAPHIC AND INDIVIDUAL-ADMIXTURE-PHENOTYPIC CONSEQUENCES OF DOMESTICATION INTROGRESSION

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The Individual Based Salmon Ecogenetic Model IBSEM has previously been used to elucidate population-level phenotypic and demographic consequences of gene-flow from domesticated Atlantic salmon in wild populations in both Canada and Norway.

Here, we present new and novel features of the model including the ability to output individual-fish phenotype (as opposed to population averages only) and individual-fish admixture. The latter feature is computed using an ancestry-tag within the model itself (thus reflecting individuals % of farm ancestry) and admixture as estimated by sets of molecular genetic markers under differing degrees of selection.

The model was re-tuned to mimic the Atlantic salmon population in the river Etne in Norway where extensive empirical data are gathered on the effects of domestication-introgression. The model was run on a time-scale (25-50 years introgression 25-50 years “recovery”) and with levels of gene-flow (via 0-25% escapees on the spawning grounds) that reproduces the introgression scenario observed in the river Etne.

The results of this work are very interesting and have significant implications for setting management limits for biological tolerance levels. Not least, this work also has important implications on how we interpret admixture estimates from empirical studies in the wild using molecular genetic markers - especially during the “recovery” phase.

All will be revealed in August ☺

THE RIVER ETNE FIELD STATION: A GLOBALLY UNIQUE SYSTEM TO STUDY INTERACTIONS BETWEEN DOMESTICATED AND WILD CONSPECIFICS

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In 2013, in response to ~3 decades of high numbers of farmed salmon escapees entering the river, a field station with an upstream fish-trap was installed in the river Etne, Norway. This trap is monitored daily through April-November by a professional staff – permitting removal and total-sampling of all farmed escapees, and non-invasive sampling of all wild fish that are thereafter permitted to enter the river to spawn. Each year, we have also conducted extensive smolt-tagging, kelt-tagging, and juvenile sampling.

We have now handled and sampled >20 **000** adult salmon hatched in the wild, all of which have been analysed for growth and age, genotyped with 31 microsatellites for individual ID/pedigree reconstruction, genotyped with 150SNPs that are diagnostic between the domesticated escapees spawning in the river 1986-2012 and the pre-aquaculture wild population. We have also genetic, disease and escape history of the escapees, and full-genome data for a sub-set of the adults from each year.

Here, we will present this unique system and what we have learned from this ~25% domestication-admixed wild population.

EFFECTS OF INCREASING SALINITY ON MARAENA WHITEFISH *Coregonus maraena* IN THREE DIFFERENT FARMING SYSTEMS

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Introduction
The present study investigated the influence of salinity fluctuations on gene-expression patterns in maraena whitefish (*Coregonus maraena*) from three aquaculture facilities. We monitored the expression of an established panel of seawater-tolerance biomarkers in four tissues of whitefish over a period of ten weeks. Fish were kept in a natural reef in the South-western Baltic Sea with locally rapidly changing salinity conditions and compared to conspecifics from a freshwater-pond system. The largely consistent expression profiles across whitefish from both habitats provided only sporadic modulations of most genes. A principal component analysis revealed that these differential expression patterns were dominated by *eef2*, *ghr* and *wasf1*. A subsequent salinity experiment was carried out in a recirculating aquaculture system under constant environmental conditions.

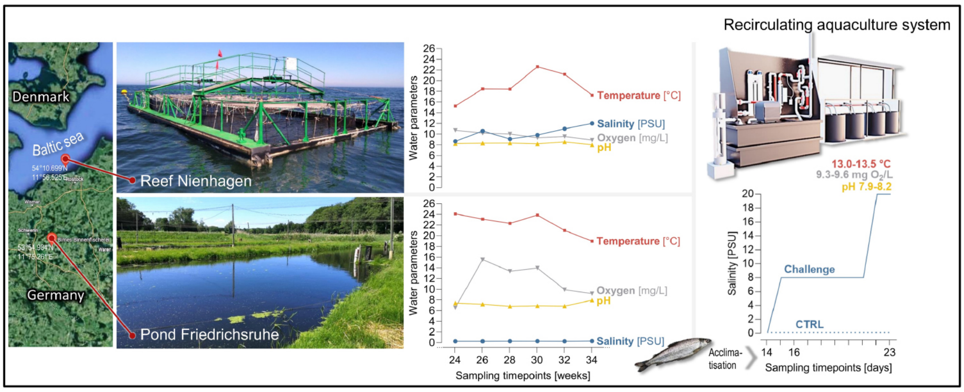

Fig. 1. Overview of whitefish sampling facilities and selected local physicochemical parameters.

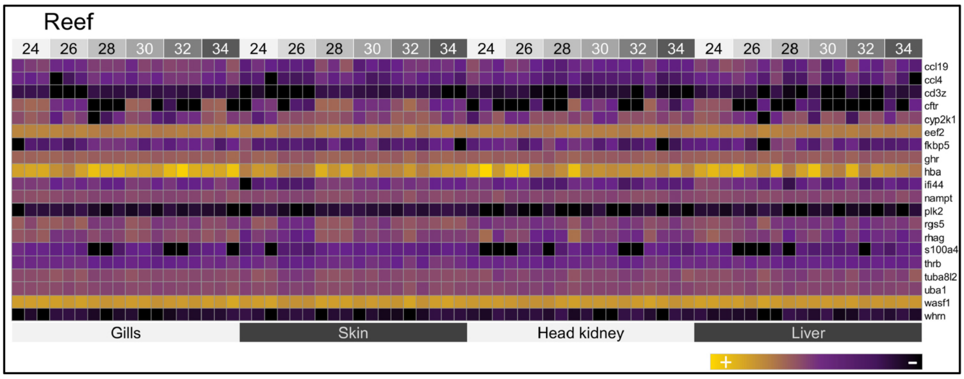

Fig. 1. Heat map (reef Nienhagen as an example) illustrating the log10-transformed transcript numbers measured in the gills, skin, head kidney, and liver of maraena whitefish kept in brackish-water conditions.

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Results
The elevation of salinity from 0 to 8 PSU was only indicated by the significantly increased fkbp5 and atp1a1a transcript levels in the gills of whitefish. The reduced levels of eef2, ghr, wasf1, nampt, and uba1 in the head kidney or skin or both tissues indicated the response mechanism to higher salinity (20 PSU). Altogether, only the quantification of the atp1a1a gene provided the most reliable evidence of the successful acclimation from low- to higher-salinity water in all three facilities investigated. Based on the overall transcriptional data and the generally good physical constitution, we assume that maraena whitefish was not negatively affected by salinities up to 20 PSU, but were capable of acclimating well to changing brackish-water conditions.

Conclusions
Based on the data obtained in the present study, we assume that European whitefish are not negatively affected by varying salinities up to 20 PSU, but are able to acclimatize well to changing brackish water conditions. During the observation period, the salinity at Nienhagen Reef in 0-5 m water depth did not exceed 12 PSU and was thus in the optimal range for coregonids (De March, 1989; Waterstraat A. and Wachlin, 2010). However, salinity is only one parameter in the spectrum of environmental conditions that can prove unfavorable for the performance of farmed fish. Good growth is crucial for the economic production of maraena whitefish. Since the start of the experiment in 2019 at the Nienhagen reef site, the maraena whitefish has shown low growth rates, indicating the presence of external stressors that have apparently not affected the parallel maraena whitefish cohort from the pond management or flow-through system.

Funding
DRM-165, LFA-MV Germany; EMFF MV-II.1-LM-010; Government of the State Mecklenburg-Western Pomerania.

MYCOBINDER PRODUCTS: DO THEY IMPACT THE GROWTH AND HEALTH OF INTENSIVELY FARMED FISH CONTAMINATED WITH MYCOTOXINS?

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This study aimed to evaluate the effectiveness of administering two different mycobinding supplement technologies in mitigating the effects of the mycotoxins FB, DON, and AFB1 on the growth and health of gilthead seabream (*Sparus aurata*). The objective was to determine whether the consumption of food contaminated with these mycotoxins could be treated using mycobinders.

Young *S. aurata* individuals (average weight 2.87±0.2g) were randomly distributed into 12 glass aquaria (125L, 25 fish per aquarium). Two types of mycobinders, each utilizing different technologies, were incorporated into the experimental diets: MycBin 1 [composed of attapulgite + oxidizing magnesium and activated carbon] and MycBin 2 [composed of a yeast strain (*Saccharomyces cerevisiae*), zeolite, and algae]. Triplicate aquaria were assigned to three experimental diets: MYC (DON, 3000 ppb; FB, 3000 ppb; AFB1, 50 ppb), A1 (MYC diet + MycBin1), and B1 (MYC diet + MycBin2). The control group (CTRL) received a mycotoxin-free diet. Fish were fed "ad libitum" twice daily, six days a week, for 12 weeks. Daily records were stored for feed intake and mortality rate. Water physicochemical parameters were regularly monitored and maintained at standard levels. At the end of the trial, morphometric characteristics were measured, and growth parameters were calculated. Liver samples were collected for histological analyses. One-way ANOVA was used to determine variance among treatments, followed by Tukey's post hoc test with a significance level set at $P < 0.05$.

The group containing mycotoxins (MYC) without binders showed lower feed consumption and delayed growth than all other groups. Both groups with the dietary addition of mycobinders (groups A1, and B1), showed significantly faster growth compared to the MYC group, therefore the addition of mycobinders had a positive effect (Fig. 1). Microscopic examination of liver samples from the MYC group revealed mild to extensive degenerative changes in hepatocytes, including hydropic and fatty degeneration as well as early necrotic changes, compared to the control group (Fig. 2A, B). In contrast, liver samples from groups supplemented with mycobinders exhibited a range of conditions, from almost normal liver parenchyma to samples displaying some pathological features (Fig. 2C, D). Specifically, liver samples from the A1 group varied, showing almost normal liver parenchyma to mild degenerative changes in hepatocytes, including mild hydropic and fatty degeneration. Similarly, liver samples from the B1 group exhibited mild-to-diffuse degenerative changes in hepatocytes, with varying degrees of hydropic and fatty degeneration. In conclusion, the dietary addition of a combination of mycotoxins negatively affected the growth and health of gilthead seabream. However, the inclusion of both types of mycobinders partially mitigated this problem, although it did not completely eliminate adverse effects.

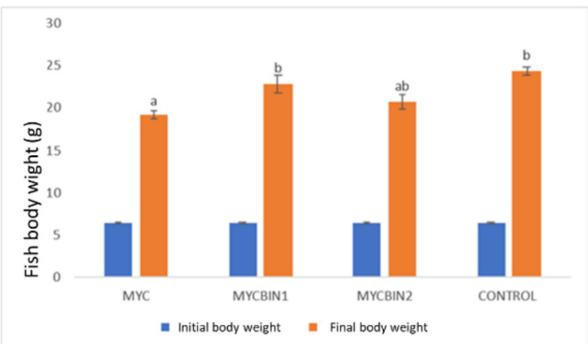

Figure 1. Final average weight per individual. Values are presented as means ± standard deviation of each treatment. In each parameter examined, symbol with a different letter indicates a statistically significant difference between treatments (P<0.05).

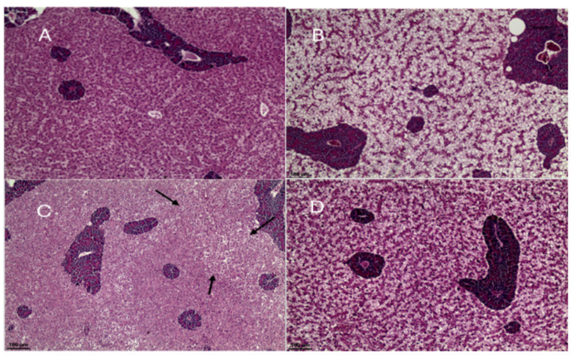

Figure 2. Photograph of a liver section. (A) Control group. Normal liver parenchyma. (B) MYC group. Severe degeneration of the liver parenchyma, with vacuolation of hepatocytes. (C,D) Groups A1, B1. Almost normal liver parenchyma. Mild degeneration in small areas of the liver.

ATTAPULGITE: A CLAY MINERAL TO CONTROL AMMONIA IN AQUACULTURE AQUATIC ENVIRONMENT

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Attapulgite is recognized as a feed additive (EU2023/1699), primarily used for technological functions in feed, such as binding, anti-caking, and mycotoxin contamination reduction. Additionally, attapulgite can perform zootechnical functions by influencing the gastrointestinal flora and improving feed digestibility in animals, which are the typical functionalities of feed additives. This study aimed to evaluate the effectiveness of attapulgite in mitigating ammonia excretion in the aquatic environment during fish farming.

The experiment was conducted in 12 x 250 L experimental aquarium facilities (EL-43BIO/exp-01) at the Aquaculture Laboratory of the Department of Ichthyology and Aquatic Environment, University of Thessaly, Greece. The system was supplied with a water flow of 33ppt salinity, filtered (through a system of mechanical and UV filters, with no biological filter in operation), oxygenated, and of temperature that was maintained at 21°C ± 1°C during the experiment. The photoperiod was maintained at a 12 to 12 h light and darkness ratio.

A total of 636 healthy gilthead seabream (*Sparus aurata*) with an average weight of 2.63 ± 0.46 g were sourced from a nearby commercial farm. Two distinct pelleted feeds were formulated at local facilities: attapulgite-supplemented (3 kg/ton) and attapulgite-free. Fish were divided into four experimental groups, each consisting of three replicates (53 fish/aquarium). The groups were as follows.
Group 1: Attapulgite-supplemented diet fed at 4% of biomass.
Group 2: Attapulgite-free (control) diet fed at 4% of biomass.
Group 3: Attapulgite-supplemented diet fed *ad libitum*.
Group 4: Attapulgite-free (control) diet fed *ad libitum*.

The experimental diets were manually administered to the fish for 15 days. The acceptance of the diets was monitored throughout the experiment. Ammonia (NH_3) levels in water were measured daily at three specific time points: before feeding, two hours post-feeding, and nine hours post-feeding. These measurements were conducted using a commercial API Ammonia Test Kit. No fish were harmed or killed during the experiment. To evaluate the data, Student's t-test was applied to compare the means among attapulgite-supplemented and attapulgite-free diets in each feeding treatment, at a significance level of $P < 0.05$.

Figure 1. NH_3 values (mean ± standard error) in treatment groups, measured before feeding, 2 hours post-feeding, and 9 hours post-feeding. Different letters indicate statistically significant differences among means within the same feeding treatment ($P < 0.05$). The absence of letters denotes no statistically significant differences between the tested groups.

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No statistical differences were observed in feeding behaviours, feed consumption, or body weight gain between the attapulgite-supplemented and attapulgite-free diet groups in both feeding treatments (4% of biomass and *ad libitum*). However, statistically significant differences were observed between the attapulgite and control groups when the fish were fed ad libitum. The attapulgite diet reduced significantly ammonia excretion in the aquatic environment by 60.58% before feeding, 47.19% two hours post-feeding, and 52.35% nine hours post-feeding, thereby mitigating the potential adverse effects of ammonia on fish health (Fig. 1). Attapulgite is a promising clay mineral that has a wide range of applications in aquaculture and aquatic environments. Nonetheless, further research is necessary to fully understand its holistic impact on fish health and welfare.

Acknowledgment: The project ‘‘Research and Educational Actions in Fish Health Protection and Welfare’’ supported the AQUA2024 presentation. This work is a part of the dissertation submitted in partial fulfillment of the requirements for the degree of MSc Course in Mediterranean Aquaculture, Department of Ichthyology and Aquatic Environment, University of Thessaly, Greece.

INFLUENCE OF MACROALGAE INCLUSION IN THE FEED ON THE GROWTH PERFORMANCE AND GUT MICROBIOME OF *Litopenaeus vannamei*

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The Pacific white-leg shrimp *Litopenaeus vannamei* is one of the most cultured species worldwide, thanks to its adaptability to environmental changes and omnivorous feeding behavior. However, ensuring sustainable growth of this industry demands continual screening for innovative aquafeed ingredients, given that the diet highly determines the animal's physiology and the business's profitability. Macroalgae have been proposed as a promising aquafeed ingredient for shrimp owing to their high content of bioactive compounds, appropriate nutritional composition, and cost-effective cultivation process. Their inclusion in diets would improve the growth performance of the shrimp, and change the microbiome composition. Moreover, studies have shown how the gut microbiota strongly influences the immune state of the animals through the direct competence of beneficial and opportunistic bacteria in the gut, keeping a healthy balance that prevents disease outbreaks. This research investigates the effect of macroalgae inclusion on the diet of juvenile *L. vannamei* in terms of growth performance and gut microbiome composition, in collaboration with the UGent Phycology lab.

This study evaluates the potential of a selection of green, red, and brown macroalgae: three species indigenous to the North Sea area (*Fucus serratus*, *Chondrus crispus*, and *Ulva* spp.) and two invasive macroalgae species (*Sargassum muticulus* and *Gratelupia turuturu*) were evaluated as sustainable aquafeed ingredient. Macroalgae were collected, rinsed, dried, grinded, and processed through cold extrusion into dry shrimp feed at an inclusion level of 5%. Liquid extracts were produced from the grinded seaweeds and screened *in vitro* for their antibacterial activity against the common shrimp pathogens *Vibrio parahaemolyticus* and *V. harveyi*. Juvenile *L. vannamei* (± 1g) were fed for 4 weeks with one of these test diets or a commercial one (control). Growth was monitored weekly and the feed conversion rate (FCR) and specific growth rate (SGR) were calculated. Shrimp were then sampled and their digestive tract was aseptically dissected for gut microbiome analysis in terms of relative abundance and richness. The nutritional and proximate composition of the macroalgae and the diets were assessed, as well as the whole-body composition of the shrimp at the start and the end of the study.

The study demonstrates the multifunctional benefits of macroalgae as aquafeed ingredients, serving as immunostimulants and nutritional supplements. Furthermore, utilizing invasive species in aquafeeds presents a dual advantage, offering a means of control while mitigating their adverse environmental impact on local ecosystems. Moreover, sustainable aquaculture practices are essential for the long-term viability of the industry, seeking to use components that have a production with a lower footprint than the traditional fish meal and oil ingredients needed for the production of carnivorous species. Seaweeds and shrimp are both low-trophic level species with short production cycles, and their integration in productive systems could be a strategy for environmental mitigation and food security efforts.

MICROPLASTIC POLLUTION BUDGET ASSESSMENT OF DIFFERENT INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) SYSTEMS

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Introduction - 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 and use of synthetic polymers over the last decades. Synthetic materials offer greater strength and durability than natural fibres for construction of ropes, infrastructures, and pipes, whilst often also being less costly and easier to handle. Broken and fragmented equipment as well as debris released from intense use are however sources of plastic emission from aquaculture operations at a local and global level, whilst accurate estimations of their contribution remain unknown. The ASTRAL project focuses on IMTA farming, aiming at defining, supporting, and promoting this type of sustainable aquaculture production across the Atlantic area. 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 impacts.

Materials and Methods -Four sites were selected within the present study: A) the coastal open low-trophic aquaculture facility 'Port-a-Bhuiltin' (Scotland); B) the coastal open multi-trophic aquaculture facility 'Lehanagh Pool' (Ireland); C) The onshore partially (50%) recirculating multi-trophic aquaculture facility 'Buffeljags Abalone' (South Africa); and D) the onshore recirculating multi-trophic aquaculture facility 'Rio Grande do Sul' (Brazil). The occurrence of plastic was investigated in the seawater at increasing distances from the open systems aquaculture facilities. In the RAS systems the sampling was performed in the water inlet, outlet, as well as in some selected areas inside the recirculating system. For the seawater collection a Compact Large Volume Microplastics sampling device was developed. Extracted samples were analysed first by μ-FTIR (Fourier Transform Infrared) microscopy and finally by GCMS-pyrolysis technique.

Results and Discussion- The evaluation of the seawater samples and sediments using a vibrational microscopy-oriented technique showed the occurrence of 29 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), polyamide (PA66) and acrylic paint (APa). The mass-based analysis confirmed the occurrence of these polymers and further detected styrene butadiene rubber as a proxy of tyre and road wear particles. The results point out a complex distribution of polymers, where the diffuse input sources in the investigated areas largely influence the observed plastic pollution distribution. The experience assessing the plastic pollution fingerprint in RAS and semi-RAS systems point out both the importance of the water quality inlet, the geographical dependent light intensity effects in plastic degradation phenomena as well as the need for an accurate replacement plan for teared plastic equipment. The ongoing work contributes 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.

Acknowledgements. EU H2020 grant agreement: 863034 - ASTRAL project (All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture.

TARGETING THE OPTIMIZATION OF GUT MICROBIOTA-HOST INTERACTION IN SALMONS: ASSESSING FUNCTIONAL INGREDIENTS IN AN EX VIVO APPROACH

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The interface between microbiota and the host mucosal functioning is a stage for numerous processes that are key for the hosts´ defensive system. In aquaculture, opportunistic microorganisms are often harbored in mucosal systems, causing disease outbreaks when fish homeostasis is disrupted. The modulation of this interface by targeting undesirable bacteria is a strategy that has been tested through nutritional approaches, such as the inclusion of functional ingredients like prebiotics. In salmon aquaculture, mucosal health and well-functioning are among the most important topics, and the implementation of novel strategies is needed to support the welfare and boost the resistance of fish. In this study, we assess the modulation of the gut mucosa microbiota and its function in two different intestinal compartments (proximal and middle intestine) by including the microalgae *Euglena gracilis*, with potential prebiotic effects in an *ex vivo* intestinal sac platform. The modulation of targeted microbial groups with opportunistic (e.g., *Vibrio*, *Aeromonas*, *Tenacibaculum*) or beneficial (e.g., *Lactobacillus*, *Bacillus*) characteristics was screened through Nanopore MinION 16S long-read bacteriome sequencing and compared between the compartments' mucosa exposed to an antibiotic treatment, or unexposed. An in-house bioinformatic pipeline was applied to retrieve the relative abundance of bacterial pathogens based on an aquaculture bacterial pathogen database with in-house modifications, and an in-house database of bacteria with reported beneficial impacts was used for comparison purposes. Moreover, the mucosa response was assessed and correlated with microbiota shifts following the transcriptional modulation of a panel of genes related to immune response, epithelium integrity, and homeostasis. Among all the identified potentially pathogenic bacteria, a group was affected by the exposure of the intestinal mucosa to microalgae, and some beneficial bacteria were also affected. The ratios Firmicutes:Bacteroidetes, and Proteobacteria:Bacteroidetes were modulated, increasing when mucosa was exposed to an antibiotic. This was evident in both compartments, and the opposite was observed when microalgae were included in the sac in one of the compartments. The expression of the acute inflammation markers indicated an impact on the mucosas´ health. Through the application of an *ex vivo* approach, it was possible to screen and highlight the impacts of a harmful compound and an algae-based functional ingredient.

Acknowledgments: This work was financially supported by the project MICROBOOST – PT-INNOVATION-0102 – funded by Iceland, Liechtenstein and Norway through the European Economic Area (EEA) grants

EXPLORING THE DIATOM *Skeletonema costatum* AS A FUNCTIONAL INGREDIENT TO BOOST ATLANTIC SALMON MUCOSA HEALTH

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The nutritional profile of diatoms has been identified as interesting for aquafeeds, and more recently, their bioactive potential for nutraceutical applications has emerged, mostly due to their immunostimulant and anti-inflammatory properties. Although diatoms present a vast biodiversity, only a very narrow number of species are currently produced at industrial scale. *Skeletonema costatum* is one of the most naturally abundant diatoms, and industrially produced biomass is commercially available. Here, we explore the potential of *S. costatum* as a mucosal health booster for Atlantic salmon (*Salmo salar*). Salmon requires support to cope with immune and production-derived insults such as sealice outbreaks.

S. costatum biomass was processed through a seven-step biorefinery pipeline where soluble and insoluble fractions were separated and stepwise subjected to different enzymatic hydrolysis processes to break down proteins and carbohydrates. The functional properties of each of the seven derived fractions were assessed on an Atlantic salmon intestinal *ex vivo* platform under previously established optimal conditions. A panel of molecular markers was used to evaluate the immune response (e.g., *IL-1B*, *IL-8*, *TGF-B*) and overall homeostasis (e.g., *HSP70*, *CAT*) in both anterior and middle intestinal explants.

Soluble fractions promoted upregulation of immune-related markers, and this was more evident in the soluble fractions retrieved after protein and carbohydrate hydrolysis. The immunostimulant effect was noticed in both intestinal segments, but the anterior intestine's response was more substantial. Intestinal homeostasis was not modulated, indicating a positive immunostimulation exerted by these fractions. These results highlight the potential of *S. costatum* as a mucosal immunity booster, indicating that its functional properties are enhanced through biorefinery processes. Biochemical characterization of the functional fractions is in course, and these will be included in an upcoming *in vivo* trial to assess its impact on Atlantic salmon resistance to pathogens and other insults.

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Bidens pilosa AS A MULTIFUNCTIONAL FEED ADDITIVE TO PROMOTE THE INNATE IMMUNITY OF PACIFIC WHITE SHRIMP AGAINST WSSV

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This study investigated the effect of dietary supplementation with an edible herb *Bidens pilosa* on the expression of non-specific immunity, and innate immunity-related genes in Pacific white shrimp (*Litopenaeus vannamei*).

B. pilosa additive (BP) was found to promote key cellular immune responses, including the production rate of superoxide anion and prophenoloxidase in haemocytes, as well as the activation of the immune genes *Toll4, TRAF6* and its downstream antimicrobial peptide genes *ALF*, *PEN2*, *PEN3*, *PEN4*, *Lyz*, and the antioxidant enzyme genes *SOD* and *GPx* in the hepatopancreas. Similar results of differential expressed genes were identified by transcriptome analysis of hepatopancreas in white shrimp after 14 days of feeding with 1% BP additive and control diet. Moreover, the immune-related Toll and IMD signaling pathway was upregulated in KEGG enrichment analysis after 14 days of feeding with 1% BP. Shrimp were then artificially infected with white spot syndrome virus (WSSV) to determine whether *B. pilosa* additive can improve disease resistance against WSSV. The *B. pilosa* supplemented group exhibited a significantly higher survival rate from 4 days after WSSV challenge. In addition, there was a significant difference in body weight between the 1% *B. pilosa* and the control without BP after 14 and 28 days of feeding.

Our findings demonstrated that dietary supplementation with *B. pilosa* as a multi-functional feed additive can enhace innate immunity and promote growth in white shrimp.

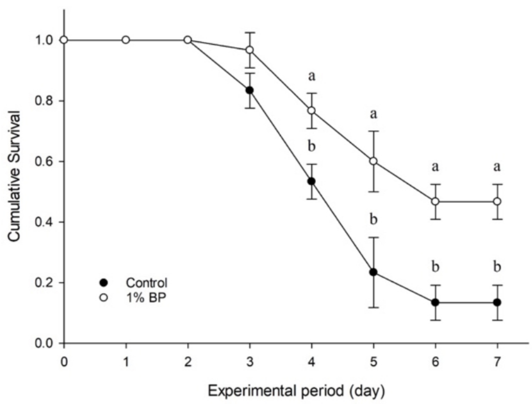

Fig. Susceptibility of Pacific white shrimp to WSSV. Cumulative survival rate of white shrimp fed with commercial feed (control group) or 1% *B. pilosa* and then infected with WSSV.

ESTABLISHMENT OF GERMLINE-SPECIFIC FLUORESCENT FRESHWATER ANGELFISH FOR TRACKING AND NUMBERS OF PRIMORDIAL GERM CELLS INVOLVED IN SEX DEVELOPMENT

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In this study, we generated the novel transgenic line, *Tg(ddx4:TcCFP13-nanos3)*, by tilapia 4.5 kb *ddx4/vasa* promoter expressing Taiwan *Acropora* coral cyan fluorescent protein TcCFP13 cDNA linked with tilapia *nanos3* 1 kb 3'-UTR to specifically label PGCs with strong fluorescent signals during embryonic development in zebrafish model and the popular Cichlid freshwater angelfish (*Pterophyllum scalare*).

We observed a complex migration process with a multistage pattern in freshwater angelfish, and a longer period for complete localization in angelfish (168 hpf) compared to zebrafish (24hpf). Notably, significant differences in PGC abundance between each embryo emerged during early somite development. We screened and counted the number of PGCs in each larva, separating larvae with fewer PGCs from those with more, and raised them until sexual maturity. We discovered that PGC abundance is correlated with sexual dimorphism in freshwater angelfish, with 90% of individuals developing into males in the PGCs-less group and 83% developing into females in the PGCs-rich group. This result is highly consistent with our previous study, where a male-biased phenomenon appeared in infertile fish with *dnd1* knockout by CRISPR/Cas9. Exploring the mechanisms of PGCs or sex development in ornamental fish, especially in freshwater angelfish, may benefit the ornamental industry's development.

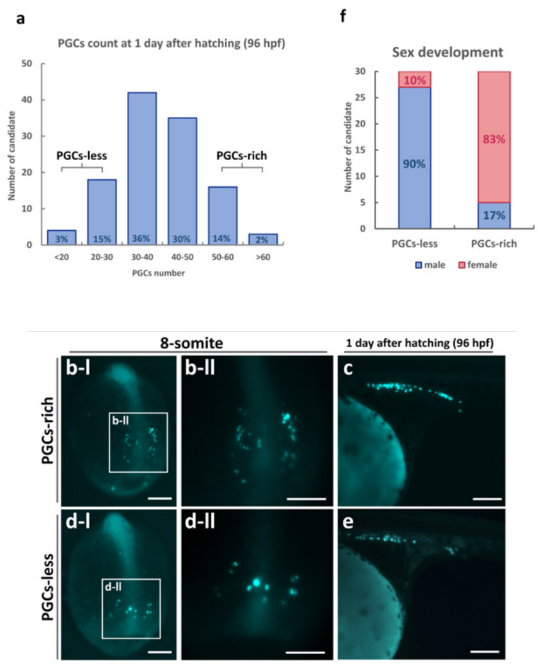

Fig. Abundance of PGCs and sex development analysis in germline-specific transgenic fluorescent freshwater angelfish.

BACK TO BASICS: IS MY TANK SELF-CLEANING? A SYSTEMS APPROACH TO SUSTAINABLE AQUACULTURE 4.0

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Achieving a self-cleaning aquaculture system is paramount to the industry's sustainability and profitability. A comprehensive understanding of particle dynamics and impact of upstream conditions on the sludge properties is critical to transform aquaculture operations into efficient and eco-friendly enterprise. By integrating advanced Industry 4.0 technologies with a coherent understanding of ecosystem dynamics, we provide tailored solutions to optimize the particle flushing from the rearing domain for better health of the overall system. This encompasses:

- **Biosolid Characterization and Management:** In-depth analysis of biosolid composition and properties is necessary to inform effective treatment and valorization pathways. Tailored solutions for biosolid reduction, recycling, and energy recovery are advised for maximizing the resource utilization and minimizing the environmental impact.
- **Precision Aquaculture Modeling:** Leveraging cutting-edge simulation tools, we create virtual representations of aquaculture systems to predict particle accumulation, nutrient fluxes, and water quality parameters. This enables proactive management strategies and identifies opportunities for process optimization.
- **Sensor Integration and Data Analytics:** Advanced sensor technologies to monitor critical water quality parameters, solid levels and fish health in real-time. Data analytics and data-driven solutions provide actionable insights to optimize feeding strategies, water exchange rates and filtration processes.
- **System Optimization and Control:** By combining our modeling expertise with real-time data, we develop advanced control systems to automate key aquaculture processes. This ensures optimal operating conditions, reduces labor costs, and improves overall system performance.

A holistic approach empowers aquaculture producers to make data-driven decisions, enhance resource efficiency, and mitigate environmental risks. By addressing the fundamental challenge of biosolid management, we contribute to the creation of sustainable, resilient, and profitable aquaculture systems.

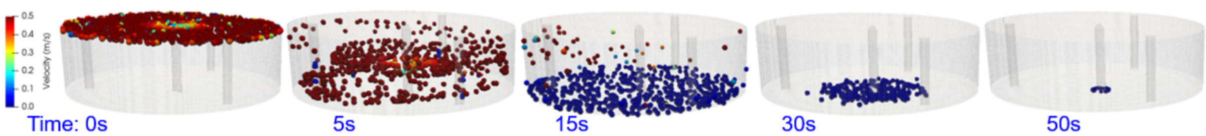

Figure 1: Proper control of biosolids in a culture tank leads to complete self-cleaning

REVITALIZING AQUAFEED SUPPLY CHAINS WITH REGENERATIVE AGRICULTURE

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The global rise in aquaculture demand has intensified environmental challenges, including deforestation, water mismanagement, and biodiversity loss, all of which are exacerbated by traditional agriculture production methods. This presentation will explore the transformative potential of regenerative agriculture in addressing these issues and revitalizing aquafeed supply chains. By adopting practices such as agroforestry, cover cropping, and reduced tillage, regenerative agriculture offers a sustainable path forward. It contributes to reforestation, water conservation, biodiversity enhancement, and soil health improvement, thereby making aquafeed production more sustainable and resilient to climate change. We will highlight how these regenerative practices not only reduce the environmental footprint of aquafeed production but also bolster ecosystem services, ensuring a sustainable future for the aquaculture industry. This approach aligns the sector with global sustainability goals, offering a model for reducing ecological impacts while supporting the growth of aquaculture.

IS GROWTH IN EUROPEAN AQUACULTURE PRODUCTION LIMITED BY HOW AQUACULTURE IS FEATURED IN MARITIME SPATIAL PLANNING? THE CASE OF IRELAND

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Coastal and maritime sectors are crucial for Europe's prosperity and sustainable growth, accounting for 75% of external trade and 37% of EU trade, and have created 5.4 million jobs and generated €500 billion annually. Maritime Spatial Planning (MSP) is essential for long-term sustainability in Europe's Blue Economy sectors, by balancing the spatial needs of maritime sectors with the need to preserve the functioning of marine ecosystems. European aquaculture is one of the major maritime economic activities (MEAs) of the EU's Blue Growth agenda. Since 2014, Member States have been legally mandated to have maritime spatial plans in place by March 2021, with the freedom to develop their plans according to national priorities and situations. This has resulted in significant variations between countries, in terms of how they approach planning generally and how aquaculture is planned in particular. Some countries have allocated zones specifically for aquaculture development, whilst others take a policy-based approach, specifying principles for various maritime sectors. In parallel with progress on MSP policy, in terms of aquaculture policy, the EU has produced Strategic guidelines for a more sustainable and competitive EU aquaculture for the period 2021 to 2030 (COM(201 /2021/236) aiming to produce nutritious, products with a low environmental and climate footprint, creating economic opportunities and jobs, and becoming a global reference for sustainability and quality. As such the guidelines identify four key areas where further work is needed. One of these relates to building resilience and competitiveness and accordingly necessitates access to space and water and a regulatory and administrative framework that is transparent and efficient.

This paper examines Ireland's policy and practices regarding aquaculture, focusing on the National Marine Planning Framework and the National Strategic Plan for Sustainable Aquaculture Development (NSPSA) 2030 assessing how these necessities are addressed. An initial examination indicates that more emphasis is placed on the aquaculture licensing system, for example, than how Maritime Spatial Planning could be better utilized to allocate space to facilitate sectoral growth whereas in other EU Member States, high-production countries have been seen to allocate dedicated zones. Good practice does exist in Ireland but in the form of Coordinated Local Aquaculture Management Schemes (CLAMS) which exist for particular bays and inshore waters. How these could contribute to the objectives of MSP is also examined in this paper. Ireland's aquaculture sector, worth €175 million in 2021, produces 40,000 tonnes of finfish and shellfish, employing 1,800 people mainly in rural areas. The sector has a high potential for sustainable jobs and growth but requires coordinated development in coastal and offshore Irish waters to avoid conflicts with other marine uses. The paper concludes with recommendations on how to modify management practices to achieve multiple policy goals.

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SMART, SUSTAINABLE, SHELLFISH AQUACULTURE MANAGEMENT: ADVANCING TECHNOLOGICAL DEVELOPMENT OF OYSTER AQUACULTURE IN THE USA

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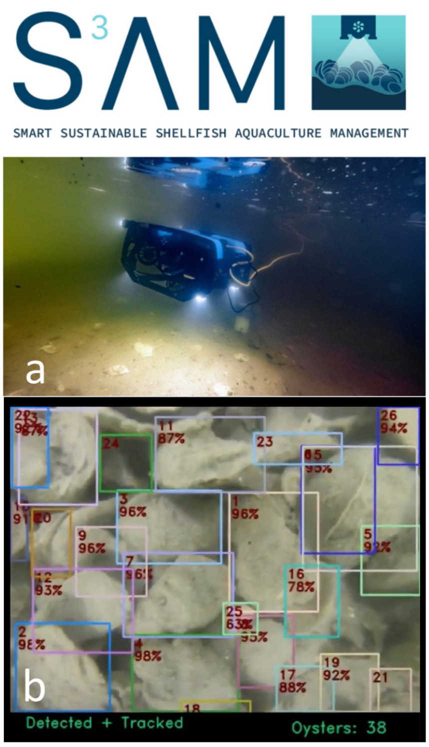
The oyster aquaculture industry has steadily grown in the United States in recent decades. Alterations in regulations and steady demand from consumers have fueled industry growth, which is principally driven by bottom-culture of crops, where oysters are planted on a bed of shells and typically harvested 2-3 years later. Although the oyster aquaculture industry has existed for centuries, the technology used to manage and harvest crops is antiquated and inefficient. Specifically, growers have used dredges to track the growth of their crops and harvest them. Dredges are inefficient at harvesting crops and may damage those left behind. To advance the technological development of the industry, we have developed technology that enables off-the-shelf robotic underwater vehicles (Fig. 1a) to help growers manage their crops and monitor lease conditions. This presentation will provide a brief overview of the Smart, Sustainable, Shellfish Aquaculture Management (S3AM) robotic system and its products that extend from a highly interdisciplinary team consisting of mechanical engineers, roboticists, computer scientists, resource economists, ecologists, and aquaculture industry extension agents.

Tracking shellfish production begins with identifying crops within the lease. Distinguishing oysters from rocks or empty shells using optical sensors in real-time poses several challenges. We used machine-learning techniques to train computers to recognize and enumerate oysters on active leases (Fig. 1b). In highly productive estuaries, such as the Chesapeake Bay, optical sensing of oysters is challenging under low visibility conditions. Therefore, we also used sonar sensors and machine-learning approaches to distinguish crops, empty shells, and bare substrates. Underwater localization of S3AM was also developed so crop information would be spatially explicit. Over time, monitoring with S3AM will provide production rates (crop growth and health) and lease water quality information, which may be useful for detecting acute environmental changes that may harm crops. To package this information, we developed an application so growers could access their crop information for greater management control. This program has leadership throughout the US and it is intended to serve the shellfish industry along all coastlines.



A NEW APPROACH TO ENVIRONMENTAL RISK ASSESSMENT IN MARINE AQUACULTURE

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A better understanding of the potential cumulative impacts of large-scale fish farming, could help marine aquaculture to become more environmentally sustainable. Risk assessment plays an important role in this process by elucidating the main challenges and associated risk factors. In Norway, impact of sea lice on wild salmonids, genetic introgression of escaped farmed salmon and poor fish welfare are identified as the main challenges to sustainable salmon production. However, also pathogen transmission to wild fish, organic emissions, use of drugs and anti-fouling agents as well as the use of wild caught wrasse for delousing plays a role in a comprehensive environmental risk assessment of Norwegian aquaculture production.

Here we describe an approach to risk assessment using Bayesian belief networks to visualise overall casual structures (Figure 1). The risk assessment process is made up of three main steps 1) defining the top node and designing a graphical structure of underlying nodes in terms of risk sources (RS´), events (A´), and consequences (C´); 2) measuring the uncertainties related to risk sources, events and consequences in terms of subjective probabilities (P) and strength of knowledge (SoK); and 3) aggregating the uncertainty measurements of each node upwards until reaching the top node. The suggested methodology is anchored in the latest thinking in risk science and has been tested in a thorough study of environmental risk related to Norwegian aquaculture. The study shows that the new methodical approach has an immanent pedagogical potential and contributes to strengthening risk understanding and risk acknowledgement among stakeholders. In conclusion, the suggested risk assessment methodology has proven a valuable tool for marine scientists in analysing, evaluating, and communicating environmental risk.

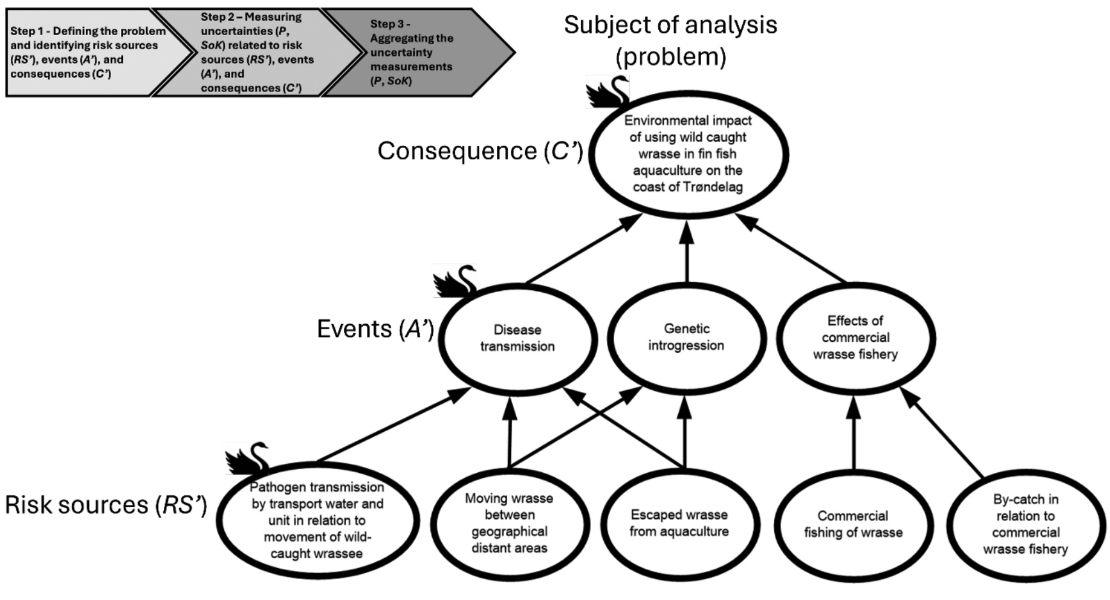

FIGURE 1: Structuring of specific consequences, events and risk sources associated with the environmental effects of using wild-caught wrasse for delousing of salmonids in fish farming. The black swans symbol visualizes the potential for surprises.

PERFORMANCE OF NEAR ZERO WASTE BIOFLOC-BASED RAS AND THE RESULTING BIOFLOC UTILIZATION – OVERVIEW, PROS AND CONS

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70%–80% of the nutrients (N, P, and C) used to feed fish and aquatic animals are released into the water as dissolved and solid excretions resulting in environmental pollution. As an alternative to the conventional nitrification followed by water exchange or denitrification, assimilation-based biofloc technology (BFT) is used to in-situ convert aquaculture excretions (with an additional external carbon source) into protein-rich microbial biofloc. BFT is primarily used in shrimp and tilapia pond culture, and was shown to enhance feed conversion efficiency, biosecurity, and wastewater recycling.

We suggested employing BFT within a side assimilation reactor to integrate the idea of ammonia assimilation into microbial biomass within intensive recirculating aquaculture systems (RAS). This reactor relies on activated sludge under microaerophilic conditions, with the fish-rearing water and solid excretion continuously circulating through it. To address the challenge of high outflowing turbidity, we introduced a submerged ultrafiltration membrane bioreactor (MBR), which led to an average turbidity in the fish of less than 25 NTU. The system offers numerous advantages over conventional RAS and traditional BFT methods. These include exceptional water reuse rates, energy conservation, and minimal waste generation. Notably, the biofloc boasts a protein content of approximately 40%, a substantial increase compared to aerobically based BFT systems which typically contain around 20% protein. Additionally, the system's low oxygen requirements have led to a remarkable reduction in daily energy demand, potentially by as much as 75%. Furthermore, comprehensive assessments have been conducted to evaluate its environmental impact and economic viability.

Dry microbial biofloc was utilized as a component in fish feed, replacing 15%, 20%, and 25% of the original feed, to examine its impact on fish growth, survival, and disease resistance. Across all groups, fish survival reached 100%. While the control group exhibited slightly higher weight gain (~15%) compared to the biofloc-fed group, the latter demonstrated significantly superior survival rates post-bacterial challenge (89% survival), contrasting with the control (58% survival, $p = 0.01$). Variations in immunity were also observed based on the origin of the external carbon source, with semolina proving more efficacious than sugar (Fig. 1). Furthermore, an economic analysis underscored the potential advantages of implementing biofloc commercially.

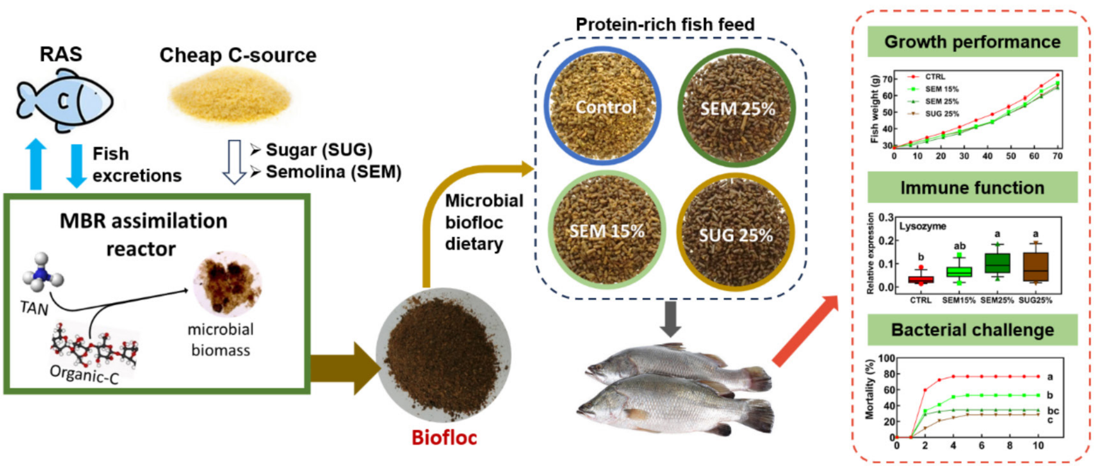

Fig. 1 Biofloc production and use in a fish feeding trial.

RESPONSES OF THE BENTHIC ECOSYSTEM TO FISH FARMING DISCHARGES IN A TROPICAL ENVIRONMENT : THE CASE STUDY OF RED DRUM FARMING *Sciaenops occelatus* IN ROBERT BAY (MARTINIQUE)

Grouazel Mélina*, Fiandrino Annie, Geoffroy Thibault, Nahon Sarah, Théo La Piana, Falguière Jean-Claude, Nicolas Desroy and Callier Myriam

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A challenge for the sustainable development of marine aquaculture is to determine the appropriate level of production that respects the ecological carrying capacity (ECC) of the receptor ecosystems. Most environmental thresholds have only been defined for temperate ecosystems, and very few studies have quantified impact thresholds in tropical environments. To fill this gap, my PhD will 1) qualify the response of benthic habitats to organic enrichment in a tropical case study, 2) identify indicators of the effects of aquaculture impact on the benthic compartment and determine thresholds via a meta-analysis, and 3) develop an impact module integrated into the MOCAA model.

This presentation exposes results of a sampling campaign conducted in 2022 in Martinique. To quantify discharges from a fish farm (*S. ocellatus*), particle traps were deployed parallel and perpendicular to the main current direction at 0, 25, 50 and 150 m from the cages. Hydrodynamics and zootechnical data were also collected to model the environmental impact of the farm with the MOCAA model. Grab samples were taken to analyze the structure and diversity of benthic communities, supplemented by sediment cores to analyze sediment physico-chemical parameters. Isotopic signatures of each compartment (food, sediment in traps, sediment) were also carried out to trace fish farming waste and determine sources of OM content.

Initial results of this study show that sedimentation rates are significantly higher at station 0 m in the main current direction, compared to other stations. Conversely, OM content in sediments was not significantly different between stations and transects. Isotopic signatures of OM from particle trap samples ranged from -22.45 ± 0.57 to -18.77 ± 0.17 ‰ ($\delta^{13}C$) and 3.18 ± 0.02 to 4.89 ± 0.29 ‰ ($\delta^{15}N$) (Fig.1). Samples follow a clear gradient along both transects, with a depletion in $\delta^{13}C$ and an enrichment in $\delta^{15}N$ at 0 m. A similar but less marked pattern was also observed for $\delta^{13}C$ and $\delta^{15}N$ values of OM from the grab samples in the main current direction. Ongoing analyses (benthic communities structure, farm waste modelling) are in progress to explain and precise these observations.

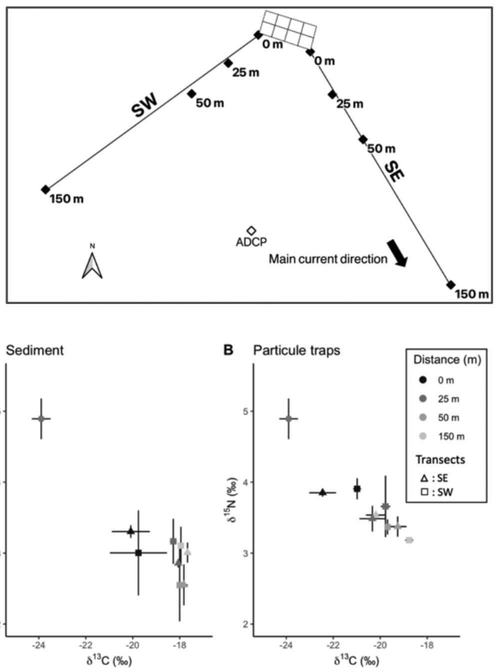

Fig.1 : Map of the study site and isotopic signatures of (A) the sediment and of (B) the water particular matter (collected with particle traps) at four distances along the two transects (SE and SW). Red dot represents the farm waste isotopic values for fish feed pellets.

ALGATEC - A PLATFORM FOR LARGE SCALE PRODUCTION OF MICROALGAE AND FOR R&D DIRECTED TO THE SCALE-UP OF PRODUCTS TO THE AQUACULTURE SECTOR

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Algatec, is a 14 ha industrial microalgae production platform, that is strategically located in Lisbon, Portugal. This first-of-its-kind facility in Europe is a product of a long-term partnership between the technology supplier A4F - Algae for Future, the landowner and producer Green Aqua Póvoa, and the commercialization partner Algikey. This unit is unique in Europe not only due to its size, with a nominal capacity of 200 ton of biomass a year but also for its synergistic integration with surrounding industries in a zero-waste and circular economy concept.

The Algatec unit is designed to meet algae requirements of aquaculture hatcheries in a timely and efficient manner with support of a close-by logistics hub. By providing a reliable and consistent supply of high-quality microalgae, in live, frozen paste, powder and extract forms, the Algatec platform aims to enhance hatchery efficiency, performance and contribute to the sustainable growth of the aquaculture industry.

With more than 20 years of accumulated experience, A4F is a leading provider of turnkey industrial algae units - DBOT (Design, Build, Operate and Transfer) - offering solutions for the decarbonization of industries, wastewater treatment, land valorization, among others through algae biomass production for several applications.

Beyond industrial technology, A4F is an R&D specialist focused on advancing algae cultivation technologies and product development for several applications. Algatec, as a platform for demonstration-scale projects, hosts activities of collaborative projects with relevant European partners such as InnoProtein, Multistream, and Asteasier with direct application in aquaculture. The general objective of these projects is to explore innovative and cost-effective approaches for algae production and biorefining at scale, ultimately driving the development of new, sustainable functional ingredients for hatcheries and feed sectors. In this presentation the highlights of those projects will be described.

Figure 1 – Algatec unit producing Nannochloropsis gaditana and Dunaliella salina for aquaculture hatcheries in 35m³ modular tubular reactors.

DIETARY PHOSPHORUS LEVELS AFFECT NUTRIENT METABOLISM BUT NOT THE GROWTH OF TAMBAQUI *Colossoma macropomum* IN THE GROW-OUT PHASE[a]

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Phosphorus (P) is one of the most important macromineral for fish due to its role on the sustainability and metabolism of aquacultured species. Therefore, P quantitative requirement has been extensively studied in several fish species, except for Tambaqui which only a handful of studies has been reported. Therefore, this study aimed to determine the available phosphorus (AP) requirement for tambaqui during the grow-out phase using several biomarkers of P adequacy.

A total of 128 tambaqui (395g ± 20) were randomly assigned to 15 1000L tanks connected to a recirculating water system and fed diets containing graded AP levels in triplicate. Fish were fed for 180 days and growth performance, bone mineralization, carcass composition and serum biochemistry were evaluated.

No mortality or apparent signs of P deficiency were observed during the growth trial. Dietary AP levels did not affect growth performance, except for phosphorus utilization (Table 1), which decreased linearly. Bone mineralization increased with dietary AP levels (Fig 1), while the scale and whole-body mineralization were unaffected. Dietary AP levels affected serum biochemistry ($P<0.05$), but not serum P and triglycerides ($P>0.05$). Based on these findings, we concluded that tambaqui in this stage developed properly without dietary inorganic P supplementation to plant-based diets. A minimum of 4.1 g/kg is needed to maintain growth parameters but leads to higher adiposity. On the other hand, the AP requirement for maximizing bone mineralization was 10.3 g/kg diet. Additionally, the level of 6.17 g/kg AP seems to be sufficient to improve immune response parameters such as total serum proteins, lysozyme, and immunoglobulins, thereby influencing the fish's resistance.

[a] This research was financially supported by Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG).

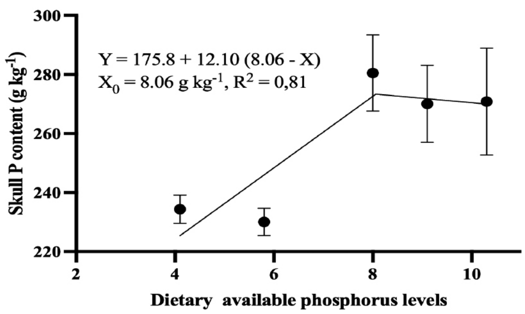

Figure 1. The effect of dietary AP levels on sull P content of tambaqui

Table 1. Daily feed intake (DFI), daily weight gain (DWG), feed conversion ratio (FCR), phosphorus utilization rate (PUR) and protein efficiency rate PER of tambaqui fed diets containing graded available P (AP) levels for 180 days.

	Dietary AP levels (g kg⁻¹)					
Variables	**4.1**	**5.8**	**8**	**9.1**	**10.3**	**P value**
DFI (g)	7.64 ±0.21	7.85 ±0.22	8.26 ±0.03	8.16 ±0.05	8.34 ±0.56	0.0938
DWG (g)	2.93 ±0.25	3.26 ±0.44	3.13 ±0.19	3.03 ±0.34	3.02 ±0.18	0.7413
FCR	1.98 ±0.04	1.92 ±0.21	2.11 ±0.09	1.93 ±0.11	1.95 ±0.30	0.822
PUR[1] (%)	1.75 ±0.14	1.34 ±0.16	0.89 ±0.06	0.76 ±0.08	0.66 ±0.07	<0.001
PER (%)	2.22 ±0.18	2.23 ±0.27	2.01 ±0.13	2.03 ±0.22	2.24 ±0.24	0.5993

Means in a row followed by the same superscript letter are not statistically different by SNK multiple range test ($P > 0.05$); [1]Linear= 2.421087-0.177693x (R^2= 0.92)

PIRARUCU REQUIRES TAURINE TO MAXIMIZE GROWTH AND ANTIOXIDANT STATUS WHEN FED DIETS HIGH IN PLANT-BASED INGREDIENTS[a]

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Taurine (Tau) is a free conditionally essential amino acid for most fish species that plays a significant nutritional role, mainly in carnivorous fish species. However, limited studies are still available regarding the role of Tau on pirarucu nutrition, an important Amazonian fish species.

Therefore, this study used diets high in plant-based ingredients to investigate the effect of Tau supplementation on juvenile pirarucu's growth, metabolism, and health (n = 115, Weight = 504.12 ± 19 g). Fish were fed five diets containing increasing levels of Tau: 0.95, 5.1, 9.6, 12.9, and 17.2 g/kg in a completely randomized design with three replicates per treatment. After a 60-day feeding trial, growth, hematology, blood chemistry, Tau content in muscle, carcass, and liver, antioxidant status, and immunological parameters were evaluated.

The control group (0.95 g/kg Tau) showed the lowest growth. According to the segmented regression model, the recommended Tau level for maximizing the development of juvenile pirarucu was 4.49 g/kg of feed (Fig 1). Additionally, fish fed the lowest dietary Tau level showed the highest ROS (reactive oxygen species) concentration (Table 1). Dietary Tau levels did not affect hematological and blood chemistry ($p>0.05$). Fish fed 9.6 g/kg of Tau showed increased catalase activity, while the control group had the worst result in NBT (nitroblue tetrazolium reduction test). Fish fed with the highest dietary Tau levels (12.9 and 17.2 g/kg) showed increased total serum immunoglobulin concentration. These results suggest that the optimal inclusion level of Tau in diets for juvenile pirarucu is 4.49 g/kg of feed and that this amino acid acts as a mitigator of oxidative stress.

[a] This research was financially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo a Pesquisa do Estado de Goiás (FAPEG).

Figure 1. Growth performance of Pirarucu fed graded Tau levels

Table 1—Antioxidant status of Pirarucu fed graded Tau levels.
According to Duncan's test (P<0.05), the rows' means followed by different letters differ significantly. ROS - reactive oxygen species, SOD - superoxide dismutase, TBARS - thiobarbituric acid reactive substances, CAT - catalase

	Taurine levels (g/kg)					
Variable	**0.95**	**5.1**	**9.6**	**12.9**	**17.2**	**P value**
ROS (pcmol /mg)	8.97 ±0.5^a	6.49 ±1.5^b	6.02 ±1.4^b	5.63 ±2.8^b	6.15 ±2.2^b	<0.01
TBARS µM of MDA /g	5.30 ±1.4	4.94 ±1.3	6.25 ±1.4	5.15 ±2.7	5.08 ±2.7	0.215
SOD (U /mg)	0.38 ±0.1	0.34 ±0.1	0.42 ±0.2	0.39 ±0.2	0.41 ±0.1	0.315
CAT (U /mg)	45.63 ±10.9^c	44.90 ±14.1^a	59.28 ±17.4^{bc}	39.27± 22.0^{ab}	44.46± 18.2^{bc}	0.008

INFLUENCE OF DIFFERENT 3'UTRS OF GENES ASSOCIATED WITH GERM CELL DEVELOPMENT ON EXPRESSION LOCATION IN ATLANTIC SALMON

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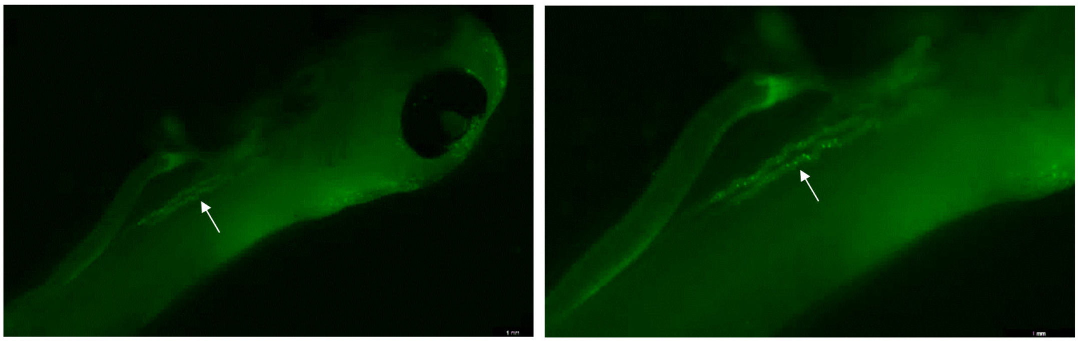
The possibilities of applications using mRNA are increasing in many fields of research. In aquaculture it could be used for vaccines, protein- and antibody delivery. Depending on the application the expression location and duration within the organism can be of great importance. mRNA stability, translational efficiency and location are to a large extent determined by their 5`and 3`UTRs. Research has shown that GFP-mRNA containing a 3'UTR from the target gene *nanos3* (Atlantic cod) results in expression in the primordial germ cells and their migration can be observed during embryonic development. *nanos3* however, is not the only gene associated with primordial germ cell development and migration and therefore other 3'UTRs from other target genes could results in a different mRNA translation and location. We investigated how different nucleotide sequences originating from salmon germ plasm mRNAs can confer and improve localization of in vitro transcribed mRNAs in the germ cells.



Example of germ cell specific expression location of mRNAs in Atlantic salmon at ~450 DD

PREDICTION OF AGE AND SEX IN EUROPEAN EEL (*Anguilla anguilla*) FROM DNA SEQUENCING BASED EPIGENETIC PROFILING

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Development of robust tools and approaches is one of the key strategies in achieving success with conservation of species threatened with the challenge of extinction. European eel is one such species listed as critically endangered on the IUCN red list and challenges conservation biologists with a complex life history. Recruitment of glass eels in natural water bodies has declined severely in the 80’s and 90’s of the previous century, demanding immediate intervention through restoration efforts. While production of hatchery reared glass eels could alleviate the fishing pressure on the natural stock, closing the life cycle through captive reproduction still remains a challenge and a pressing issue to address. Furthermore, limited data available on the age, sex and genetic diversity makes it near impossible to efficiently assess the stock. Unfortunately, the current approaches employed in determining the age and sex of these individuals are lethal and especially aging require specific training and equipment and even then, the precision of ageing is low. Development of a non-lethal strategy to identify the sex and age of individuals is a requirement that the we aim to address in the current study.

DNA methylation data in addition to providing an epigenetic landscape of an individual, has been proven to serve as a biomarker to predict the sex and relative age of an individual. Within the current study, we aim to develop a robust DNA sequencing based methylation pattern analysis across a range of eel individuals to benchmark biomarkers for sex determination as well as assessment of age. The approach employs non-lethal sampling of tissue (small fin clips). The DNA extracted from these fin clips is subsequently sequenced using the Oxford Nanopore sequencing technology. Since this technology is able to infer base modifications such as methylation directly, we can directly infer fin clip tissue profiles for this epigenetic mark. Fin clip based methylation profiles have been shown to correlate well to age of the fish in previous studies. Since sex-hormones are known to affect epigenetics in various body tissues as well, we expect similarly to infer correlations for this trait too. Nanopore long read sequence data will be generated for around 100 individuals of different age groups including yellow and silver eel individuals from different geographical locations. Differential DNA methylation patterns across these individuals will be correlated with age estimates generated using traditional otolith and histology based age and sex determination approaches respectively. The expected outcome will be a set of methylation biomarkers that are reliable, cost effective and can be routinely used to monitor stocks and potentially help resolve issues associated with captive reproduction. My presentation aims at discussing the results obtained through analyzing Nanopore sequencing data generated at approximately 30x genome depth across multiple individuals.

SENSORY QUALITIES AND METABOLITE PROFILE IN FILLET OF RAINBOW TROUT *Oncorhynchus mykiss* FED A DIVERSE SET OF ALTERNATIVE FEEDS

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Alternative protein sources for fish feed is a highly active topic, and the list of contestants keeps growing. This study takes a phylogenetically wide scope of protein sources ranging from filamentous fungi (*Paecilomyces variotii,* SCP), to marine filter feeding blue mussel (*Mytilus edullis*, MM) and sea vase (*Ciona intestinalis*, CIO), to terrestrial insects in yellow mealworm (*Tenebrio molitor*, MW) and black soldier fly (*Hermetia illucens*, BSF). All with an extra purpose in recycling nutrients back to our food production systems (Albrektsen et al., 2022). However, if the quality of the final product is subpar, is it worth the effort? The sensory aspects are always present, and the feed we use can affect the final product. Therefore, we set aim to compare the sensory attributes of rainbow trout fed a broad group of promising protein sources, using ^{1}H NMR as a potential translator between metabolome- and sensory characteristics.

Five alternative- and one control diet (soybean, CTRL) were given to rainbow trout of 579.5 ± 89.4g (mean ± SD). After 18 weeks, four fish from each tank (with two replicate tanks for each treatment; n = 8) were gutted, rinsed, stored on ice, and transported for sensory evaluation. All 48 fish were sampled for muscle tissue and diced before serving. Each assessor (n=27) was served 12 bowls of tartar (one per tank). The detected sensory attributes of each fish was assessed by four to eight judges using rate-all-that-apply (RATA). Samples were analysed with targeted NMR-based metabolomics, and 45/58 metabolites passed quality control. Four outlier samples were also removed (n=44). ANOVA and sPLS-DA were the main statistical tools used.

Using a 95% CI for the sPLS-DA model, two metabolome cluster pairs showed to be different: CTRL-BSF; and CTRL-MW. Only minor overlaps are evident in BSF-MM, CTRL-SCP, and MM-MW. Six metabolites of particular interests were: 2-aminobutyrate; hypoxanthine; trimethylamine N-oxide (TMAO); betaine; glycine; and o-acetylcarnitine as they were among the most contributing sPLS-DA loadings and showed significant differences in concentrations between treatment groups ($p < 0.05$). Four sensory attributes showed differences between treatments: colour; salmon colour; aroma; and root vegetable taste intensity ($p < 0.05$), but the intensities were low and no clustering differences were found.

All diets produced similar sensory characteristics as the control and the differences found were of low to moderate intensity, emphasising the soft sensory space of rainbow trout. Nonetheless, the fish tends to develop more root vegetable taste under the SCP diet. The metabolites behind a sensory attribute is complex, but some links are worth highlighting. Higher levels of hypoxanthine and uracil shows to be related with higher aroma intensity. These are not responsible for the aroma, but correlate positively with loss of freshness (Chang et al., 2020). Hypoxanthine concentrations were higher for the CTRL-treatment than for BSF, MM, and MW, which could indicate that these diets may prolong freshness. Also, TMAO is converted to TMA during post-mortem bacterial spoilage, causing a characteristic “fishy”-odour (Olafsdottir et al., 1997). TMAO levels were higher in the MM-treatment which could indicate slower spoilage.

Albrektsen et al. 2022. Reviews in aquaculture, 14(4), 1790-1812;
Chang et al. 2020. Analytica Chimica Acta, 1127, 98-105;
Olafsdottir et al. 1997. Trends in food science & technology, 8(8), 258-265.

AUTOMATIC WEIGHT IDENTIFICATION OF SALMON UING 2-D IMAGE BASED CONVOLUTIONL NEURAL TECHNIQUES

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Salmon fish (Salmonidae specie) is a critical component of the trophic dynamics within Scandinavian aquatic ecosystems, especially in Norway. This region has witnessed the proliferation of aquaculture facilities along its coastline, reflecting the industry's significance. These aquaculture enterprises meticulously record mortality rates and the mass of individual salmonids, either expressly or on average. Manual execution of these tasks, however, is labor-intensive and prone to inaccuracies. Various scholars have advocated for the utilization of three-dimensional (3-D) imaging technologies for mass estimation, yet the economic implications render this approach less feasible. Concurrently, attempts to employ two-dimensional (2-D) imagery for this purpose have been made, albeit with limited success in achieving precise mass estimations.

This investigation proposes a methodology for estimating the mass of salmon utilizing two-dimensional (2-D) imagery. Initially, a dataset comprising over 800 images of salmon, with known lengths and masses ranging from 1 kg to 6.5 kg, was compiled as Figure 1. The average mass of the Salmonidae samples was recorded at 4.28 kg. A Mask-Region based Convolutional Neural Network (CNN) algorithm was employed to develop a segmentation model, trained on 250 annotated images of salmon. Notably, during annotation, fin regions were excluded from segmentation due to potential damage or absence, ensuring model robustness. Post-segmentation, the model was evaluated using the remaining images (Figure 2), where the pixel area and length of each fish were determined. Additionally, the ratio of area to length was calculated.

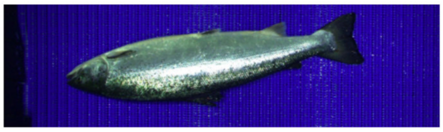

Figure 1. Sample image

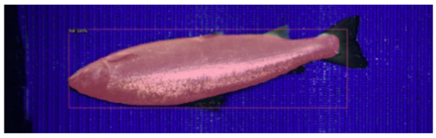

Figure 2. Segmentation results

Subsequently, a simplistic neural network architecture, consisting of two layers, was developed. This network was trained and validated using features such as pixel length, area, and the previously calculated area-to-length ratio, aiming to predict the actual mass of the fish. The predictive model was tested on data from 762 salmon, achieving an average accuracy exceeding 98.2%. The model exhibited a mean error of 77.8 grams, with the minimum and maximum errors being 23 and 165 grams, respectively. Figure 3 illustrates the correlation between the actual and predicted masses of the salmon.

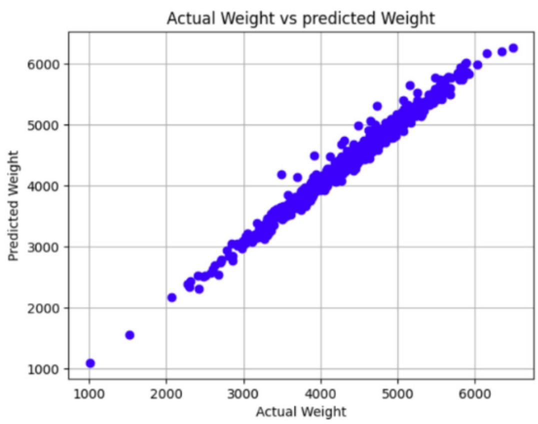

Figure 3. Actual Vs. Predicted Weight

ASTRAL POOL OF TECHNOLOGIES: A TECHNOLOGY VALIDATION PERSPECTIVE WITHIN THE ATLANTIC AREA

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Introduction

Integrated multitrophic aquaculture (IMTA) has garnered growing interest in enhancing sustainability through the sequestration of waste and subsequent primary production. Additionally, it promotes a circular economy and boosts ecosystem services associated with lower trophic-level cultures (Sanz-Lázaro, and Sanchez-Jerez, 2020). IMTA systems encounter several challenges, including the necessity for vigilant monitoring and safeguarding of animal welfare across different trophic levels. There is also an imperative need for technological innovations to ensure climate-resilient cultivation systems capable of monitoring acute changes in key physico-chemical parameters and addressing from traditional threats (e.g., Harmful Algal Blooms) to emerging pollutants (e.g., microplastics). The All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture (ASTRAL) project aims to define, support, and promote IMTA across the Atlantic area. The present work aims to explore the ASTRAL Pool of Technologies from an end-user perspective, considering technology validation feedback within IMTA labs located in Brazil, South Africa, Scotland, Ireland and Argentina.

ASTRAL Pool of Technologies

The ASTRAL Pool of Technologies is designed to enhance IMTA applications with various advanced components. This includes cost-effective Internet of Things (IoT) kits that can incorporate a wide range of commercially available sensors for monitoring critical water quality parameters, vision-based sensors for Harmful Algal Bloom (HAB) monitoring and non-invasive biomass estimation, and biosensor technologies that use bivalve behaviour as a proxy for identifying concerning environmental conditions. Additionally, a fluorometer-spectrometer system allows real-time monitoring of a wide spectrum of physico-chemical parameters and nutrients. These ASTRAL technologies are integrated into an Artificial Intelligence Data Analytics Platform (AIDAP), following a digital twin-style paradigm (Figure 1). It allows for closed-loop feedback and enables virtual replicas of the farming environment, species and facilities to support decision-making better. ASTRAL technologies have been validated within ASTRAL IMTA labs from Brazil, South Africa, Ireland, Scotland and Argentina. The present work further explores technology validation feedback, main challenges and value for aquaculture production.

Conclusions

The ASTRAL pool of technologies provides a complete solution for IMTA lab applications. Technology validation has taken place within ASTRAL IMTA labs to ensure technology readiness level and assess user feedback. AIDAP enables technology integration for continuous and autonomous monitoring. It provides easy data and model sharing towards thread monitoring within different IMTA labs. Feedback on technology validation within ASTRA

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.

References

Sanz-Lázaro, C., & Sanchez-Jerez, P. (2020). Regional Integrated Multi-Trophic Aquaculture (RIMTA): Spatially separated, ecologically linked.. Journal of environmental management, 271, 110921 . https://doi.org/10.1016/j.jenvman.2020.110921.

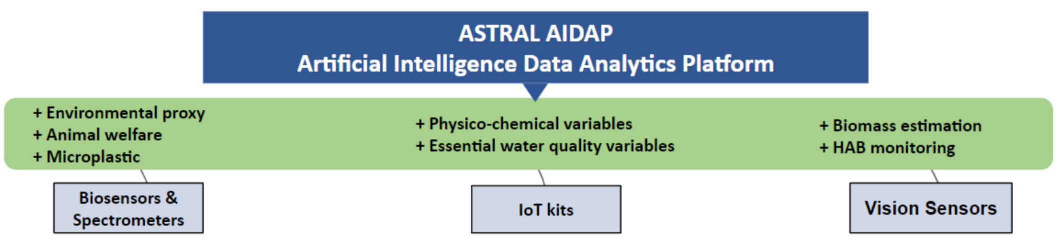

Fig. 1 - Overview of ASTRAL Pool of Technologies

PLANKTOSCOPE-BASED TECHNOLOGY: ADDRESSING MICRO-SCALE MONITORING NEEDS IN IMTA SYSTEMS

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Introduction

Integrated multitrophic aquaculture (IMTA) has garnered growing interest in enhancing sustainability through the sequestration of waste and subsequent primary production. Additionally, it promotes a circular economy and boosts ecosystem services associated with lower trophic-level cultures (Sanz-Lázaro, and Sanchez-Jerez, 2020). IMTA systems encounter several challenges, including micro-level threads such as Harmful Algae Blooms (HAB) and emerging microplastic (MP) pollution. MPs have captured global attention following reports on massive trash islands within all ocean gyres, suggesting they are polluting aquatic ecosystems on an unprecedented scale (Connan et al., 2021). MPs can harm IMTA systems through various pathways, such as releasing toxic compounds and distributing potentially harmful non-native microorganisms. In addition to causing rapid oxygen depletion within aquatic environments, HABs may release toxic substances and cause serious environmental impacts in aquatic ecosystems ZOHDI and ABBASPOUR, 2019). FlowCam[1] is a well-established technology for micro-scale assessment based on flowcytometer. However, it is an expensive and bulky equipment, which makes it impractical for low-cost and large-scale applications. On the other hand, PlanktonScope[2] is an affordable and modular flow cytometry platform tailored for citizen oceanography. It is a versatile and economical device that can be easily adapted for many field applications. The All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture (ASTRAL) project aims to define, support, and promote IMTA across the Atlantic area. The present work explores possible adaptations to the PlanktoScope technology to meet IMTA lab needs regarding micro-scales water sample assessment and its validation for HABs and MPs monitoring within a lab-based setup and a Brazilian IMTA cultivation system.

Modified PlanktoScope unity and technology validation

The proposed technology offers an economical solution by adapting the PlanktoScope unit for use in micro-scale monitoring of phytoplankton and microplastics, leveraging Artificial Intelligence (AI) within IMTA systems. Figure 1 provides a visual representation of the technology's validation in a relevant setting, specifically within a Brazilian IMTA laboratory. End-user feedback underscored the technology usefulness in meeting the specific requirements of IMTA operations.

Conclusions

The present work presented a cost-effective solution based on the PlanktoScope open-hardware and open-software solution for addressing micro-scale microplastics and phytoplankton monitoring at IMTA farms. Technology validation and user-feedback assessment at a Brazilian IMTA lab highlited technology usefulness for both application.

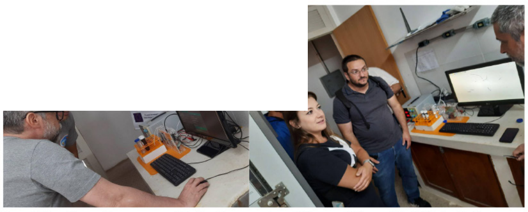

Fig. 1 - Technology validation at a Brazilian IMTA lab system.

(Continued on next page)

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.

References

CONNAN, Maëlle et al. The Indian Ocean 'garbage patch': Empirical evidence from floating macro-litter. Marine Pollution Bulletin, v. 169, p. 112559, 2021.

ZOHDI, E.; ABBASPOUR, M. Harmful algal blooms (red tide): a review of causes, impacts and approaches to monitoring and prediction. International Journal of Environmental Science and Technology, v. 16, p. 1789-1806, 2019.

https://www.fluidimaging.com/

https://www.planktoscope.org/

MICROBIAL CONTRIBUTION TO FOOD DIGESTION IN THE GUT OF ALGIVOROUS SEA URCHIN: A NOVEL RESOURCE OF ALGAL POLYSACCHARIDES DEPOLYMERIZING BACTERIA

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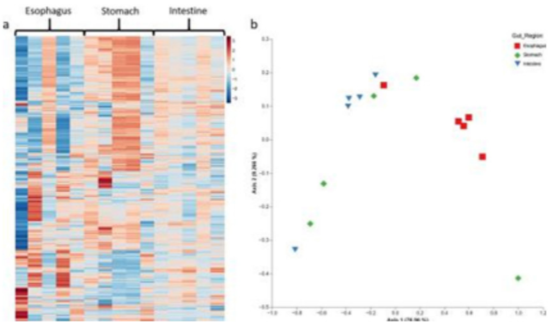
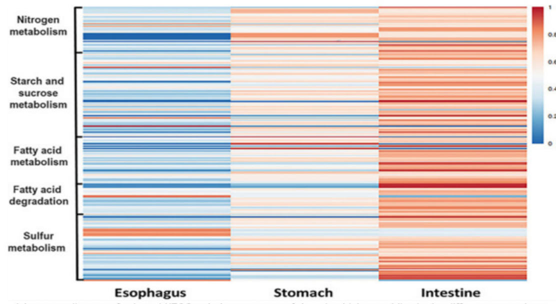
An ecological insight into the spatial arrangement in the gut bacterial community of the algivorous sea urchin *Tripneustes gratilla elatensis* will improve our knowledge of host-microbe relations concerning the involved taxa, their metabolic repertoire, and the niches of activity. We hypothesized that alterations in the bacterial compositional structure under different diets and along the gut are associated with the potential contribution to food digestion. Toward this goal, we investigated the bacterial communities in the esophagus, stomach, and intestine of the sea urchin when fed a mono-specific diet of either *Ulva fasciata* or *Gracillaria conferta,* or an algal-free aquafeed. The study combined 16S rRNA amplicon sequencing, followed by bioinformatics analyses of community structure, interactions, and the prediction of their functional genes.

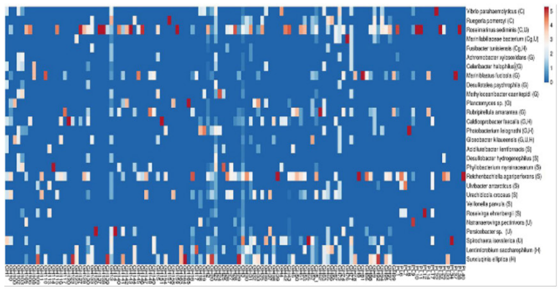
Sea urchins fed with *U. fasciata* grew faster and their gut microbiome network was rich in bacterial associations (edges) and networking clusters. Bacteroidetes was the keystone phylum in the gut with few microbes of this phylum being central hub nodes that maintained community connectivity, while others were driver microbes that led the rewiring of the assembly network based on diet type. Spatial distribution was evidenced by communities with distinct features in the esophagus and intestine. Bacteria that can contribute to *Ulva* digestion were common in the stomach and intestine and consisted of genes for carbohydrate decomposition, fermentation, synthesis of short-chain fatty acids, and various ways of N and S metabolism. The various bacterial genes for the degradation of algal polysaccharides may be valuable for biomass biorefinery processes.



A comparative analysis of the KOs in the gut microbial assemblies of T. gratilla elatensis. (a) A heatmap diagram of the KEGG orthologous genes in the three main gut regions of the esophagus, stomach, and intestine. Each gene orthologue is colored according to its number of KOs in each of the five biological replicates (for each gut region); color varies from dark blue (0) to dark red (20,098). (b) A principal-coordinate analysis demonstrates the dissimilarity between the microbial assemblies in the different gut regions regarding their potential functions based on the measured, weighted UniFrac indices (n=15).



A heatmap diagram of selected KEGG orthologous genes of the microbial assemblies in the different gut regions of *T. gratilla elatensis*. The map highlights the occurrence and the cumulative number of copies after vector scaling in the microbial assembly of the esophagus, stomach, and intestine gut regions.



A heatmap diagram of the variation in content and number of copies of glycoside hydrolase (EC 3.2.1.-) and polysaccharide lyase (EC 4.2.2.-) genes in the genome of the identified key microbes that fell into the determination of core (C), core-generalist (Cg), generalist (G), specialist (S), or unique (U) microbes, and the keystone hub microbes in the microbial networks (H)

ADAPTIVE MECHANISMS OF NILE TILAPIA *Oreochromis niloticus* EXPOSED TO UNIONIZED AMMONIA

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Fish are often challenged to survive when exposed to unionized ammonia (UIA) by displaying some physiological and behavioural coping mechanisms like reduction in metabolic rate. In response to ammonia toxicity, some fish species detoxify ammonia to urea or glutamine or excretes ammonia through the gills to the aquatic environment. Does Nile tilapia also use the same coping mechanisms as other fishes when exposed to UIA? To investigate this, different Nile tilapia groups were exposed to two UIA (7 and 61 µM) levels with a control (0 µM) by measuring haematological parameters, observing the histological changes in the gills and also measuring levels of urea as detoxification mechanism in the fish. The experimental fish were placed in different PVC tubes. Eight fish (BM 303.0±34.0 g) were used for the control treatment.

After 24 h, all the fish were anesthetized. After which liver, blood, gill and white fish muscles were sampled from each fish. For treatments 7 and 61 µM-UIA, three different Nile tilapia groups for each treatment were exposed to the UIA concentrations for 24,72 and 168 h. After each exposure period, the same parameters for the control groups were repeated. Sublethal effect of UIA resulted to a significant rise of plasma NH_3 and Na^+ levels after exposing fish to 61 µM. Liver and muscle urea decreased significantly from the control fish group with a significant increase in excreted urea only after 24 h of exposing fish to 61 µM-UIA (Table 1). In this experiment, gill remodelling occurred in Nile tilapia in response to ammonia toxicity; interlamellar cell mass heights at all exposure periods reduced from the control fish group but significant reductions were observed after 24 and 72 h of 61 µM exposures. This experiment revealed that, Nile tilapia detoxify ammonia to urea. However, the urea is not retained in the tissues of the fish but mostly excreted into the environment.

Table 1: Urea excreted in Nile tilapia tissues exposed to different UIA levels at different exposure times

Urea	Period of exposure (h)	NH_3 (µM) 7	61
Urea in water (mM kg^{-1} h^{-1})	0	0	0
	24	0.30 ± 0.00 ^{a}	3.93 ± 0.00 ^{b}
	72	0.09 ± 0.01 ^{a}	0.32 ±0.34 ^{a}
	168	0.02 ± 0.02 ^{a}	0.21 ±0.33 ^{a}
White muscle urea (mM/g tissue)	0	0.93 ± 0.34 ^{a}	0.93 ± 0.34 ^{a}
	24	0.83 ±0.43 ^{b}	0.34± 0.47 ^{b}
	72	0.91 ± 0.03 ^{a}	0.83 ± 0.34 ^{a}
	168	0.81 ± 0.41 ^{a}	0.70 ± 0.43 ^{a}
Liver urea (mM/g tissue)	0	2.13 ± 1.05 ^{a}	2.13 ± 1.05 ^{a}
	24	2.04 ± 0.60 ^{a}	1.09 ± 0.35 ^{b}
	72	2.71 ± 0.70 ^{a}	1.77 ± 0.95 ^{a}
	168	2.83 ± 0.76 ^{a}	2.57 ± 0.46 ^{a}

EMBRACING MICROALGAE: A SUSTAINABLE SHIFT FROM FISH OIL TO CIRCULAR ECONOMY CO-PRODUCTS FOR OMEGA-3 FATTY ACIDS IN AQUACULTURE

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As the demand for Omega-3 fatty acids continue to rise, the depletion of traditional sources like fish oil poses significant environmental challenges and a significant bottleneck to the growth of the Aquaculture industry. In addition, climate change is leading to increasingly erratic supply patterns for the fish oil and fishmeal industry which only serves to increase uncertainty in the industry. In this scenario, microalgae has moved from a promising alternative to an essential element of the raw material basket offering a growing sustainable solution for meeting the nutritional needs of the aquaculture industry. This presentation explores the transformative potential of microalgae adoption, particularly focusing on its role in mitigating carbon emissions and advancing towards a circular economy model.

By harnessing microalgae, the aquaculture industry is increasingly reducing its reliance on fish oil, thereby alleviating pressure on marine ecosystems. The next step to a more sustainable model is the integration of microalgae production with co-products from a circular economy model in order to shift to a more holistic approach towards sustainability. This shift not only addresses the environmental concerns associated with traditional Omega-3 sources but also fosters resource efficiency and waste reduction.

This presentation aims to delve into the practical implications of incorporating microalgae into aquafeed formulations and discusses economic viability, and the environmental footprint of microalgae-based Omega-3 in aquaculture feeds. Additionally, case studies and success stories from pioneering initiatives will offer insights into the scalability and real-world applications of this paradigm shift.

Embracing microalgae as a source of long-chain Omega-3 fatty acids not only ensure the health and welfare of farmed fish but also propels the aquaculture sector towards a low-carbon trajectory, guided by principles of circularity and environmental stewardship.

PROTEIN SKIMMERS IN RAS – INDUSTRY KNOWLEDGE GAINED AND FUTURE OUTLOOK

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Protein skimmers, or foam fractionators, are gaining interest in large RAS, both for freshwater and saltwater. The function of the protein skimmer may serve different purposes when elaborating on detailed water quality parameters and will most often be used to 'improve water quality'. Protein skimmers can have positive effect on water quality parameters linked to removal of organic matter and particles – mostly related as Total Suspended Solids (TSS), dissolved organic matter (DOM) or Biological Oxygen Demand (BOD). Removing organics can lead to a reduced competitive advantage of heterotrophic bacteria and reduce free swimming bacteria, while also reduce off flavor issues – which can have huge implications for RAS growing fish directly for consumption.

To understand protein skimmer function in RAS better and dimension larger skimmers into RAS removal metrics and dynamics should be understood along the chemical and physical properties inside the foam chamber and the interconnections to usage of ozone in the skimmer. Ozone can severely improve skimmer performance with partial oxidation of dissolved organic molecule, activating potential for removal in the foam cloud, and can be integrated in protein skimmer air stream.

Practical observations point towards a general lower ozone usage compared to industry standards or applied in traditional ozone side streams in RAS – potentially due to the combination of partial oxidation, removal of organics in the foam and the high-water volume in contact with the ozone.

Studies and practical experience also point towards reducing retention time in skimmers from the traditional 1,5-2 minutes, and rather inject ozone into a larger water stream, ultimately gaining better treatment results in the system from the same volume as earlier intended.

Dimensioning large protein skimmer into RAS from an early phase gives the opportunity to direct large water flows into the reaction chamber by gravity, which in combination with lower ozone/oxygen consumption will pose a possibility to reduce both OPEX, footprint and system complexity with pumps, pipes, and automation.

Protein skimmers with ozone inection cause a steady foam formation oxidizingm entrapping and removing dissolved organics from the water loop.
Graphic: Philip H. Gyldenkærne

INNOVATIVE HIGH-DENSITY SHRIMP CULTURE TECHNOLOGY: TRANSFORMING SHRIMP AQUACULTURE INTO INDOOR, CONTROLED SYSTEMS

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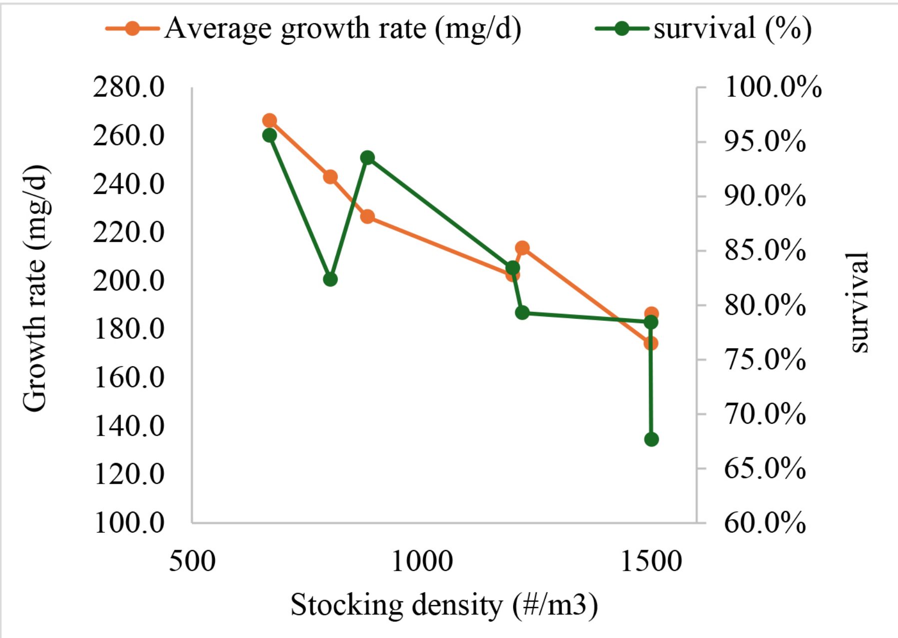
The global shrimp aquaculture industry predominantly relies on extensive outdoor systems located around the tropical belt. Europe, a significant consumer of shrimp, has a total annual consumption of approximately 700,000 tons, with *L. vannamei* accounting for around 450,000 tons. However, European shrimp production is currently only 200-250 tons per year, a figure that does not meet local food security policies, especially in light of geopolitical challenges such as the COVID-19 pandemic and wars that disrupt international trade.

The economy and regulations in Europe necessitate efficient indoor production facilities with minimal environmental footprints. To achieve this goal, the productivity of these facilities must be high, at levels significantly above current industry standards.

This presentation introduces an innovative method for high-density shrimp culture in large-scale Recirculating Aquaculture Systems (RAS), aimed at addressing these challenges. Our approach hinges on the principle that shrimp tank productivity is a function of both tank volume and surface area. The newly developed shrimp tank features an extended surface area, enabling shrimp culture with a target production of more than 10 kg/m³. Essential components for maintaining such densities include efficient organic waste removal, uniform feed distribution, and mechanisms for the removal of shells and dead shrimp, all supported by advanced monitoring and control systems.

Despite these advancements, growing shrimp at increasing densities of up to 1,500 shrimp/m³ has been shown to negatively impacts their growth and survival rates (table 1). The underlying causes of these declines remain unclear. This session will provide an in-depth exploration of the transformative potential of high-density shrimp culture technology, drawing on eight years of research. We will discuss the practical applications, benefits, challenges, and future prospects of indoor shrimp culture, offering insights into how we can collectively advance towards more sustainable and profitable shrimp aquaculture practices.

Table 1: effect of shrimp density (number of shrimp/m³) on survival and growth rates in intensive culture tanks. The data was obtained from 5m³ tanks. The shrimp were cultured from size of about 5gr to approximately 15gr.



TRANSFER AND BIOACCUMULATION OF CHEMICAL AND BIOLOGICAL CONTAMINANTS, AND RETENTION OF PRIONS AFTER SHORT-TERM EXPERIMENTAL FEEDING WITH BRAIN HOMOGENATE FROM SCRAPIE INFECTED SHEEP, IN *Hediste diversicolor* AND *Hermetia illucens* WHEN REARED ON SALMON AQUACULTURE SLUDGE

Andreas Hagemann*, Arne M. Malzahn, Veronika Sele, Ikram Belghit, Jojo Tibon, Hanne Nilsen, Hilde Sindre, Nina S. Liland, Sylvie L. Benestad, Linh Tran, Kristin S. Pettersen, Anne-Katrine Lundebye, Pedro Araujo, Ali Asim, Arne Holst-Jensen

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There is an urgent need to find new, sustainable protein and lipid sources for aquafeeds which are not in direct competition with existing food production value chains and human nutrition. Aquaculture farming entails discharges of large volumes of waste products, ascribed as fish sludge, which consist of feed spill, respiratory products and faeces. Utilizing fish sludge in the circular bioeconomy to feed low-trophic species such as marine polychaetes and insects may provide a sustainable feed chain, however, food security must not compromise feed or food safety, and the use of fish sludge as feed is currently not permitted in the European Union. Integration of fish sludge in the circular bioeconomy requires knowledge of potential chemical and biological risks involved.

The SecureFeed project has investigated the fate of different biological and chemical hazards in fish sludge and their potential fate via ingestion by invertebrates. To test the potential of the polychaete *Hediste diversicolor* and black soldier fly *Hermetia illucens* larvae (BSFL) to bioaccumulate, sustain or expunge known fish pathogens and heavy metals, two 2-week long feeding experiments were conducted during which the animals were fed on a standardised aquaculture sludge mix spiked with different concentrations of two different viruses and two different bacteria. Moreover, juvenile polychaetes and BSFL were exposed with prions from brain homogenates of scrapie-positive sheep, mixed or not mixed with aquaculture sludge, to assess, for the first time, whether *H. diversicolor* and *H. illucens* can serve as potential vectors for prion diseases.

None of the viruses or bacteria could be detected in the polychaetes or BSFL after 14 days of exposure. Polychaetes did not accumulate heavy metals from the aquaculture sludge exceeding levels set by current EU regulations for animal feed ingredients, while BSF larvae bioaccumulated some undesirable substances including Cd, Hg and dioxins. Polychaetes and BSFL orally inoculated with a substantial dose of prions retained detectable levels of prions using the ultrasensitive amplification method (PMCA) indicating that these organisms can potentially serve as mechanical vectors for prion diseases.

This work was funded by a grant from FHF – Norwegian Seafood Research Fund (“SecureFeed”, grant no. #901732). The prion experiments were carried out at the Norwegian Veterinary Institute (Norwegian National reference laboratory for TSE in animals, WOAH Reference laboratory for CWD, Ås, Norway), SINTEF Ocean’s national research infrastructure “Norwegian Center for Plankton Technology (#245937/F50)” and at IMR’s ISO-certified laboratories (NS-EN ISO/IEC 17025). The authors thank Protix for supplying BSFL for the experiments and Dr Olivier Andreoletti from INRAe / ENVT Toulouse, France, for kindly providing us with brain tissues from sheep naturally infected with scrapie.

REPRODUCTIVE AND PHYSIOLOGICAL RESPONSES OF BLUE GOURAMI *Trichopodus trichoprerus* TOWARDS HIGH ENVIRONMENTAL TEMPERATURES

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The Blue Gourami (Trichopodus trichopterus) stands as a beloved ornamental freshwater fish originating from Southeast Asia. Given its habitat in pelagic freshwater ecosystems, the escalating threat of climate warming, particularly in tropical regions, raises concerns for the welfare of gouramis and their natural habitats. Elevated atmospheric temperatures can potentially impose significant challenges on the physiology of gouramis, affecting their survival, reproduction, and growth. Understanding how gouramis adapt physiologically to fluctuating environmental temperatures is crucial for their long-term viability.

In response to this pressing issue, several experiments have been undertaken. Initially, experiments were conducted under normal environmental temperatures, with an average of 27°C, followed by trials under elevated temperatures of 32°C and 34°C, aiming to assess the effects of high-water temperatures on feeding and reproductive performance (Objective 1). Additionally, the impact of temperature variations on embryonic development and larval performance (Objective 2) was investigated.

Further research endeavors are underway to delve into the influence of temperature on the biochemical composition of bubble nest structures, aiming to gain comprehensive insights into the adaptive mechanisms of gouramis in response to changing environmental conditions.

EFFECTS OF EGG INCUBATION TEMPERATURE ON CARDIAC FUNCTION AND MORPHOLOGY IN ATLANTIC SALMON LARVAE

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Introduction: Welfare concerns in Atlantic salmon aquaculture have garnered increasing attention in recent years. Deviating heart morphology is frequently observed and is believed to be associated with mortality. Recent studies have indicated that lower freshwater temperature yields more robust smolts with lower prevalence of heart deformities. However, it is unknown whether decreased temperatures during egg incubation affect cardiac morphology and function in a similar manner. Here, we aimed to investigate possible impacts of altered egg incubation temperature (4°Cvs 8°C) on heart function and morphology in Atlantic salmon larvae.

Methodology: Atlantic Salmon hearts are transparent during the first period after hatching, and functional cardiac measurements were obtained and compared at this stage. Following fertilization, Atlantic salmon eggs were incubated at two temperatures: 4°C (cold) and 8°C (warm). After hatching, temperatures were gradually adjusted to 6°C and larvae were sampled 19 and 47 days post-hatching. Larvae were imaged with a light microscope to assess *in vivo* cardiac function. Then, larvae were euthanized to obtain body weight and length, and hearts were dissected out for detailed *ex vivo* examination of cardiac morphology.

Results: Larvae from warm incubated eggs had more macroscopical deficiencies, such as scoliosis (~10%) and a higher proportion of inward curvature of the ventroventricular ridge (29% and 6% in warm and cold incubated group, respectively). Relative ventricle size was smaller and stroke volume and cardiac output were also lower in the warm group 19 days post-hatching. These differences persisted at 47 days post-hatching, albeit with maintained cardiac output due to a higher heart rate in the warm group. *Ex vivo* analyses revealed that larvae of the warm group had rounder ventricles and larger bulbi.

Conclusion: Our results indicate that elevated egg incubation temperature impairs proper cardiac development and function lowering temperature during egg incubation would improve early cardiac development of farmed Atlantic salmon. How this influences individuals later in life remains to be elucidated.

HIGH GROWTH RATE OFATLANTIC SALMON *Salmo salar,* IS LINKED TO TISSUE OXIDATION, CONSUMPTION OF ATNIOXIDANTS AND RISK OF OXIDATIVE STRESS

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In plants and animals, growing tissues and growth zones generally contain more H_2O_2 and are more oxidized than non-growing tissues (Corcoran et al., 2013, FEBS J. 280, 944). Therefore, we proposed the hypothesis that growth is connected to increased tissue oxidation also in Atlantic salmon.

In the first study, salmon post-smolts, which had been fed two levels of vitamins C+E, were implanted with growth hormone (GH) and held alongside an untreated control group on the two diets for 42 days. Figure 1 shows that the levels of antioxidant vitamins in the fish tissues followed a dose response to the dietary levels and were reduced in response to GH. Several genes which regulate redox metabolism were modulated by GH, especially in fish fed the low vitamin levels. This indicates that the high vitamin level protected against the oxidative effect of GH (Yin et al., 2022, Antioxidants, 11, 1708).

In the second study, salmon post-smolts were exposed to a natural simulated photoperiod during spring. This led to increased growth, consumption of antioxidant vitamins, more cataracts and increased tissue oxidation, as indicated by relevant enzyme activities and the redox transcriptome. Temperature was constant at 9ºC, thus the change in photoperiod alone induced the physiological changes which lead to tissue oxidation and growth stimulation (Yin et al., 2023, Antioxidants*,* 12, 1546).

These studies support the hypothesis that growth is connected to oxidation in tissues of Atlantic salmon post-smolts. It is important to supplement enough antioxidant vitamins in periods of rapid growth, to balance oxidative stress. Perhaps other antioxidants, for example polyphenols from plant extracts, can help alleviate such oxidative challenges, but this needs to be further elucidated.

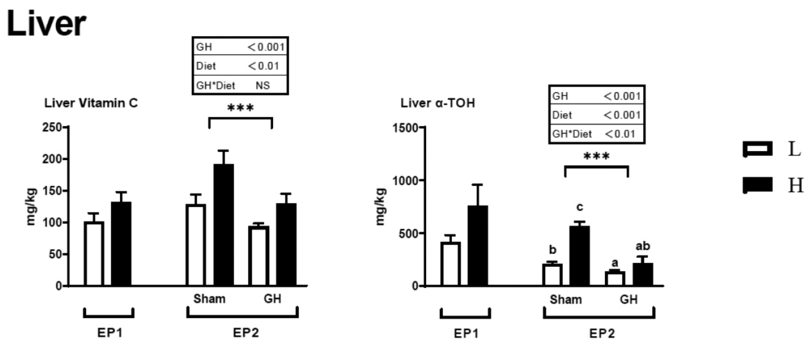

Figure 1. Liver vitamin C and E in Atlantic salmon fed two levels of vitamin C+E, implanted with GH and held for 42 days after implantation (EP: Experimental period 1 and 2, before and after GH implantation, white and black are low and high dietary vitamins, respectively).

ENVIRONMENTAL IMPACT ASSESSMENT OF MUSSEL FARMING IN LIMFJORDEN, DENMARK

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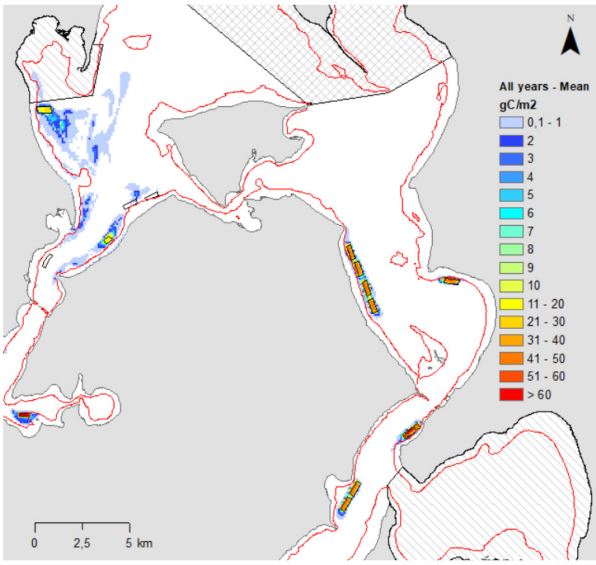
A preliminary environmental impacts assessment of future production sites of mussels on the seabed (culture banks) or in the water column (mussel farms) in the Danish strait, Limfjorden, has been conducted by DTU AQUA in collaboration with DHI A/S. The assessment was based on a modelling tool developed to assess if mussel productions sites may have a potential significant impact on the nearby Natura 2000 areas or nearby stone reefs, biogenic reefs and eelgrass beds.

The modelling tool is used to simulate the dispersal of faeces from the mussel production sites using a dispersal modelling framework based on DHI's integrated hydrodynamic and ecological modelling system MIKE 3 FM ECO Lab / ABM Lab. Preliminary results of the model calculations show that the direct sedimentation from the mussel farms takes place just below the farm and within approx. 100 m from each farm. Resuspension of mussel faeces due to wave strikes and currents under mussel farms and from culture banks can spread faeces to a larger area. For areas close to production sites with the highest exposure to currents and wave strikes, the calculations show that there may probably be some accumulation of organic matter of up to between 10-20 g C/m2 on average at a distance of 100-500 m from the production sites, depending on the location. In general, the accumulated material will occur in an area from the outline of the production site to deeper water, where the general sedimentation conditions favor sedimentation, or along the depth curve in the dominant current direction along the coast. For comparison, the natural average accumulation of sedimented material from the water column in Limfjorden is estimated to 10 g C/m2. Based on expert judgement, oxygen consumption associated with the settling material can be assumed to be very

local under or within the production sites but may also occur locally and briefly outside the primary impact zone and will not in itself give rise to oxygen depletion in the water column. The calculations in the analysis can be assumed to be generally conservative in the sense that there will be a greater accumulation of material on the seabed and a greater oxygen consumption in the model than can be expected in reality. Positive effects of mussel farming on general sedimentation and basin-scale oxygen consumption have also not been included in the calculations. Also, no cumulative impacts including existing mussel production sites were included. These considerations are currently being addressed in ongoing studies.



Accumulated organic C (gC/m2) as mean of means of May and October 2014 - 2016.

SCHIZOCHYTRIUM-DERIVED OIL AS A FISH OIL ALTERNATIVE IN ATLANTIC SALMON DIETS: IMPACT ON FISH GROWTH AND PRODUCT QUALITY

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Identifying alternatives with nutritional profiles similar to fish oil represents a major challenge for the Atlantic salmon farming industry. Such alternatives should be capable of overcoming issues like declining levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in farmed salmon fillets, a consequence of incorporating terrestrial plant oils into salmon diets. These plant oils are inherently low in omega-3 fatty acids, leading to a notable gap in the supply of omega-3 EPA and DHA. An ideal alternative, to overcome this nutritional deficiency, is the oil derived from *Schizochytrium* sp. In our feeding trial, we elucidated the effects of *Schizochytrium* sp.-derived oil (Veramaris*® oil, VMO)* on the growth and product quality of Atlantic salmon reared from their freshwater life phase to their harvest size in seawater.

A two-phase feeding trial was conducted at the research facility of LetSea AS in Dønna, Norway. During the 68-day freshwater (FW) phase, 4 experimental groups were offered one of four diets: 1) a control diet with 5.1% fish oil and 12% vegetable oil (0VMO); 2) a diet with 2.5% fish oil, 13.4% vegetable oil and 1.2% VMO (50VMO); 3) a fish oil-free diet, and containing 14.9% vegetable oil and 2.3% VMO (100VMO) and 4) the diet 100VMO, in which synthetic astaxanthin was replaced with algal astaxanthin (AA) (100VMO+AA). Fish with an average initial weight of 46 g were fed the experimental diets until they reached an average weight of 111 g. After this freshwater phase, the fish from all the groups were PIT tagged and randomly allocated to two groups of the seawater phase. These fish were held in two sea cages (400 fish/cage) and fed either the 0VMO diet or the 100VMO diet, adapted to the nutrient needs of the respective life stage. At the end of the 18 months experimental period, biometric data of all fish were recorded. Thereafter, 12 fish from each of the FW phase groups were sampled to assess fillet proximate and fatty acid composition. The effects of *Schizochytrium* sp.-derived oil incorporated salmon diets on product quality were also evaluated to assess it impact on the harvested fish.

Our results revealed that *Schizochytrium* sp. derived oil is as effective as fish oil, based on the data of feed intake, final weight, condition factor, specific growth rate, thermal growth coefficient and survival rates of the 0VMO and 100VMO groups. Fillet color analysis conducted with a Konica Minolta chroma meter provided information regarding the comparable levels of yellowness (b*) and lightness (L*) across the study groups. There were also indications that the diet containing *Schizochytrium* sp. oil, fed during the seawater phase, had a positive effect on the redness (a*) of the fillet of fish that had been fed the 100VMO+AA diet during the freshwater phase. Nevertheless, the SalmoFan™ measurements revealed that all the groups exhibited values within a narrow range of 26 to 26.5. Statistical analysis did not detect any significant differences in flesh texture or melanin spots. Our findings underscore the efficacy of a sustainable alternative to fish oil that is now considered a viable feed ingredient for salmon feeds.

PARTICIPATORY ASSESSMENT OF AQUATIC SNAILS FOR PRODUCING IN INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) SYSTEM WITH FRESHWATER PRAWN IN BANGLADESH

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IMTA utilizes the byproducts, such as waste, from one aquatic species to serve as inputs for another. In this system, by integrating fed aquaculture with inorganic extractive components (e.g. aquatic plants) and organic extractive components (e.g. aquatic mollusks), farmers develop balanced systems aimed at environmental remediation. Aquaculture, while crucial for meeting the growing global demand for seafood, is facing increasing criticism for its contribution to greenhouse gas (GHG) emissions and climate change. Adopting IMTA in fish farming has been proven to minimize GHG emissions where one of the required organic extractives is aquatic mollusk.

This study involved two stages: first, gathering the community's perception of snails in prawn farming using participatory appraisal tools like focus group discussions (FGDs), seasonal calendars, and ranking exercises, and second, identifying snails through direct field observation using the quadrate sampling method. This two-part research was conducted during the monsoon season (July-October) because snails thrive in water during this time, and people's awareness of them is heightened. Three FGDs were conducted in the three zone according to the degree salinity such as low saline zone (Rangpur), intermediate saline (Botiaghata) and high saline zone (Dumuria) in Khulna district of Bangladesh. From each of the sampling sites, three different types of waterbodies such as, gher (used for prawn and shrimp), aquaculture ponds (used for prawn and finfish culture) and non-aquaculture waterbodies (canal, wetlands, beel, derelict ponds, etc.) were selected. In each waterbody, at least three quadrates, each of $1m^2$, were placed in a zig-zag pattern and the number and types of snail species within each quadrate were recorded.

The identified species through participatory appraisal tools were *Macrochlamys sp., Bellamya sp., Neritina sp., Brotia sp., Pila sp., Lymnaea sp., Lamellidens sp., Sulcospira sp., Sulcospira sp., Sulcospira sp.,* and *Melanoides sp.* According to people's perception, five species of mollusk, such as *Bellamya sp.,* and *Pila sp., Neritina sp., Lamellidens sp.,* and *Sulcospira sp.,* were available in the prawn farming region during the longest period of the year. However, in quadrat sampling method, two species such as *Bellamya sp., and Pila sp.,* were found most abundant irrespective of zone of salinity and waterbodies. Participants in the FGDs reported that these two species can be produced in IMTA ponds along with prawn, that may be used as animal and fish feed. Since there has not been much research on mollusk in Bangladesh, molecular genetic tools can be used for the advanced species-level identification of all those mollusks.

Table 1: Scoring of snail species in terms their abundance as perceived the participants in FGDs

Snail species	**Score**
Macrochlamys sp.	▣▣, ◈◈◈, ◉◉◉
Bellamya sp.	▣▣▣▣▣, ◈◈◈, ◉◉◉◉◉
Perna sp.	▣
Neritina sp.	▣, ◈
Brotia sp.	▣▣
Pila sp.	▣▣▣▣, ◈◈◈◈, ◉◉◉◉◉
Lymnaea sp.	▣
Lamellidens sp.	▣, ◈, ◉◉◉◉
Sulcospira sp.	▣▣▣, ◈◈◈◈◈, ◉◉◉
Melanoides sp.	◈◈◈◈◈

▣= Dumuria; ◈=Botiaghata; ◉= Rongpur

PATHOGEN FREEDOM IN OPEN ENVIRONMENTS: WHAT'S POSSIBLE?

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Demonstrating pathogen freedom in open water environments is challenging as agent and host status may abruptly change. The level of confidence, especially over time, in data obtained from open settings drives both surveillance design and interpretation. How long is too long between surveys, and how much time is required at each refresh to remain confident that pathogen status is as previously described? We propose a method to assess pathogen freedom probability based on surveillance data coupled with information about pathogen introduction risk and apply that method to an open water mollusc farming environment on the Pacific Coast of the United States. We estimate assurance in absence of several mollusc pathogens inferred from surveillance data gathered over the course of three years; and we estimate absence of new introductions from a model derived by expert panel elicitation. We then derive risk-revised probabilities of freedom, to consider the joint probability of the two. Results provide strong evidence to suggest continuous absence of the target pathogens - *Marteilia refringens*, *Marteilioides chungmuensis*, Ostreid herpesvirus 1, *Perkinsus marinus*, and *Perkinsus olseni* - for the host species, region, and time-period studied. Our field example shows the feasibility and utility of considering introduction risk in open water surveillance evaluations. Findings demonstrate (1) the ability to retain science-based confidence in freedom for select pathogens through time in an open system, (2) the limitations of sample volume, and the relative importance of sample frequency, for pathogens that are deemed higher introduction risks, and (3) expert elicitation is a generalizable and valid alternative to formal site-specific risk assessment. Statistical models to formulate and simplify application are under development.

COMPREHENSIVE AQUACULTURE HEALTH PROGRAM STANDARDS (CAHPS)

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U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), in collaboration with the National Aquaculture Association (NAA) with have developed a framework for aquatic livestock health inspection and verification. CAHPS is based on the fundamentals of early detection, targeted surveillance, and risk-based biosecurity practices to ensure livestock health and provide confidence that the health status of the livestock is accurate and protected. Participants in CAHPS develop their own livestock health and biosecurity plans based on the needs or requirements for live animal movement, trade, or marketing. CAHPS is a voluntary health improvement program designed to help farmers reduce the burden of repeated health testing and ensure biosecurity practices are adequate to prevent the introduction of unwanted pathogens.

A POSSIBLE LINK BETWEEN INBREEDING DEPRESSION AND REDUCED WELFARE IN TRIPLOID ATLANTIC SALMON *Salmo salar*

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Genetic impacts of escaped farmed salmon on wild salmon populations is still a big challenge to the sustainability of the salmon aquaculture industry. Farming sterile salmon would eliminate genetic interactions between farmed and wild salmon. To date, the most cost-effective and consumer-accepted method of inducing sterility is triploidisation. Triploidy involves a pressure shock to a fertilised egg, causing the retention of the second maternal polar body. The resulting fish contains two copies of DNA from the maternal line. But the use of triploids in commercial farms has been limited due to concerns over the poorer welfare triploids display compared to standard diploid salmon.

Triploids generally exhibit a dosage effect on the second maternal chromosome set that regulates gene expression. Most often this translates to an additive effect with more influence from the maternal line on the phenotype. We hypothesise that the doubling of the maternal line may cause an inbreeding effect in triploid offspring, and the heterozygosity level of the dam may explain the welfare challenges documented in triploids. Inbreeding can affect several production-related traits like growth rate, survival and developmental rate, and these negative effects could be compounded in triploids with a double maternal load.

To test this, we have followed standard diploid, standard triploid and gynogenetic diploids (individuals with two copies of maternal DNA only) from 12 half-sib families by measuring individual growth, survival, and welfare over an entire production cycle from fertilisation to harvest. A genetic sample from each fish and parent has been taken and will allow us to link individual growth and performance over time to family-level heterozygosity and explore how the maternal genetic load affects welfare.

Our findings could help the industry move toward a more sustainable production by implementing breeding programs specifically designed for triploid salmon production and help mitigate the welfare issues documented on farms at present.

STUDYING THE EFFECTS OF *Hermetia illucens* MEAL ON GROWTH, GUT HEALTH, AND MICROBIOTA IN GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Aquaculture is steadily increasing in the food industry, but a major challenge in aquaculture is to make the production process more environmentally sustainable (Naylor et al. 2021). This means that we need to find ways to protect nature while farming fish and other aquatic life. In recent years, the use of FM and FO in feed has decreased due to the high environmental impact and increased market prices. Therefore, alternative feed ingredients are being sought to meet nutritional needs while minimizing environmental impact and costs. One of the promising alternative nutrient sources for fish feed is insect meal, with *Hermetia illucens* meal (HIM) being particularly valuable. It contains biologically active compounds such as chitin, antimicrobial peptides, and short-to-medium chain fatty acids (FAs). These compounds enhance the innate immune response of fish and alter the composition of the gut microbiome, in which has a positive effect on gut health (Terova et al. 2019). Although many studies have explored the use of insects as sustainable protein sources in fish feed, most have been conducted on a small-scale and in controlled environments. There are only a few large-scale studies. Therefore, the current study investigated the effect of replacing FM with HIM in the diet of gilthead seabream (*Sparus aurata*). Growth performance, gut health and gut microbiota profiles were assessed to evaluate the health of the fish and their response to the diet.

Materials and methods

Approximately 60,000 gilthead seabream with an average initial weight of 131±1.4 g were randomly allocated to four coastal cages (two cages per diet). They were fed either a control diet (FM) or an experimental diet (HIM), with 11% defatted HIM replacing the FM by 35%. Feeding occurred twice daily, six days per week, at rates between 0.6 and 1.3% of biomass. A total of 100 fish per cage were collectively weighed to monitor biomass. After 25 weeks, 88 fish per diet were sacrificed to measure growth parameters. Liver samples from 24 specimens (12 fish per diet, six per cage) were weighed to calculate the hepatosomatic index (HSI). In addition, liver and intestinal samples (proximal and distal parts) from ten fish were used for histologic analysis. For the analysis of gut microbiota, ten fish per diet were sacrificed and their intestines (excluding pyloric ceca) were collected. The digesta and mucosal microbiota were collected and mixed. High-throughput sequencing of 16S rRNA gene amplicons (MiSeq platform, Illumina) was used to characterize the gut microbial community profile, as described in detail in (Rimoldi et al. 2019)with increasing levels of insect meal inclusion (10%, 20%, and 30%. Fecal samples were collected from nine fish per tank for volatile fatty acid analysis. Statistical tests, including Student's t-test or Mann–Whitney U-test for data distribution, were used to compare growth performance, alpha diversity indices, and bacterial abundance between groups. PERMANOVA was used to assess variations in beta diversity. Statistical analyses were performed using Past4 v. 4.02 software, with significance set at $p < 0.05$.

Results and Discussion

The 25-week feeding trial showed that growth parameters, including final body weight, specific growth rate, feed conversion rate, and HSI, were not affected by the diet. In addition, gross examination of the gastrointestinal tract and liver showed no signs of inflammation, indicating that the overall health of the gilthead seabream was not affected by the experimental diets.

Analysis of the gut microbial community profile by high-throughput sequencing showed consistent dominance of phyla Proteobacteria, Fusobacteria, and Firmicutes in the gilthead seabream gut microbiota, regardless of the diet administered. Despite the diet variations, the assessment of alpha diversity showed no significant differences between the two feeding groups. Analysis of the Venn diagram revealed a common core microbiota comprising only eight species, indicating a stable microbial community structure in all groups (Figure 1). In particular, there was a significant decrease in the abundance of *Cetobacterium* and an increase in the relative abundance of genera *Oceanobacillus* and *Paenibacillus*, indicating a modulation of gut microbial populations by the diet.

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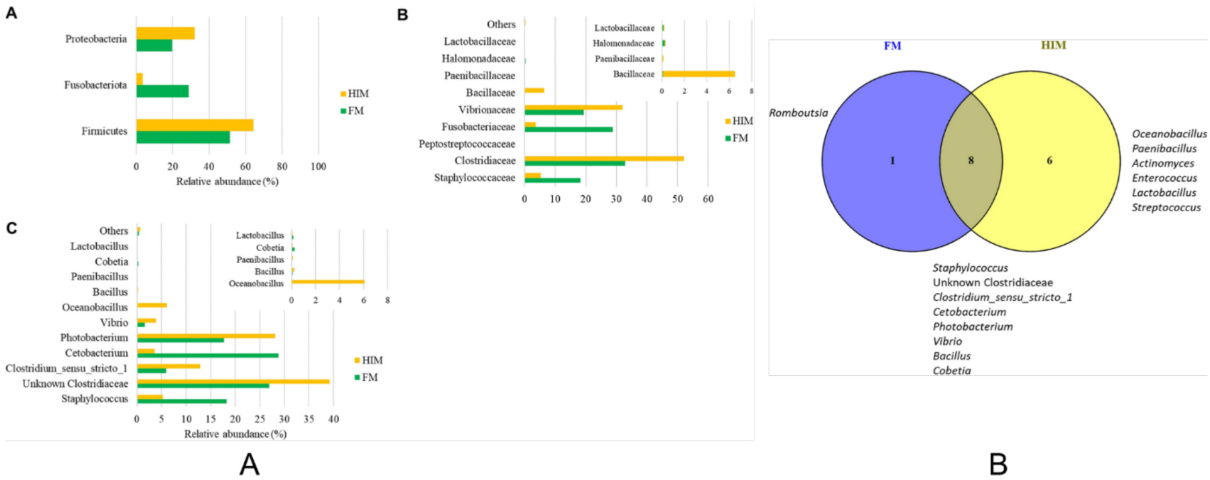

Figure 1. (A) Profiles of the gut microbiota of gilthead sea bream fed two tested diets. The relative abundance of the most common bacterial phyla (A), families (B), and genera (C) are indicated. Bacterial taxa with lower abundance (< 0.5%) are summarized and labeled as "other". **(B)** Core microbiota. Venn diagram showing unique and shared taxa between two experimental groups

Further analysis using multivariate permutational analysis revealed statistically significant differences in gut bacterial communities between the two feeding groups, highlighting the effects of dietary interventions on microbial composition. Estimation of the metabolic activities of the microbial community using the PICRUSt software revealed that fish fed the insect diet had an increased number of genes associated with bacterial chemotaxis, motility, and the two-component signal transduction system. This suggests a possible role of insect meal in modulating microbial metabolic pathways associated with host-microbe interactions.

Moreover, insect consumption in the HIM group resulted in a significant increase in short-chain fatty acids (SCFAs), particularly acetic and propionic acids, which play a critical role in host energy metabolism and gut health, compared to the FM diet. In contrast, no significant differences were found in the levels of butyric acid and isobutyric acid in the fecal samples between the two experimental groups, indicating an effect of dietary measures on SCFA production. Overall, the results suggest that the consumption of HIM positively influences the composition of the gut microbiota and metabolic activities in gilthead seabream, possibly contributing to better nutrient utilization and gut health.

Conclusions

Our results show that the inclusion of HIM as an alternative animal protein source has a positive effect on the gut microbiota of sea bream. This includes an increase in the abundance of probiotic and chitinolytic bacterial genera, leading to improved nutrient utilization and production of short-chain fatty acids (SCFAs) that are absorbed by the host. These changes contributed to the energy metabolism of *Sparus aurata* in coastal farms.

Acknowledgements

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References

Naylor, Rosamond L., Ronald W. Hardy, Alejandro H. Buschmann, Simon R. Bush, Ling Cao, Dane H. Klinger, David C. Little, Jane Lubchenco, Sandra E. Shumway, and Max Troell. 2021. "A 20-Year Retrospective Review of Global Aquaculture." *Nature* 591 (7851): 551–63. https://doi.org/10.1038/s41586-021-03308-6.

Rimoldi, Simona, Elisabetta Gini, Federica Iannini, Laura Gasco, and Genciana Terova. 2019. "The Effects of Dietary Insect Meal from Hermetia Illucens Prepupae on Autochthonous Gut Microbiota of Rainbow Trout (*Oncorhynchus mykiss*)." *Animals* 9 (4): 143. https://doi.org/10.3390/ani9040143.

Terova, Genciana, Simona Rimoldi, Chiara Ascione, Elisabetta Gini, Chiara Ceccotti, and Laura Gasco. 2019. "Rainbow Trout (*Oncorhynchus mykiss*) Gut Microbiota Is Modulated by Insect Meal from Hermetia Illucens Prepupae in the Diet." *Reviews in Fish Biology and Fisheries* 29 (2): 465–86. https://doi.org/10.1007/s11160-019-09558-y.

RISING CHALLENGES IN FRESHWATER PRAWN HATCHERY OPERATIONS: EVIDENCE FROM BANGLADESH

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Shrimp farming in Bangladesh started in eighties through the conversion of rice fields within the coastal polders to mangrove areas. Since the start of the shrimp farming, this industry predominantly centers on native species of shrimp (*Peneaus monodon*) and freshwater prawn (*Macrobrachium rosenbergii*) and in earlier stage for the farming of both species the country heavily relies on wild-caught post larvae (PL). In the early 2000s, 90% of PL for prawn and more than 50% for shrimp (an estimated two billion) originating from natural habitats. Nonetheless, the Bangladesh government's imposition of restrictions on harvesting wild PL in 2000 has had notable repercussions on the stock, ecology, and biodiversity of coastal and marine fish and other aquatic species. Consequently, there has been a growing scarcity of wild PL relative to farm-level demand, accompanied by a substantial rise in the price of harvested PL, prompting a surge in shrimp hatchery development across saline zones of Bangladesh (Cox's Bazar and Khulna) in following years. The success stories of prawn hatcheries, evidenced by a 25-fold increase in post larvae (PL) production from 2004 to 2012 alongside a threefold rise in hatchery numbers, and in case of shrimp a 4.6-fold increase in PL production from 2005 to 2018 with a consistent number of operational hatcheries, have become the home of livelihood for thousands of coastal marginal incoming people. However, the recent decline in PL production was noticeable in prawn hatcheries compared to shrimp. While the production rate of PL in the hatcheries of native prawn experienced a significant decline of 18.6-fold, conversely, in shrimp hatcheries, this reduction was notably less pronounced, at only 1.7-fold in 2022. Even in 2012, the count of private prawn hatcheries stood at 53, yet presently, a mere six hatcheries endure, and others have compelled going out of business. This decline of production and number of prawn hatcheries is attributed to various factors, including the quality of inputs such as probiotics, feed, and chemicals, disease outbreaks, the technical proficiency of hatchery operators, and external influences such as weather conditions, all of which impact the survival rates of PL at hatchery level. Disease and climatic factors are identified as primary drivers of PL mortality in prawn hatcheries, particularly due to the prevalence of diseases such as White Spot Syndrome Virus (WSSV) in warm, shallow waters. The reliance on wild-caught broodstock from the Bay of Bengal exacerbates this risk, with significant viral presence observed during periods of rising sea temperatures. Concerns have escalated in recent years regarding various viruses' impact on early- and post-larval stages, compounded by their genetic adaptability, transmission patterns, and lack of effective antiviral treatments. Notably, viruses like Macrobrachium Muscle Virus (MMV) and Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV) exhibit resilience to coastal environments, necessitating heightened vigilance in climate-responsive prawn hatcheries. Additionally, bacterial adaptation to tropical conditions, driven by factors like quorum sensing and environmental stress, poses further challenges, leading to water quality deterioration and increased susceptibility to disease outbreaks, ultimately impeding prawn hatchery production in Bangladesh.

MOLECULAR DELINEATION, IMMUNE EXPRESSION ANALYSIS, AND ANTIOXIDANT FUNCTIONAL ASSESSMENT OF CATALASE (CAT) FROM CHUB MACKEREL *Scomber japonicus*

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Catalase (CAT) is one of the crucial antioxidants and immunologically important enzymes that regulate host redox homeostasis through the breakdown of cellular H_2O_2 into water (H_2O) and oxygen (O_2). Intensive aquaculture practices of chub mackerel (*Scomber japonicus*) are always challenged by the oxidative and immunological stressors. The identification of multifunctional genes can be one of the promising ways to enhance their stress tolerance ability. Thus, the vital antioxidant and immunological functions of catalase from *S. japonicus* (SjCAT) were investigated under this study.

First, a group of healthy fish were reared, and their tissue samples were collected for the transcriptome establishment and the tissue specific expression analysis. Immune challenge experiment was performed to observe the expression of *SjCAT* upon the immunostimulants and the immune organs such as blood and spleen were collected. All the extracted tissues were subjected to RNA extraction and the cDNA was synthesized to observe the tissue specific and temporal expression. Catalase from *S. japonicus* was identified from the transcriptome and subjected to the *in silco* analysis. The identified SjCAT coding sequence (CDS) was cloned into pMAL-c5X, pcDNA3.1$^{(+)}$ vector for the recombinant protein synthesis and cellular culture-based assays.

The CDS from *S. japonicus* was comprised of 1584 bp encoding for 527 amino acids in length with a molecular weight of 59.99 kDa. The *in-silico* analysis revealed that the active site signature motif and the heme-binding ligand were highly conserved among the teleost fish and all the other identified vertebrate counterparts. The highest evolutionary identity (96%) and similarity (97.9%) relationship of SjCAT were indicated with *Thunnus maccoyii*. The highest *SjCAT* mRNA expression was observed in blood and followed by brain, heart, muscle, and liver. Furthermore, significant modulation of *SjCAT* expression was observed in blood and spleen upon the immunostimulants of Polyinosinic: polycytidylic acid (poly I: C), lipopolysaccharide (LPS), *Vibrio harveyi* (VH) and *Streptococcus iniae* (SI). The recombinant SjCAT (rSjCAT) protein functions were also characterized by catalase activity variation upon pH and temperature changes, ABTS radical scavenging assay, colony counting assay, and the catalase activity assay. In addition, the cell viability assay, and the apoptotic suppression upon H_2O_2 stress, reduction of the metal cation generated ROS production and the NO scavenging assay further confirmed the antioxidant activity of SjCAT. Altogether, results of the study suggest that SjCAT is a potent antioxidant and immunologically important gene for the host survival during the redox unstable environments and critical pathogenic interventions.

RAS FEED EXTRUSION: CAUSALITY & CRITICAL PARAMETERS

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Recirculating Aquaculture Systems (RAS) represent a pivotal milestone in the evolution of aquaculture, offering a controlled environment where water is continuously recycled and cleaned. Unlike conventional farming methods reliant on open water systems, RAS minimizes environmental impact, enhances biosecurity, and maximizes resource efficiency. This innovation will be instrumental in addressing the sustainability challenges facing the aquaculture industry, reflecting a concerted effort to advance practices towards a more sustainable future.

In response to the increasing demands of RAS, there is understandably emphasis on optimizing nutrient density, digestibility, and understanding fish metabolisms within feed formulations. Achieving high nutrient density and digestibility in RAS feeds is crucial for maximizing feed (and biofilter) efficiency and minimizing fecal waste to the water. However, ensuring technical quality remain a challenge due to the stringent nutritional and raw material requirements. For water quality aspects, the need for high technical quality of the feed remains universal for the success of RAS operations:

- Minimal leaching of lipids
- Minimal post-manufacturing dust formation
- High water stability

Employing advanced manufacturing processes and implementing rigorous quality control measures are pivotal in mitigating the risk of feeding with feed of poor technical quality. A notable advancement in this regard is the integration of novel twin screw technology with specially developed screw profiles, aimed at enhancing the cook, homogeneity, and consistency of feeds. This technological innovation ensures that feeds maintain both their nutritional integrity and physical properties, making a significant difference in the impact of RAS and aquaculture.

By prioritizing technical quality and utilizing cutting-edge technologies, producers can optimize feed performance and contribute to the long-term sustainability and success of RAS aquaculture ventures.

Figure 1: High performance RAS feed result in significantly cleaner water.

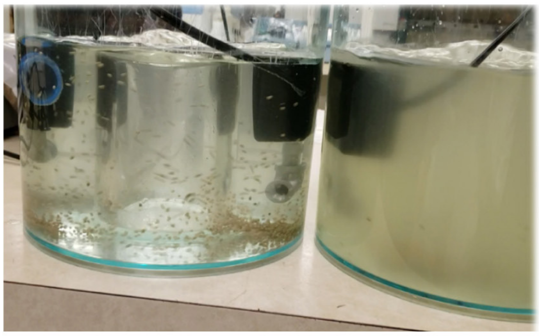
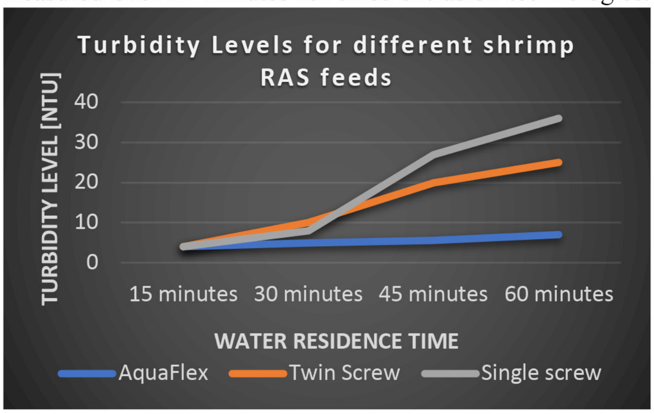


Figure 2: Laboratory scale RAS testing. Turbidity levels measured over 60 minutes for three extrusion technologies.



MAPPING COMPONENTS FOR SUSTAINABLE AQUAFEED PRODUCTION: FACTORS NEEDED TO MAXIMIZE NET RESSOURCE EFFICIENCY

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To enhance net resource efficiency in aquatic feed production, it is important to understand and quantify contributions from all significant factors, including nutritional and technical quality, energy usage, water usage, equipment wear, and emissions. Drawing parallels with industry standards from pet food manufacturing (FEDIAF), traits of environmentally sustainable yet economically viable aquafeed manufacturing can be identified.

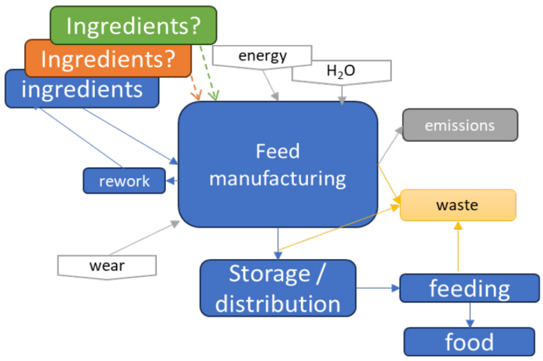
Recent years of global events brings massive market pressure for producing high quality feed for the aquaculture industry at decreasing operational costs. Aquafeed manufacturing faces constant changes in consumer demands and fluctuations in the raw material market. Therefore, flexible, resilient and agile extrusion and drying systems are needed, to be able to deliver on nutritional- and technical qualities, while at a minimum wear and utility expense. These systems are characterized by high up-times, meeting required thermal/electrical energy inputs, as specified from formulation and raw materials, and ultimately yield low variability on moisture and nutrient density.

The optimization of net resource efficiency in aquafeed processing is driven by nutritional quality, technical quality, wear, as well as processing efficiency for utility consumption. In this context, recent advances in extrusion and drying systems will be discussed. This includes capabilities of unique screw designs, mapping of extrusion variables for technical quality as well as a display of how drying parameters can be fruitfully optimized and automated.

Establishing and mapping these components can form the basis for a methodology one can follow to determine where to focus efforts. This approach aligns with cradle-to-grave system boundaries, enabling a comprehensive evaluation of environmental footprint. Consequently, a proposed investment's impact on both net resource efficiency and environmental sustainability can be better understood.

Figure 2: Flexibility and resilience are important attributes in aquafeed extrusion innovation. Reduced wear and waste and increased raw material flexibility allow for improved net resource efficiency.

Figure 1: Feed manufacturing attributes are central for net resource efficiency in aquaculture. Adapted from FEDIAF.



ADVANCEMENTS IN TWIN SCREW TECHNOLOGY FOR AQUAFEED PRODUCTION: A COMPARATIVE STUDY

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Extrusion technology plays a pivotal role in the manufacturing of feed for aquaculture. Traditional single screw (SX) and twin-screw (TX) technologies have been widely employed, each with their strengths and limitations. Motivation is to be able to produce feed at high technical quality to minimize feed footprint, yet being as close as possible to the least cost formulated feed, that meets all nutrient requirements.

It is the intent in this study to quantify the effect of recent innovations made to the design of twin screw extruders, focusing on quality, -effectiveness, and handling of oil and water addition. Newest twin screw technology (AF) use fundamentally different screw elements, built to be resilient to wear as well as being flexible towards raw materials and processing conditions. Latter implies operator skillset to be lesser of a factor as well as having the ability to operate with higher oil addition to the extruder itself.

Objective was to assess which extrusion system produces better quality feed at comparable density targets, is more cost-effective for utility consumption, and handles oil and water addition more effectively. Runs were done using a salmon feed formulation. 5 runs at different processing conditions was done on each of the three extrusion platforms. Mechanical and thermal energy input as well as water and oil additions were the variables used across the 5 different runs. For all runs, same density target was sought to be obtained.

Results indicate that the new twin screw technology outperforms traditional single screw and traditional twin-screw technologies in several key aspects. Specifically, it demonstrated:

- Superior quality and higher uniformity at lower water levels
- More consistent density output across different production conditions
- Creation of more circular pellets, facilitating more accurate bulk density assessment as well as allowing for deeper oil penetration.

These findings underscore the potential of the new twin screw technology, specific for aquaculture, to offer improved and more consistent feed quality while addressing key challenges in traditional extrusion systems – at wear cost comparable to single screw systems.

Table 1: Result overview – feed produced on three extrusion platforms

	AF	TX	SX
Pellet CV(D) [%]	2.94	4.84	5.85
Circularity [%]	95.8	95.9	90.3
Durability [%]	84.8	89.3	69.8
Avg. water inclusion[%]	31.9	32.8	33.7
Min-max ρ across runs [g/L]	378-409	380-469	320-431
Wear cost assessment (experience) [$/ton]	1.35	1.95	1.25

AQUASTAR: AUTONOMOUS UNDERWATER SYSTEM FOR TARGETED ASSESSMENT & REPAIR

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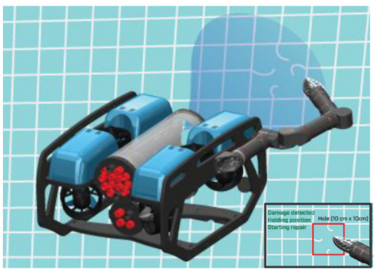
The AQUASTAR project targets combined autonomous inspection and repair of holes in aquaculture fish cage nets. Holes in the net represent the main reason for fish escape and is a challenge in aquaculture fish farming in traditional net-based net pens.

To reduce the risk of escapes in Norway, recent regulations state that inspections must take place before, during, and after operations that involve the net. This has led to more frequent net inspections, but closer investigations have revealed several occurrences where both small holes and large tears have gone undocumented. This creates a need for enhanced inspections and, most preferably, immediate repair of holes.

Today, net inspections are carried out manually and include a remotely operated vehicle (ROV) and an ROV pilot. However, manually inspecting repeating net patterns for hours on end may be tedious and can increase the risk of missing holes in the net. Detected holes are often reported by denoting the hole's approximate size, depth, and compass heading. Next, a report stating needs for repairs is issued to companies that perform hole repair using divers. However, the time between a hole is detected and the repair is done may range between hours to days, leaving a time window for holes to grow in size and for fish to escape. Moreover, while it may be expensive to hire divers for hole repair, divers are exposed to risks that may lead to both permanent injuries and even death. This strongly advocates for both enhanced hole detection systems and alternative net repair solutions.

The AQUASTAR project will develop solutions for ROVs with manipulators enabling automatic hole localization, automatic vehicle positioning relative to holes, and hole repair. This will serve as a tool for ROV pilots to achieve enhanced net inspections and the opportunity to immediately repair holes in the net, both manually and autonomously. The project will build upon previous developments within net- and object-relative navigation systems and vehicle-manipulator operations. Moreover, AQUASTAR aims to develop and continuously update models for detecting holes in the net, and to publish open-source datasets for hole detection. The models and datasets will include several varying conditions present in the aquaculture industry such as illumination conditions based on time of day, season and weather, varying particles in the water, varying levels of biofouling, and more.

The AQUASTAR project is an industrial research and collaboration project between Njord Aqua, Måsøval, Sintef Ocean, and NTNU, and is supported by the Research Council of Norway.



UTILISATION OF AN ENERGY RELATED INDEX FOR ASSESSING AQUACULTURE SITES IN EXPOSED WATERS

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When moving from a very sheltered aquaculture site to a very exposed oceanic site, the energy increases proportionally in a continuum. Lojek et al. (2024) considered the primary influential parameters (water current, waves period, water depth) which dictate the candidate species, structure, technology, methods, and operational aspects of any aquaculture endeavour and investigated six possible indices which cover these variables. Added to advanced computer modelling, assisted by detailed and constant environmental monitoring, it may be possible to refine site selection, structure and design decision, species selection, equipment and logistic considerations and health and safety requirements. This paper will discuss the benefits of two selected indicative indices from the potential equations and compare them with known operational aquaculture sites highlighting present structural capability and limitations. The two indices are also utilized to reflect on their suitability for assessing sample sites with respect to biological, technological, operational or maintenance aspects of aquaculture activities.

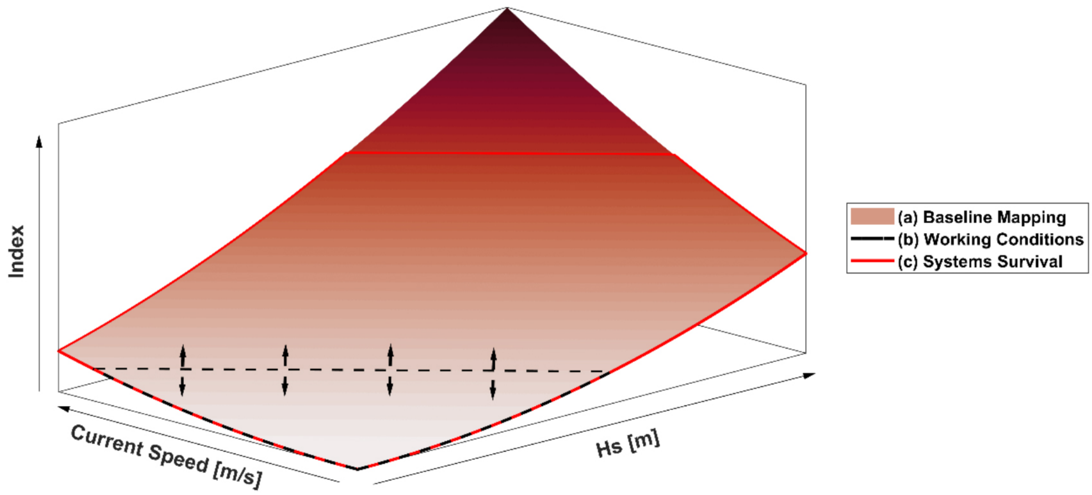

Figure 1: Surface graph to illustrate the magnitude of the index value as a function of the flow velocity (v) and the wave height (H_s) for a given wave period (T_p). The red line is intended to illustrate the limit of the structures used ("system survival"), the black dashed line ("working conditions") shows the limit of the operation conditions, which can vary depending on the type of work (black arrows). Modified after Heasman et al. 2024, Frontiers in Aquaculture.

INFLUENCE OF INCREASING WAVE ENERGY ON AQUACULTURE STRUCTURES, OPERATIONS AND MAINTENANCE

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To address concerns regarding food security, aquaculture is venturing into exposed and/or distant ocean areas, necessitating innovative solutions to thrive in high-energy environments. *Mytilus edulis* and *Perna canaliculus* remain the primary targets for commercial exposed bivalve aquaculture. The aquaculture industries and researchers are actively developing structures and methodologies for such conditions however there is still some work to do. For seaweed cultivation, such as *Saccharina latissima*, *Laminaria digitata*, *Lessonia variegata*, longline setups are commonly used, but refinement is needed to withstand harsh environments and improve productivity. Innovative systems like offshore ring systems and whale-safe composite farms showcase resilience in exposed conditions, despite the operational challenges posed by high-energy environments. In finfish aquaculture, such as *Salmo salar* and *Rachycentron canadum,* three primary design categories for open ocean net pens are identified: flexible gravity pens, rigid megastructures, and submersible pens. Each category offers distinct advantages and disadvantages, impacting their suitability for different situations.

As aquaculture ventures into more demanding environments, a concerted focus on operational efficiency is imperative to reduce effort while increasing productivity. Continued research and innovation will be critical for the successful expansion of aquaculture in exposed ocean conditions, thereby fostering sustainable food production and positive environmental outcomes.

This paper considers the commercial and research progress in aquaculture expansion in exposed seas, with a particular focus on the farming of macroalgae, bivalves, and finfish cultivation.

APPLYING SCOPE FOR GROWTH ESTIMATES TO COMPARE THE SUITABILITY OF FEEDS OF THE WHITE SHRIMP *Penaeus vannamei*

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Introduction

The Scope-for-growth (SFG) of an organism is the amount of assimilated energy available for individual production e.g. growth or reproduction after maintenance requirements of organisms have been met. This method applies an energy balance equation based on an experimentally determined rate of physiological processes. The SFG of *P. vannamei* was calculated as the difference between absorption (A), and the sum of the respiration
(R) and excretion (U) [SFG = A - (R+U)]. In the present study, we investigated the effects of two commercial diets: one used as a pre-growing diet for marine fish and juveniles (diet A) and a second specially designed for shrimps (diet B) on the SFG of *P. vannamei* to compare their suitability.

Materials and methods

Each diet trial was performed on 10 specimens of average wet weight of 13.99 ± 2.74 g.
The food consumption as well as faeces excretion rate were determined by weight, ammonia excretion rate was determined using the salicylate method and the respiration rate was measured using an optical oxygen electrode.

Results

P. vannamei fed on diet B were characterized by a higher food consumption rate as well as assimilation efficiency (5.0 %) in relation to diet A. In the case of diet A, the average proportion of energy allocated to production was 74.3%, energy allocated to respiration was 24.9%, and excreted as ammonia was 0.8% in relation to the amount of energy assimilated from the food. Whereas for diet B the average proportion of energy allocated to production was 81.6%, energy allocated to respiration was 17.9%, and excreted as ammonia was 0.5% in relation to the amount of energy assimilated from the food. The estimated SFG of cultured shrimps was 8% higher when fed on diet B with regard to diet A.

Discussion and conclusions

A commercial diet designed for shrimp cultivation seems to be more suitable for *P. vannamei*. Thus higher SFG values were recorded in shrimps fed on diet B due to the higher amount of assimilated energy, with the lowest energy expenditure on metabolic processes. It can be assumed that the composition of diet B, was more attractive and easier digestible for *P. vannamei* than feed composed for marine fish and juveniles. Such differences between diets might suggest the relevance of diet composition and the need for further optimization to achieve high production in white shrimp aquaculture.

THE USE OF PASSIVE ACOUSTICS IN RAS FOR MONITORING OF FISH AND SYSTEM OPERATION

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Recirculating aquaculture systems (RAS) are complex production facilities that require close monitoring of process and farmed fish. The high level of automation has been achieved in RAS, however real-time solution for monitoring of fish is still lacking. In addition, improved monitoring of system operation can reduce risks that can lead to unexpected mortalities or reduced fish welfare. Passive acoustic monitoring (PAM) is technology that can potentially be used to monitor sounds produced by fish and the equipment in RAS, which was tested in this study.

Hydrophones (AS-1, Aquarian Audio) were singularly deployed in eight 0,5m^3 small scale RAS tanks (Nofima Research Station, Sunndalsøra, Norway), to record sounds during system operation. Raven Pro 1.6.4 software (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology, 2023) was used inspect the audio and produce spectrograms of periods of interest.

Increased occurrence of drum filter flushing was identifiable by sounds with most energy concentrated in low frequencies (Figure 1A), and emergency oxygenation caused signals over a broad frequency range (Figure 1B). Sounds sharing similarities with previously reported salmonid sounds such as fast repetitive ticks (FRT) were identified in the frequency range between 2-8 kHz but were difficult to distinguish during periods of emergency oxygenation. Our results show that PAM can be used to identify changes in the soundscape caused by system operation and fish, however individual sounds might be difficult to distinguish when they overlap in time and/or frequency range.

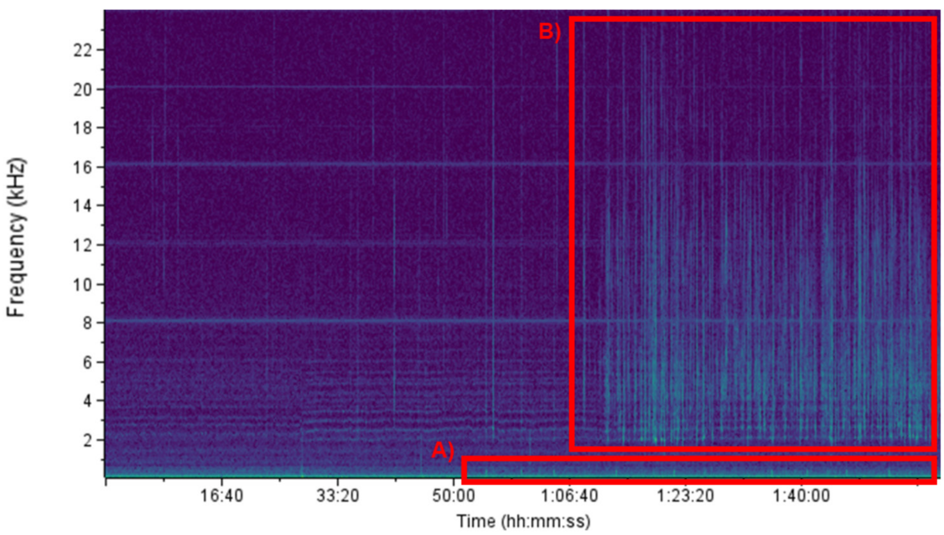

Figure 1. Spectrogram (FFT size 1024 samples, FFT overlap 50 % and Hann window) showing 2 hours of sound recorded from a tank with Atlantic salmon highlighting a change from normal operation (up until 50:00), followed by a period with **A)** increased occurrence of drum filter flushing, and **B)** frequent dosing of emergency oxygen.

SAFE AND HEALTHY SEAFOOD PRODUCTION IN NORWEGIAN AQUACULTURE

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The safety of fish produced in aquaculture is continuously debated; is it safe, as free of undesirables / pollutants as possible, and of course below upper limits set by EU. Due to this EU and Norway demand analytical values to prove that the situation is within these boundaries. The Institute of Marine Research receives, blinds, and performs analyses of Norwegian produced aquacultured fish, on behalf of the Norwegian Food Authorities NFSA, and reports the results on a yearly basis. In addition, data is put on our free online Seafood database, seafood data, as soon as the results are reported to the NFSA. Analytical results will be presented in this poster.

Results are reported to the NFSA, and published in the seafood database is Seafood data | hi.no,

In addition, results are important when it comes to the nutrient profiles, to establish recommendations on nutritional security. Data is needed to secure that the population can plan for a diet that meet their nutritional needs. The seafood database therefore holds data also on nutrients; and it covers wild fish and other seafood sources in addition to the results on farmed fish.

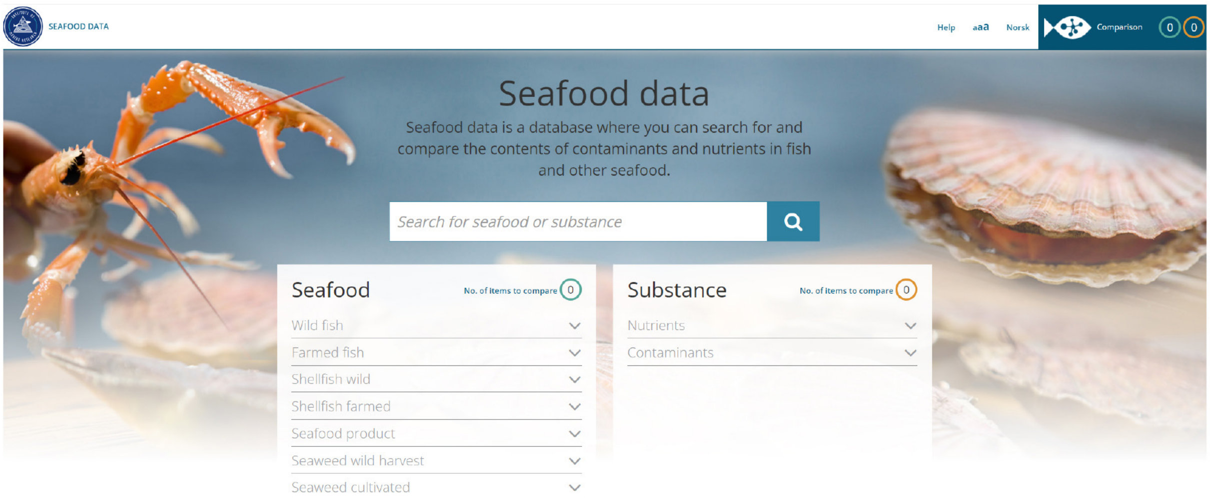

Figure 1. The seafood database contains data on farmed and wild seafood, undesirables and nutrients.

EFFECTS OF UNPREDICTABLE REPEATED STRESS ON RAINBOW TROUT (*Oncorhynchus mykiss*) IMMUNE RESPONSE AGAINST THE FISH PARASITE *Ichthyophthirius multifiliis*

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Aquaculture is providing increasing proportion of fish for human consumption, due to the decline of wild stocks. During the production cycle, fish are regularly subjected to various stressors due to farming practices and their effect in the context of a disease outbreak remains unknown. Our experiment evaluated the effects of unpredictable repeated stress in rainbow trout challenged with the ciliate *Ichthyophthirius multifiliis*, causing white spot disease in freshwater fish.

Prior to the pathogen exposure, fish were stressed once a day and post pathogen exposure twice a day with a rotation of three different stressors (chasing, air exposure and transfer). At 7 days post infection (dpi), the parasite burden was evaluated in fish and in the tank water (environmental DNA), and the local (gill) and systemic (spleen) immune response was investigated. The fish mortality was recorded from 0 to 12 dpi when all the fish from both infected groups died.

Mortality was partially delayed for the fish subjected to stress (Figure 1A). There was no statistical difference in parasite burden between the stressed and unstressed infected fish groups. The immune gene expression analysis suggested an organ-dependent bimodal immune response. In spleen, a type I immune response was initiated whereas in gill, it was a type II immune response. The unpredictable repeated stress induced mainly upregulations of immune genes (e.g. *cat-1*, *hep*, *il-10*) in gill and downregulations (e.g. *il-2*, *il-4/13a*, *il-8*) in spleen (Figure 1B). Our results suggested that the stress protocol did not immunocompromise the fish against *Ichthyophthirius multifiliis*.

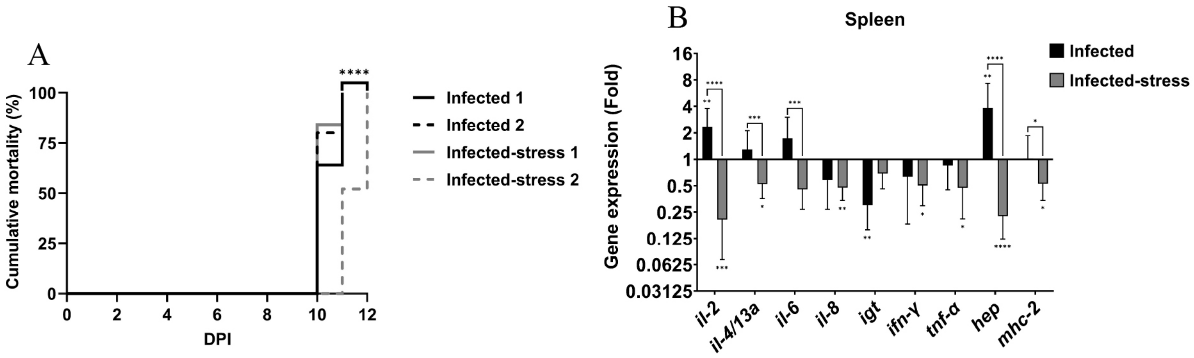

Figure 1. Mortality recorded from 0 to 12 dpi in the challenge fish groups (A). Immune gene expression of the infected fish groups at 7 dpi in spleen (B). * $p<0.05$. ** $p<0.01$. *** $p<0.001$. **** $p<0.0001$.

TOWARDS A SINGLE-CELL ATLAS OF ATLANTIC SALMON ORGANOGENESIS

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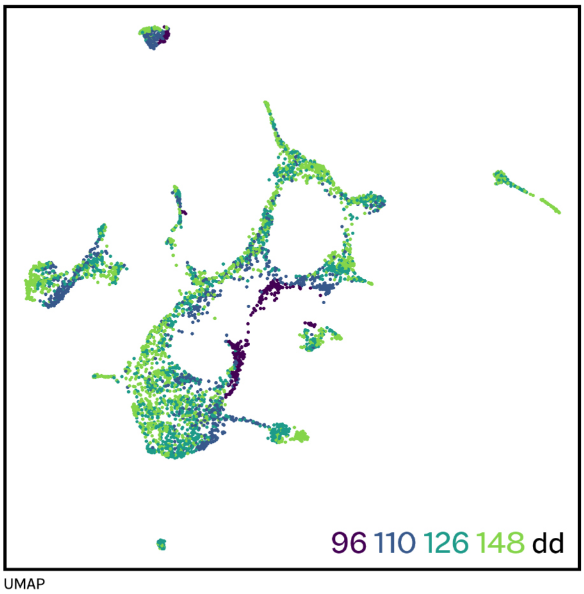
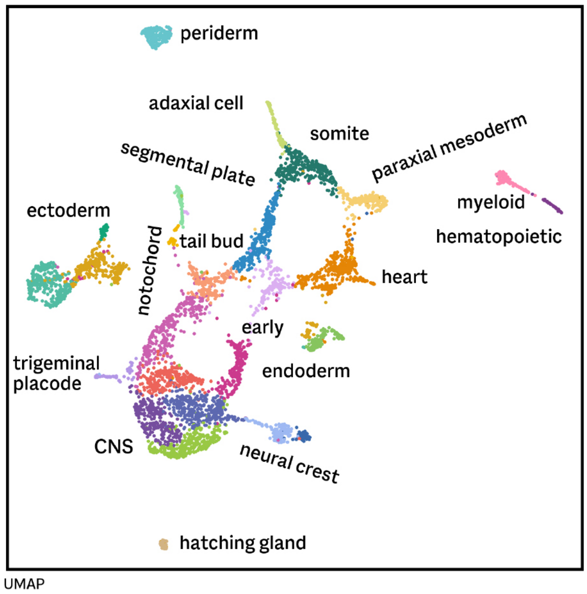
As they develop in eggs exposed to the environment, fishes are especially susceptible to external influences during embryogenesis. These include temperature and light, which have been shown to have long-term fitness effects. In the SALMOCODE project, we will use single-cell transcriptomics to investigate these vulnerabilities in the Atlantic salmon embryo.

As a first step, we have studied the earliest phases of organogenesis in Atlantic salmon using single-cell transcriptomics. By isolating nuclei from four early developmental stages (96–148 day degrees, corresponding to the interval between gastrulation and mid-somitogenesis) and profiling them using a split-pool barcoding protocol, we have collected a dataset for 4864 embryonic cells (figure left, UMAP projection).

During gastrulation only four cell types are represented (endo-, meso-, and ectoderm, and enveloping layer), however these rapidly differentiate into at least 25 distinct cell types in the last sampled stage (figure right). The high temporal resolution enables the identification of clear differentiation trajectories for the emergence of future tissues and organs, including the neural crest, the heart, and pronephros. In addition, the dataset already allows the discovery of key regulatory genes involved in the earliest cell fate commitments for these tissues.

We are currently expanding this effort to become a reference atlas of the salmon embryo from gastrulation up until hatching. In addition, we are using CRISPR/Cas9 gene editing to verify the involvement of several genes we identified in heart development.





TOWARDS A SINGLE-CELL ATLAS OF EEL DEVELOPMENT: LESSONS FROM SALMON

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All animals develop from a single fertilized egg into juveniles and adults consisting of a multitude of specialized cells. In recent years, single-cell transcriptomics (scRNA-seq) has emerged as the leading technology for studying this developmental process. By measuring the expression of all genes in individual cells dissociated from a tissue, it creates thousands to millions of gene expression profiles. These can be clustered into profiles specific for cell types and states, as well as into trajectories that reflect developmental change. This has enabled the mapping in exquisite detail of all cell types emerging during embryo development in zebrafish, frogs, and mice.

Single-cell transcriptomics is relevant for aquaculture, as it also allows the precise identification of the onset of developmental defects. These can have both external and internal causes. As they develop in eggs exposed to the environment, fishes are especially susceptible to external influences during embryogenesis. In addition, egg quality has a strong influence on embryogenesis. Finally, a single-cell atlas identifies when, and in which cell types, genes exert their function, and could therefore inform marker selection for breeding programs.

In Norway, we have recently started to generate a single-cell atlas of organogenesis (early embryogenesis) in Atlantic salmon. This reference will be used to diagnose the quality of embryos reared under a wide range of environmental conditions, with the aim of optimizing production conditions towards better organ health in adults.

A similar effort would highly valuable for understanding the early development of the European eel. Eel embryogenesis often aborts before hatching, and scRNA-seq could be used to identify the mechanisms involved, as well as their vulnerable time-windows. Such diagnostics could also help to decipher the influence of broodstock quality, which is a strong predictor of successful embryogenesis. As a first step towards a single-cell atlas of eel development, we have optimized the isolation of viable nuclei from individual embryos, which can be used for the affordable and large-scale scRNA-seq protocols.

This abstract is based upon work from COST Action EEL SUPPORT, CA22163, supported by COST (European Cooperation in Science and Technology), and the SALMOCODE project, supported by the Norwegian Seafood Research Fund (FHF grant 901864).

INFLUENCE OF FEEDING PROTOCOLS ON GROWTH AND SKELETAL ANOMALIES IN ATLANTIC COD LARVAE (*Gadus morhua*)

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Introduction

Atlantic cod aquaculture bottlenecks can be partially tackled through optimized rearing and feeding protocols. Enriched rotifers and Artemia are commonly used as main source of live feed in fish larvae rearing, although studies have explored the inclusion of natural zooplankton in Atlantic cod diets, resulting in higher growth rates and viability (Øie et al., 2017). Other marine organisms with a balanced nutritional profile such as Barnacles, have also been successfully tested in feeding protocols for other marine species. Despite the extensive work carried out on larvae nutritional requirements, there are still knowledge gaps on species specific nutritional preferences. Therefore, it is paramount to develop customized microdiets once they influence appetite, growth, digestive tract maturation, and skeletal development of Atlantic cod larvae. The present study evaluated the potential of a feeding protocol with plankton eggs and barnacle nauplii, and compared the effect of two microdiets on growth performance and skeletal anomalies in Atlantic cod larvae.

Material and methods

The trial aimed at testing a feeding protocol with Barnacles and evaluating the effect of two different microdiets on Atlantic cod growth and skeletal anomalies. Larvae from Ode AS (Norway) were randomly distributed in triplicate 400 L tanks at 2 days post-hatch (dph) and kept until 66 dph (trial end). The light regime was 24h and temperature ranged from 8.8 to 10.8 ± 0.2°C. Fish groups and feeding protocols were as follows: control group (SK) was fed rotifers, large barnacle nauplii (Cryo-L) and a commercial microdiet. The two experimental groups (D1 and D2) were fed rotifers, plankton eggs (Cryo-µ), small barnacle nauplii (Cryo-S), Cryo-L and one experimental microdiet each (D1 and D2, respectively). Sampling occurred at 3, 30, 50 and 66 dph and comprised the analyses of several parameters including standard length (SL) and Skeletal anomalies.

Results

At 66 dph, no significant differences were found on SL between the two experimental treatments - D1 and D2 (Figure 1, A). Furthermore, both experimental groups showed lower incidence of skeletal anomalies. In particular, D1 group presented lower incidence of skeletal anomalies ($P<0.05$) than the control group (SK), as shown in Figure 1 (B).

Discussion and Conclusion

The effect of the feeding regime on growth and skeletal anomalies of Atlantic cod larvae was evaluated in the present study. There was no obvious effect of the experimental feeding regimes on growth (Figure 1, A), whereas the control group performed better after 30 dph, potentially due to the different physical properties of the microdiets. Standard length was in the same range or higher as previously observed with protocols including natural zooplankton in the live feed phase (Folkvord et al., 2017). Regarding skeletal anomalies (Figure 1, B), group D1 exhibited a significantly lower incidence of skeletal anomalies, including severe anomalies, which could suggest that such occurrence can also be related to the dietary PL source as well as essential fatty acids (EFA) supplied as dietary PL (Hamre et al. 2011). This corroborates results from Hansen et al. (2011), where Atlantic cod fed a microdiet rich in marine PL had a higher prevalence of skeletal anomalies at 75 dph. Furthermore, the authors also reported that scoliosis occurrence was significantly lower in the vegetable PL group, similarly to what was found in the present study. Overall, the experimental group fed rotifers, Cryo-µ, Cryo-S, Cryo-L and microdiet D1, related lower incidence of skeletal anomalies in cod larvae, including severe anomalies. These results highlight the potential of optimizing feeding protocols for Atlantic cod larvae and the positive effects this may bring on larval and juvenile quality.

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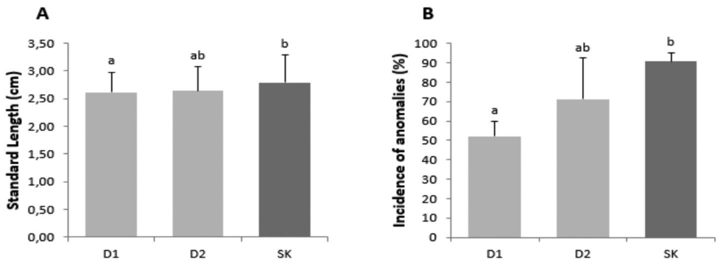

Figure 1. Standard Length (A) and Incidence of skeletal anomalies (B) in Atlantic cod (66 dph) fed two experimental (D1 and D2) and one commercial (SK) microdiets. Results are expressed as means ± standard deviation. Different lowercase letters indicate significant differences between the dietary treatments (P<0.05).

Acknowledgements

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References

Øie, G., T. Galloway, M. Sørøy, M. Holmvaag Hansen, I. A. Norheim, C. K. Halseth, M. Almli, et al. 2017. Effect of Cultivated Copepods (Acartia tonsa) in First-Feeding of Atlantic Cod (Gadus morhua) and Ballan Wrasse (Labrus bergylta) Larvae. Aquaculture Nutrition 23 (1): 3–17.

Engrola, Sofia, Luís Figueira, Luís E.C. Conceição, Paulo J. Gavaia, Laura Ribeiro, and Maria Teresa Dinis. 2009. Co-Feeding in Senegalese Sole Larvae with Inert Diet from Mouth Opening Promotes Growth at Weaning. Aquaculture 288 (3–4): 264–72.

Folkvord, A., R. Koedijk, O. Grahl-Nielsen, S. Meier, B. Rydland Olsen, G. Blom, E. Otterlei, and A. K. Imsland. 2018. You Are What You Eat? Differences in Lipid Composition of Cod Larvae Reared on Natural Zooplankton and Enriched Rotifers. Aquaculture Nutrition 24 (1): 224–35.

Hamre, Kristin, Manuel Yúfera, Ivar Rønnestad, Clara Boglione, Luis E.C., Hansen, Øyvind J., Velmurugu Puvanendran, Jens Petter Jøstensen, and Christian Ous. 2011. Effects of Dietary Levels and Ratio of Phosphatidylcholine and Phosphatidylinositol on the Growth, Survival and Deformity Levels of Atlantic Cod Larvae and Early Juveniles. Aquaculture Research 42 (7): 1026–33.

Hansen, Øyvind J., Velmurugu Puvanendran, Jens Petter Jøstensen, and Christian Ous. 2011. Effects of Dietary Levels and Ratio of Phosphatidylcholine and Phosphatidylinositol on the Growth, Survival and Deformity Levels of Atlantic Cod Larvae and Early Juveniles. Aquaculture Research 42 (7): 1026–33.

EVALUATING THE PERFORMANCE OF A MATURE NITRIFYING BIOFILTER IN A RECIRCULATING AQUACULTURE SYSTEM (RAS) BY FLOW CYTOMETRIC ANALYSIS

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In a mature nitrifying biofilm, heterotrophic bacteria are located in the outer layer of the biofilm and protect the nitrifying bacteria located at the inside of the biofilm both from shear stress and environmental challenges. Since heterotrophic bacteria grow faster than the autotrophic nitrifying bacteria, changes in diversity and productivity in the water phase may reflect changes in the environment faster than analyzing the biofilm. Our hypothesis is that the microbial productivity in the water phase inversely correlates with the nitrifying activity of the mature biofilm in the nitrifying biofilter of a recirculating aquaculture system (RAS).

Establishing effective management of the microbiome requires technologies that can monitor it in a high-throughput and timely way. Kytos' innovative microbial fingerprinting technology can rapidly analyze thousands of microbes within minutes. The analysis results are translated into a set of easily understandable microbiome metrics (e.g. diversity, bacterial activity, etc.) that can be coupled to system performance (e.g. overfeeding, change of salinity etc.). By establishing knowledge on the RAS site specific microbial activity both in the biofilm and water phase, these metrics can be used as a management tool for actively steering the microbial community in the nitrifying biofilters.

Therefore, this study aimed at quantifying the relationship of microbial diversity and heterotrophic bacterial productivity found in the water phase and the biofilm of the biofilter. Furthermore, quantifying the heterogeneity of the samples taken at several locations of the same biofilter at the same timepoint was investigated to provide insight into the necessary sampling number and frequency. Mostly, it is more convenient for RAS farmers to take water samples rather than sampling the actual biofilm in the biofilter. Therefore, the parameters diversity, heterotrophic bacterial productivity and viable cell number were investigated in both the water and biofilm phase.

Initial results show significantly higher microbial diversity in the biofilter water samples compared to the associated biofilm samples. At the same time, the bacterial productivity and cell loads were significantly higher in the biofilm samples compared to the water samples.

DEVELOPING A ZOOPLANKTON CHALLENGE MODEL FOR ATLANTIC SALMON

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Harmful gelatinous zooplankton, especially those of the phylum Cnidaria such as jellyfish, can threaten farmed fish through various mechanisms, including both physical damage and venomous effects, and are significant contributors to gill disease development. However, there is a lack of scientific and technical understanding of the interactions between gelatinous zooplankton and fish gills and the use of laboratory based challenge models, using lab-reared organisms, can elucidate the gaps in our knowledge. Models using laboratory reared Cnidarians have not been previously reported, and the aim of this study was to develop these.

Jellyfish polyps of *Aurelia aurita* were successfully reared under laboratory conditions, and by artificial strobilation, juvenile medusae (1-2 weeks old, ~1 cm in diameter) were produced, transported and used to challenge Atlantic salmon (*Salmo salar*). A preliminary exposure of the salmon gills with juveniles of *A. aurita* was carried out through direct contact with the gills. The challenge experiment involved the use of 3 replicate control tanks and 3 replicate challenge tanks. In each tank, 20 Atlantic salmon weighing between 100 to 400g were anesthetised, and young medusa were then directly applied to the gill tissue using a syringe. For control groups, a syringe containing tank water instead of jellyfish was used. To assess the effects of the challenge, 5 fish per tank per sample point (-1, 1-, 3-, and 20-days post-challenge) were randomly selected, euthanised, and sampled for histopathology and PCR analyses.

In the gross examination of gills, small necrotic patches were noted in 30% of the fish challenged on the 1st and 3rd day post-challenge. In the histopathological exam, focally extensive necrotic, inflammatory, and haemorrhagic pathology was observed in 60% of the challenged fish 1-day post-challenge. Additionally, these lesions were associated with the presence of moderate levels of filamentous and short rod bacteria in two fish gills (see Figure). Localised moderate lesions persisted in subsequent sampling points, although the number of lesions decreased, and they showed signs of repair.

These preliminary results successfully demonstrated the presence of gross gill and histopathology lesions following contact with juveniles of lab-reared *A. aurita*. Additionally, they highlighted, for the first time, the rapidity with which bacterial colonization occurs following a primary cnidarian-induced lesion in laboratory conditions.

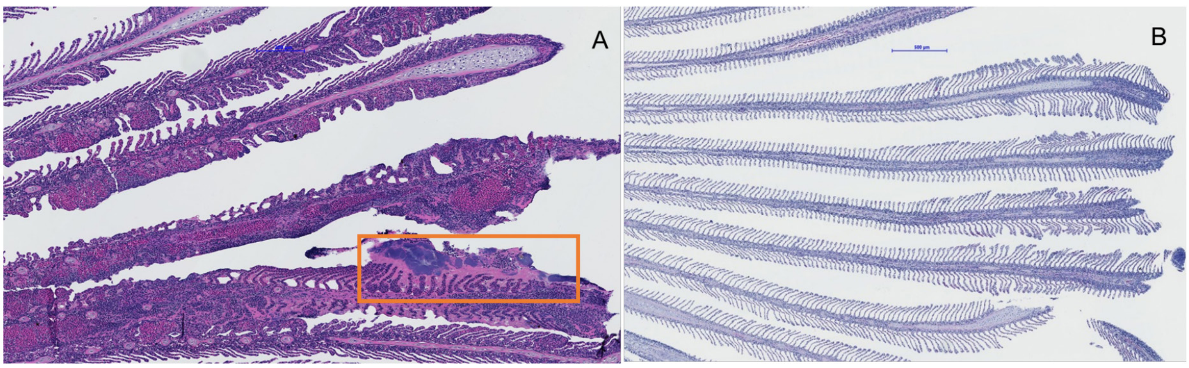

Figure. Gills of Atlantic salmon 1-day post-challenge (A) exposed to jellyfish and (B) from control tanks. Note the bacterial colonisation (orange box) in the jellyfish-induced lesion.

ENVIRONMENTAL EFFECTS OF AQUACULTURE AND OYSTER RESTORATION IN THE NORTH SEA UNDER DIFFERENT CLIMATE CHANGE SCENARIOS

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Over the coming decades, changes in the North Sea are expected due to climate change and planned human activities, including offshore aquaculture, and oyster restoration efforts. One of the goals of the EU-funded FutureMARES research project was to examine these effects and their impact on each other. To achieve this goal, we used a state-of-the-art North Sea model to simulate marine conditions under optimistic as well as pessimistic climate change scenarios. Dynamics of seaweed, mussels and oysters were explicitly computed, as well as their feedbacks on the environment. To investigate effects on the environment, we compared simulations without aquaculture/oyster restoration, to simulations with large-scale seaweed cultivation only, large-scale mussel cultivation only, co-cultivation of seaweed and mussels, and aquaculture and oyster restoration combined (e.g. Figure 1). In the model, seaweed and mussel farms were located in future Dutch offshore wind farms suitable for cultivated species. Oyster bed locations were chosen based on areas with a high habitat suitability and where fishing efforts are expected to be excluded.

For all scenarios, the model results estimate that seaweed aquaculture will reduce the local winter nutrients and thereby also the surface chlorophyll-a concentrations, as seaweed builds up its nutrient reserves before the spring bloom. This is visible not only in the vicinity of the farms but also far downstream. Mussel aquaculture will reduce chlorophyll-a concentrations in the vicinity of the farms, since they directly feed on phytoplankton. It will also likely increase local nutrients due to their excretion/increased re-mineralization. When restored oyster beds were confined to more offshore locations, where habitat suitability is the highest, they have a small effect on the local nutrients and do not interact much with the aquaculture, which is likely to occur closer to the coast. However, when oyster beds are also restored in more moderately suitable areas, closer to the coast, they have a larger effect on the marine environment and aquaculture in neighbouring farms (especially mussel yields).

Restoring oysters would be a slow process as the most suitable, offshore areas have limited primary production and therefore food availability for the oysters, as well as heavy stratification, leading to very low growth rates. Model results suggest that the most optimal location to start with the oyster restoration process would be in the Frisian front.

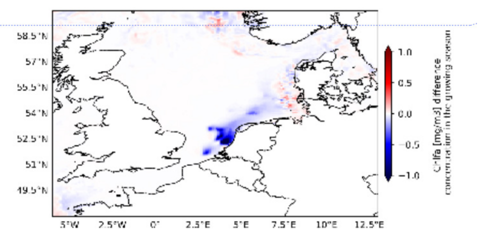

Figure 1: Difference in predicted surface chlorophyll-a concentrations between simulations with large-scale aquaculture and oyster restoration and without, in 2050 under the most pessimistic climate change scenario

EVALUATION OF METHODS FOR SINGULATING GREENSHELL™ MUSSEL (*Perna canaliculus*) SPAT FROM SPAT-COLLECTOR ROPE

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The early stages of mussel farming are often extremely inefficient with a large proportion of seed (spat) typically lost early in the production cycle. An effective approach for decreasing such losses is to grow spat to larger sizes in nursery systems prior to seeding. However, for such an approach to be commercially viable, spat must first be separated from their settlement substrates into a single seed format, i.e., singulated. This experimental study sought to determine whether the use of chemical treatments (i.e., chlorine and protease enzymes) at various concentrations and exposure times, or altering the holding conditions of the spat (i.e, salinity, air exposure, starvation and oxygen deprivation), could be an effective approach for singulating juvenile Greenshell™ mussel (*Perna canaliculus*) spat from spat-collector ropes. The proportion of mussels singulated from spat-collector ropes after treatment were measured then reintroduced into seawater. After 24h of rearing, the survival and recovery rate of spat was also measured.

Chemical treatments involving proteases (PAP, PXT, and BAP) and chlorine demonstrated varying results. Proteases, along with chlorine, were capable of singualting up to 65% (PAP) and 61% of larger spat, while for smaller spat, singualtion rates reached 85% (PXT) and 79%, respectively. However, these treatments were associated with elevated spat mortality, particularly with increased concentrations and exposure times, resulting in recovery rates as low as 34% for large spat and 26% for small spat. On the other hand, alterations in environmental conditions showed singualtion rates ranging from 28.5% to 80.2%, with the highest singulation rate observed in tanks without aeration and without food for a period of 24h. Despite these variations in singulation rates, survival remained consistently high (>80% survival) across all treatments, leading to the highest mean recovery of 65% for spat of all sizes.

These findings shed light on ongoing efforts to enhance the efficiency and sustainability of mussel farming practices. They underscore the potential utility of chemical treatments or modifications to spat holding conditions for singulating spat from settlement substrates before transferral to nursery systems for further growth. Notably, the study reveals a tradeoff between survival and singulation efficacy, with chemical treatments showing higher singulation rates but increased spat mortality compared to alterations in environmental conditions. Moreover, considerations regarding the timing and practicality of these methods warrant further exploration in optimizing mussel farming operations.

LEVEREGING GENOMICS FOR PRECISION FARMING: BUILDING SEX-SPECIFIC HIGH-DENSITY LINKAGE MAPS OF BARRAMUNDI *Lates calcarifer* FOR ACCURATE BREEDING PROGRAM SIMULATIONS

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Barramundi (*Lates calcarifer*) as an important tropical aquaculture candidate is increasingly being subjected to genetic improvement programs. Whilst there are currently some genomic resources available for the species, to date there has been no high-resolution genetic linkage map produced that is useful to understand recombination rates within the genome and for QTL mapping. Here we genotyped 41,000 SNP in two barramundi families (n= 266 progeny total) to develop the highest-resolution linkage map for the species thus far and the first constructed based on Australian *L. calcarifer.*

The linkage map shows substantial regions of male-only and female-only recombination zones across the chromosomes (Figure 1), a striking finding given the protandrous nature of this species (fish are born as males and some transition to females later in life but have the same genome). One of the motivations for constructing the linkage map was to accurately simulate inheritance of chromosomes across generations in “digital twin” simulations of barramundi breeding programs, to evaluate the cost benefit of technologies such as genomic selection. The varying recombination rates between genders in our linkage map suggest the need to consider sex-specific inheritance patterns in such breeding program simulations.

The new linkage map offers valuable insights into recombination mechanisms and will be crucial for genomic selection efforts for *L. calcarifer*, improving breeding programs and enhancing our understanding of its biology.

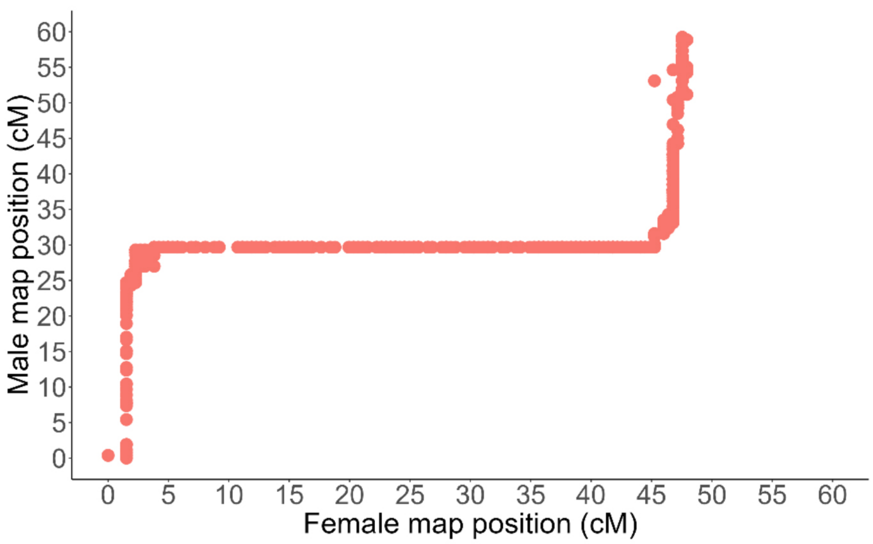

Figure 1: The largest linkage group in the barramundi genome. The x- and y-axis show the female and male frequencies of recombination, respectively, in centimorgans across the linkage group. From this plot, we can see there are male-only recombination regions at the telomere regions of the chromosome and female-only regions of recombination near the centromere.

FUNCTIONAL DIETS BASED ON MICROALGAE CAN BOOST IMMUNE RESPONSES IN GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES

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Gilthead seabream (*Sparus aurata*) is a crucial species for Mediterranean countries' aquaculture. Global changes encompass various large-scale environmental transformations, which result from anthropogenic activities, linked to the rearing conditions. One of the strategies to ensure animal welfare is proper nutrition, crucial for improving the animal's performance and immune status. Fortified feeds, regarded as those with selected additives added, can trigger the immune system and thus are important in certain periods of fish farming (e.g., high densities, grading, transportation). Fish resilience against pathogens or other stressors can be enhanced, diminishing the impact of disease outbreaks, mortality, and overuse of chemicals to restore the stock.

This work aimed to evaluate the effect of including microalgal extracts as functional dietary supplements to enhance the resilience of gilthead seabream (*Sparus aurata*) early juveniles to acute stress. A feeding trial was conducted for 3 weeks, where fish were fed one of the four experimental diets: a commercial-like diet (CTRL), two diets supplemented with a microalgal (*Tetraselmis chui*) aqueous extract: high level (HIGH) and low level (LOW), and a third diet with commercial product with β-glucans from algae (BG). At the end of the feeding period, key performance indicators were evaluated and fish were sampled (S1). Subsequently, fish experienced a repeated acute stress involving handling, crowding, and transport, culminating with a bacterium challenge (*Photobacterium damselae* sp. *piscicida*). After 6 hours, fish were sampled again (S2). Fish were measured, and blood, tissues, and caudal fin were collected for physiological and molecular biomarkers related to immune functions (peroxidase, immunoglobulins) and stress-related (cortisol). Fish mortality was also tracked for two weeks. The head-kidney was selected to study a panel of 20 immune-related genes: cytokines (*il1β*, *il10*, *il8*, *tnfα*), antimicrobial peptide (*hep*), T-cell markers (*cd3x*, *cd8β*), chemokine receptors (*ccr3*, *cxcr4*), pattern recognition receptors (*tlr2*, *tlr5*, *tlr9*), and other relevant immune-related proteins (*tgfβ*, *mchII*, *mmp9, csfr1*, *cas3* and *c3*).

Data from this study showed that seabream juveniles fed HIGH and BG diets presented normal performance and higher survival rates. Plasma peroxidase levels suggested that the inclusion of microalgae bioactive compounds has an immunomodulatory effect. As expected, cortisol levels increased from S1 to S2 in all treatments. In terms of the regulation of head kidney immune genes, seabream fed HIGH presented an increase in *cxcr4*, *cd*8*β*, *hep* and *c3* at the end of the feeding period. After stress (S2), fish fed BG presented a decrease in *cxc*r*4*, together with a tendency of upregulation of *cd8β* and *hep* genes. Preliminary data suggest that *Tetraselmis chui* aqueous extracts and *β* glucans seem to be promising candidates for inclusion in diets for gilthead seabream juveniles, mainly for stressful periods. Further analyses are still ongoing.

EVALUATION OF THE NUTRIENT MATRIX VALUES FOR PHYTASE IN TILAPIA *Oreochromis niloticus*

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Effective phytate destruction by phytase will release not only phytate-bound phosphorus, but also other nutrients in the diet that chelate with phytic acid under gastric conditions. To achieve the full economic benefits from phytase, a matrix can be applied to the diet formulation to account for this release of additional nutrients. However, as the dose response curve is not linear, the appropriate matrix values must be assigned to phytase when the inclusion rate varies. The objective of the current study was to validate the phytase (Quantum Blue, AB Vista, Marlborough, UK) matrix for aqua by applying 1500 FTU and 2500 FTU matrix values for available phosphorus (avP), calcium (Ca) and protein to the diets of tilapia.

A total of 750 male tilapia (*Oreochromis niloticus*; average weight 20g) were allocated to one of 5 diets, with 4 replicate 100L tanks per diet and 32 fish per tank. Water temperature was set at 28±1°C. Plant-based diets were fed to satiation (3 meals a day) over a 12-week period. Treatments comprised a positive control diet (PC) with 0.95% total P, 0.74% Ca and 35.2% protein, a negative control diet with a formulated reduction in 0.20% avP, 0.15% Ca and 0.56% protein (NC1), NC1 plus 1500 FTU/kg phytase (NC1+1500), a negative control diet with a formulated reduction in 0.24% avP, 0.18% Ca and 0.65% protein (NC2) and NC2 plus 2500 FTU/kg phytase (NC2+2500). Productive performance was measured over the 12 weeks and retention was determined through carcass analysis at the end of the study. Data was subjected to ANOVA using the fit model platform in JMP Pro 15.1, with treatment means separated using Student's T-test.

Feed analysis revealed that the expected downpsec in avP was achieved in NC1 and NC2. Dietary Ca levels were slightly higher than predicted targeted in NC diets, reaching an average 0.15% reduction across all NC diets. Dietary protein was more severely reduced in NC diets than expected, reaching a minimum decrease of 1.4%. Performance of tilapia was worsened with each nutrient downspec ($P<0.05$; Table 1). Addition of phytase to each respective control increased weight gain of fish and reduced FCR to a statistically similar level as the PC.

Retention of nitrogen and P are shown in Table 1. Nitrogen retention worsened with each nutrient downspec, with NC2+2500 getting back to the PC ($P<0.01$). Phosphorus retention of phytase fed fish increased beyond all the control diets ($P<0.01$).

In conclusion, performance of tilapia could be maintained when avP, Ca and protein were reduced in the diet by supplementing phytase at the appropriate dose, thereby validating the matrix values for the commercial phytase tested in tilapia.

Table 1. Productive performance and nutrient retention

Treatment	BWG, g/d	FCR	N retention, %	P retention, %
PC	1.46^{a}	1.30^{b}	35.8^{a}	44.9^{b}
NC1	1.20^{ab}	1.46^{ab}	31.6^{b}	37.4^{b}
NC2	1.01^{b}	1.59^{a}	27.1^{c}	40.3^{b}
NC1+1,500	1.35^{a}	1.33^{b}	31.7^{b}	61.0^{a}
NC2+2,500	1.35^{a}	1.32^{b}	33.5^{ab}	69.7^{a}
SEM	0.094	0.058	1.17	5.08
P-value	0.03	0.015	0.002	0.002

^{a,b} For a specific additive, means having different superscripts differ significantly ($P<0.05$)

SUBMERGED FARMING FOR IMPROVED FISH HEALTH AND WELFARE

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ScaleAQ, a technology supplier for the aquaculture industry, acknowledges the need to improve the fish welfare, reduce mortality and improve the production through the development of technology for sustainable aquaculture. The fish health report (2023)[1] gives every year the status on Norwegian aquaculture regarding fish health. The report shows the highest mortality rates ever recorded and highlights three health issues, i.e. injuries from mechanical delousing operations, complex gill diseases, and bacterial diseases (winter ulcers). It further highlights jellyfish as one of the top ten health challenges. Increasing global sea temperatures and marine heat waves and low oxygen conditions could also have a negative impact on future aquaculture production.

ScaleAQ is currently developing and testing a submerged production concept (SubSea) in collaboration with a Norwegian fish producer. The function and goal of SubSea is to improve fish health by avoiding surface challenges such as lice, algae, jellyfish, high and low temperatures, as well as forces from waves and currents on moorings and floating collars. The concept is intended for use in exposed and existing (deep) locations. The depth and water quality of the location must be suitable for deep-sea operations.

ScaleAQ's comprehensive design for submerged operations has been extensively tested in tank simulations. The Subsea system consists of a floating collar with a circumference of 120-200 meters and a cylindrical net pen with a net roof. The net is lowered to a depth of 25 m, significantly reducing the risk of infestation with sea lice and avoiding other surface challenges. In submerged operations, fish are prevented from going to the surface, filling their swim bladder with atmospheric air, as well as engaging in jumping and other surface-related activities. To compensate for this, an air-filled dome is installed in the net roof, providing fish with the opportunity to fill their swim bladder. Lights are mounted underneath the net roof, as well as around the air dome, to illuminate the area. Cameras are installed to observe fish behaviour and feeding activity, and oxygen sensors are employed to monitor oxygen levels. Winches are mounted on the floating collar to ensure safe and efficient lowering and raising of the net pen.

Submerged systems and related technology on a commercial scale has been tested over the years through development licenses and continued in regular production at multiple sites. Published summaries of full-scale operations are available (Warren-Myers et al, 2021), indicating reduced lice infestation, normal swimming behaviour, and impact on animal welfare due to suboptimal environmental conditions. Scientific efforts to document the effect of submerged concepts on health, welfare, and production biological parameters have been ongoing intensively for a decade, although trials of submerged concepts date back to the 1970s (Sievers et al, 2021[2]).

At the conference we will present the work that ScaleAQ is doing on subsea farming, and present the status regarding sealice infestation, growth and welfare, as well as technical results.

Fiskehelserapporten 2023 (vetinst.no)

INTERORGANIZATIONAL ACCIDENTS IN NORWEGIAN SALMON FARMING

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The increased production of Atlantic salmon in Norway has led to a need for more efficient production methods. One consequence is an increase in the use of specialized services and companies that are hired to assist in fish farming operations. These are mainly vessel-based operations such as delousing and counting fish, and maintenance operations of the fish farm equipment. This presentation discusses the results from an analysis of occupational fatalities and a recent OHS survey in the Norwegian fish farming industry, with a focus on the accidents that happen in the interorganizational interface between fish farms and independent vessels.

Findings from registered fatal accidents in the Norwegian fish farming industry and a digital OHS survey sent to fish farming employees in the period May to August 2023 are presented.

In the period 1982-2022, 38 fatal accidents were registered by SINTEF Oceans in the fish farming industry (Holmen and Holen, 2023). Figure 1 shows that in the two ten-year periods from 1982, the majority of the fatal accidents happened during transportation. In the last ten-year period, 2012-2022, lift operations and maintenance are more prevalent. These operations are often performed in collaboration between operators on fish farms and hired, independent service vessels.

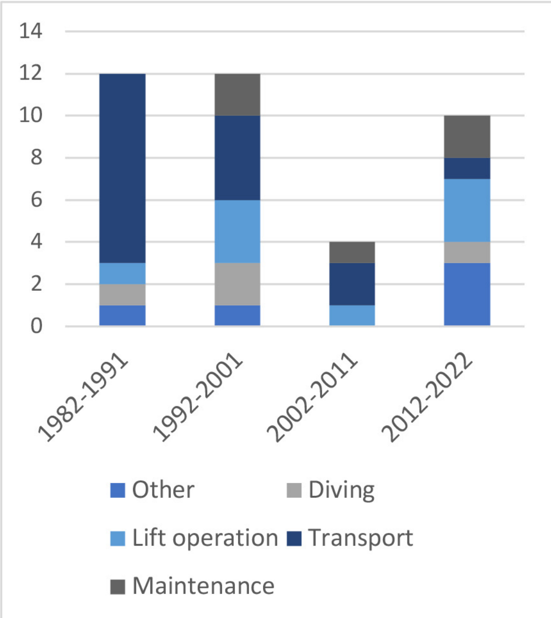

Figure 1 Fatal accidents in Norwegian fish farming and work operations at the time of accident in the period 1982-2022 (Holmen and Holen, 2023).

In an OHS survey among fish farm workers, 66% of the respondents working on independent vessels think that inadequate collaboration with fish farmers is a threat against safety (Thorvaldsen *et al*., 2023). 56 % of the employees on vessels answer that the fish farmers' demand for efficiency sometimes leads to breaking of safety procedures.

These findings indicate a need to focus on safety in the operations where fish farmers and vessels collaborate. Learning from other industries points towards disorganization, dilution of competence and economic pressures as important factors to monitor (Milch and Laumann, 2016).

Literature

Holmen, I.M. and Holen, S.M. (2023) Occupational accidents in fish farming - analysis of registered occupational accidents on fish farms and vessels. (Arbeidsulykker i havbruk - Analyser av registrerte personulykker på havbruksanlegg og -fartøy). 2023:01396. Trondheim.

Milch, V. and Laumann, K. (2016) 'Interorganizational complexity and organizational accident risk: A literature review', Safety Science, 82, pp. 9–17. Available at: https://doi.org/10.1016/j.ssci.2015.08.010.

Thorvaldsen, T. et al. (2023) The HSE-survey in fish farming 2023 (HMS-undersøkelsen i havbruk 2023). 2023:01362. Trondheim.

BEHAVIORAL TESTING OF STRESS HABITUATION TO HIGH REARING STOCKING DENSITIES IN GILTHEAD SEA BREAM (*Sparus aurata*)

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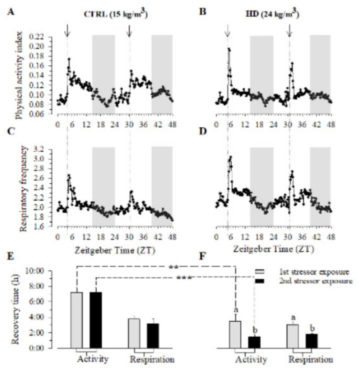
Stress initiates adaptive processes that allow the organism to cope with real or perceived threats. However, the initially "adaptive" stress responses can arise as "maladaptive" because of a persistent activation of stress effectors. This undesirable situation is controlled by the process of stress habituation, which refers to the physiological adaptation to recurrent exposure to persistent or intermittent stressors. There is now evidence in farmed gilthead sea bream that relatively high stocking density triggers a number of adaptive responses in nature, including changes in physical activity and social behaviour, metabolic homeostasis, muscle growth-regulatory mechanisms and microbial communities on the gut and skin surfaces (Holhorea et al., 2023: doi.org/10.3389/fphys.2023.1272267). Moving forward, the aim of this study was the behavioural testing of the stress habituation of fish kept at different stocking densities, using a compression test as a recurrent stressor with focus on the stress recovery phase.

Two-year old fish (425-440 g) were grown up from June to August at two different stocking densities (CTRL 10-15 kg/m^3; HD, 18-24 kg/m^3) in a flow-through system (3,000 l tanks, IATS aquaculture infrastructure), following the natural changes in day-length and temperature that increased from 21ºC to 29 ºC over the course of the trial. Water [O_2] was maintained always regulated, varying between 5.2-4.2 ppm and 4.2-3.2 ppm in CTRL and HD fish, respectively. At the end of the trial, randomly selected fish from CTRL and HD fish were taken for blood haematology and skeletal muscle transcriptomic analyses. Fish images were also taken for scoring external tissue damage. Then, four days later, fish were subjected to a compression test in two consecutive days with a 75% reduction of the available space for fish free displacements during 45 min (https://vimeo.com/927085326). Behaviour was continuously monitored by means of the AEFishBIT data-logger, externally attached to the operculum for the simultaneous individual measurements of physical activity and respiration.

HD fish showed a reduction of voluntary feed intake associated to a significant reduction of growth rates and haematopoietic activity, as a result of an inferred hypo-metabolic state that is prone to use metabolic fuels for exercise rather than growth in comparison to CTRL fish. Otherwise, HD fish showed signs of skin erosion and epidermal/muscle injuries that would promote a shift of the muscle transcriptome pattern towards muscle reparation rather than muscle protein accretion by itself. In line with all this, the recovery phase for the behavioural measurements of activity and secondly respiration rates after stress testing were shortened in HD fish. This became especially evident after the second stressor exposure, with a shortened stress recovery time after the repeated stressor in HD fish (2 h), while it remained almost invariant in CTRL fish (6-8 h). Altogether, this study provides new insights to infer the complex interplay of stress habituation and adaptive responses to high stocking densities in farmed gilthead sea bream.

This work was supported by AQUAEXCEL3.0 (H2020; 871108) and Next-Generation GVA-ThinkInAzul (THINKINAZUL/2021/024) projects.



MICRO-PLASTICS IN COMMERCIAL IMPORTANT FINFISHES AND SHELLFISHES OF BANGLADESH

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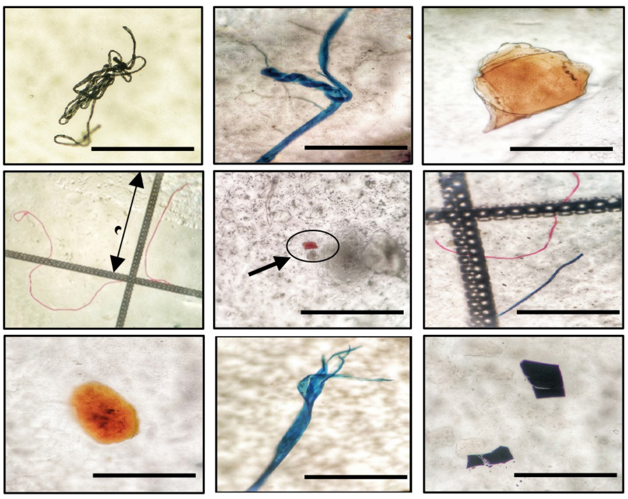
Plastic pollution especially microplastics (MPs) (<5 mm) in aquatic ecosystem is an ongoing global issue that posing significant threats to aquatic species consequences coastal and marine environment and human health. The present study aims to investigate the presence and abundance of MPs in commercially important six finfish and two shellfish. Finfish species included Tuna (*Katsuwonus pelamis*), Hilsa (*Tenualosa ilisha*), Seabass (*Lates calcarifer*), Ribbon fish (*Trichiurus lepturus*), Bombay duck (*Harpadon nehereus*), and Jewfish (*Johnius argentatus*) species and shellfish included shrimp (*Macrobrachium rosenbergii*) and Prawn (*Penaeus monodon*). Both shellfish samples were collected from wild (capture) and farmed (culture) sources in Chittagong, and finfish samples were collected from the fish landing center of Kuakata Bangladesh. Each finfish and shellfish samples were of n=10, where shellfish samples were in equal representation from wild and farmed sources. Then the muscle, gill, gut/gastrointestinal tract (GIT) of each sample were examined for MPs. For extraction of MPs, two-step digestion methods were applied including alkali (KOH) and peroxide (H_2O_2), followed by vacuum filtration and microscopic identification. Fourier-Transform Infrared Spectroscopy (FTIR), heatmap, cluster analysis and principal component analysis (PCA) conducted to identify polymer compositions and correlation among MPs.

The result revealed that in case of finfish a total of 7085 MPs were identified where tuna contained the highest and bombay duck had the lowest. On average the MPs abundance per finfish samples were ranged from 1.56±0.39 to 7.16±1.36 MPs/g in muscle, 1.91±0.32 to 4.46±0.75 MPs/g in the gut, and 2.36±0.24 to 6.53±1.58 MPs/g in gill. In case of shellfish, MPs contents were lower in prawn than the shrimp for both wild and cultured samples. Among the examined organs, GITs were more affected by MPs, followed by gills and muscle of the shellfish, irrespective of the culture and capture sources. Among distinct groups, 1-5 mm sized (33.33-62.78%), white/transparent colored (18.45-54.63%), filament shaped (75.00-94.71%), and fiber types (73.21-94.71%) were dominant. FTIR test confirmed 58.89% polyethylene, 21.67% polypropylene, 17.22% polyester, and 2.22% of non-plastic variants in MPs. Multivariate analysis showed four clusters: clusters 1 and 3 on their own, and 2 and 4 cluster combined two species based on the 50% similarity matrix. In addition, PCA percentage varied from 46% to 69.4% in PC1, and 14% to 22.9% in PC2 revealing the dominance of the factorial dimensions by showing positive-negative distance. The findings provided a comprehensive understanding of MPs extent, source, and characteristics in commercially significant finfish, and shellfish from the coastal area of Bangladesh, thus effective policy implications demanded to avoid plastic pollution in consequences mitigate the potential risks to human health at local and global level.



PUBLIC HEALTH RISK ASSOCIATED WITH CHEMICAL CONTAMINANTS IN FRESH AND PROCESSED FISH OF BANGLADESH

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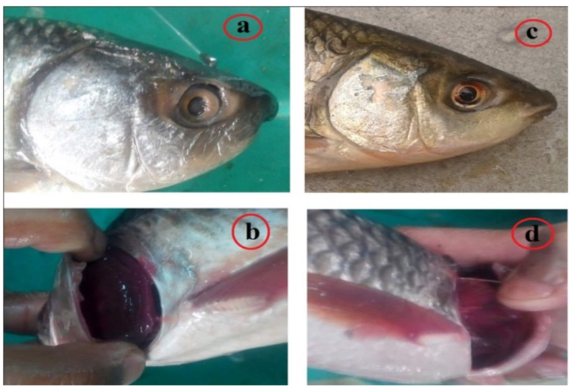
The chemical contaminants in fresh fish and processed fish are of great food safety concern and emerging public health issue in Bangladesh and global aspects. The aim of this study was to assess the public health risk associated with exposure to formalin in fresh fish, and pesticides (organochlorine and organophosphorus) and heavy metals (lead, mercury, cadmium, chromium, arsenic) in dried fish. Formalin concentrations (mg/kg) were determined (n=3) in tilapia, Indian major carp rui, Chinese carp and a minor carp from local market and in laboratory simulations (0.5, 1.0, 2.0 and 4.0% formalin solution for 5, 15, 30 and 60 min). For pesticides and heavy metals, dried fish (Bombay duck, ribbon fish, silver jewfish, shrimp, Chinese pomfret) were collected from Cox's Bazar, Chittagong, Bhola, Patuakhali, Khulna of Bangladesh. Fresh fish and dried fish consumption data were collected from adult respondents (100 from each district) using a food frequency questionnaire (FFQ). Formalin was measured using HPLC method, and pesticides residues were determined using QuEChERS extraction coupled to GC-GCMS, and heavy metals were estimated using atomic absorption spectrophotometric method.

The results revealed that the formalin treated fish with increasing concentrations and contact time showed increased trends of formalin acquisition irrespective to fish species and analytical methods used ($p<0.05$). Formalin exposure of fish consumed was lower than acceptable daily intake (ADI) (0.2mg/kg BW.day) and tolerable daily intake (TDI) (0.15mg/kg BW.day) for both "total population (400 respondents, both consumer and non-consumers)" and "consumers" as determined using spectrophotometry method. Maximum exposure of formalin (0.28 mg/kg BW.day) was for tilapia (consumers) under HPLC method. Margin of exposure (MoE) provides high priority (<10,000) for tilapia and Indian major carp rui at P99 under spectrophotometric analysis where under HPLC analysis tilapia had MoE values more below 10,000 at P99, P95 and P90 (both total population and consumers). In case of dried fish, frequency and amount of dried fish consumption was highest for Bombay duck in Cox's Bazar (11.57g/capita/day) and ribbon fish (12.10g/capita/day) in Chittagong. The estimated daily intake (EDI) and harmonized risk indicator (HRI) values expressed no health risk from pesticide residues in all the positive samples. For heavy metals, target hazard quotients (THQ) for non-carcinogenic health risk were below 1, indicating no health risk for all samples. However, carcinogenic risk R value indicated a potential health risk for chromium, and carcinogenic R_T value indicated a potential health risk for all the metals. In view of food safety, exposure to chemicals associated with fresh fish and processed fish consumption is public health concern in Bangladesh and it has to be taken into consideration when prioritizing risk management strategies. Thus, the study suggests fisher's training on food safety, consumer's awareness, and policy implications to establish risk management strategy that control chemical contaminants in fresh and dried fish consequences ensure safe food for local and global consumers.



ASSESSING THE ENVIRONMENTAL IMPACTS AND NUTRITIONL OUTCOMES OF TILAPIA FARMING IN BANGLADESH

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There is an urgent need to transform global food systems into sustainable models which provide affordable, healthy, and micronutrient rich foods for all. This requires data-driven interventions and policies guided by rigorous food system performance assessments. Life Cycle Assessment (LCA) modelling is increasingly being used to evaluate the combined environmental and nutritional performance of food systems, known as nutritional Life Cycle Assessments (n-LCA). This study utilised novel n-LCA methodology to assess tilapia aquaculture and integrated agriculture-aquaculture systems in Bangladesh. The environmental and nutritional performance of fishponds, rice-fish co-culture, and poultry-fish co-culture was assessed by combining a nutrition metric, the Potential Nutrient Adequacy (PNA) metric, with LCA methods. Micronutrient affordability assessments were also performed to further evaluate the nutritional performance of the farming systems. Although poultry-fish farms were found to have the highest nutritional quality, stand-alone fishponds were found to have better environmental performance overall compared to the rice-fish and poultry-fish farms when nutrition was combined in the LCA. Results show feeds, fertilisers, energy, and chemical inputs have higher environmental impacts compared to other material inputs across all farm types. Results from the affordability assessment identified tilapia and two other fish species (*Cirrhinus mrigala* and *Esomus danricus*) as the most affordable sources of essential micronutrients. These three fish species have a better environmental footprint compared to the other 17 fish species across the three farming systems. This study provides an important example of how nutritional impact can be combined with Life Cycle Assessments and offers insight into the way nutrition metrics can effect the overall results of environmental performance assessments. Utilising the Potential Nutrient Adequacy metric as a nutritional functional unit provides a more transparent approach to food system LCAs, although further development, testing, and validation of n-LCA methodology is needed to refine the process of combining performance assessments. The nutritional quality of tilapia has been undervalued in the literature, but it is shown here that tilapia can provide sustainable, affordable nutrition to populations across Bangladesh.

THE PHOTORECEPTIVE PINEAL ORGAN OF THE ATLANTIC SALMON

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The seasonal light periodicity is a major regulator of numerous important biological processes and life history transition stages in Atlantic salmon such as growth, smoltification and reproduction. The light period is reflected internally by the time keeping hormone melatonin which signals "darkness" with high blood plasma levels during the night and low blood plasma levels during the day. Artificial light conditions are often used in the Atlantic salmon aquaculture to promote growth. However, very little is known about the effects of such light regimes, like for example keeping the fish in constant light conditions. In teleosts, the pineal organ is directly photoreceptive and responsible for both light detection and melatonin synthesis. This organ is located dorsally in the brain where the skin is less pigmented and the skull is quite transparent, providing a window for light to enter. The photoreceptive cells of the pineal organ express opsins that are G-protein coupled receptor proteins capable of receiving light information which in turn translates to melatonin blood plasma levels by regulation of melatonin synthesis of the cell. However, which photoreceptive input is responsible for the circadian melatonin rhythmicity is still unknown.

In this study, we have mapped several opsins and melatonin synthesizing factors of the pineal organ by using RNA sequencing, *in situ* hybridization and immunohistochemistry both in first feeding larva and at the parr stage of Atlantic salmon. We are also in the process of analyzing CRISPR/Cas9 opsin mutants.

The results revealed that seven opsins are expressed in the pineal organ, including *exorhodopsin*, *parapinopsin*, *peropsin* and *teleost multiple tissue opsin*. Many of the opsins are expressed in cells towards the lumen of the pineal organ, but we also find one opsin located in the dorsal sac (**Figure 1**). Many of the opsins are found both in the first feeding larvae and at the parr stage. We also find important factors of the melatonin synthesis in the photoreceptive cells of the pineal such as serotonin, aryl-alkylamine N-acetyltransferase 2 (*aanat2)* and acetylserotonin O-methyltransferase (*asmt)*.

The location of the studied opsins and the melatonin synthesizing factors suggest a link between specific opsin activation and melatonin synthesis. Importantly, an in-dept analysis of the Atlantic salmon pineal organ provides the basis for understanding the impact of light environment on life history transitions such as smoltification and hold the potential to increase animal welfare and yield of the aquaculture industry.

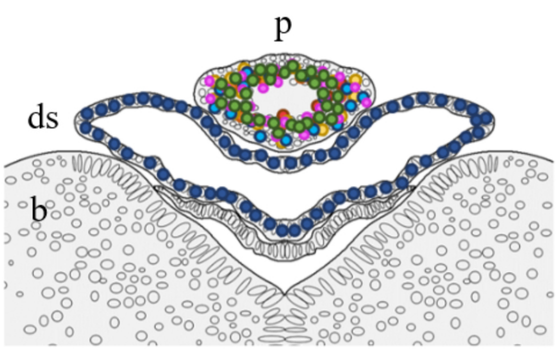

Figure 1: Schematic illustration of a histological section of the brain region of the pineal organ from the first feeding larva Atlantic salmon. The majority of the opsins are expressed towards the lumen, while one opsin (dark blue) is expressed in the dorsal sac. p = pineal organ, ds = dorsal sac, b = brain.

POST-HARVEST LOSS OF PRAWN IN DIFFERENT STAGES OF DISTRIBUTION CHAIN IN SOUTH-WEST REGION OF BANGLADESH

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Fisheries and aquaculture sector in Bangladesh consistently faces substantial post-harvest quantitative and qualitative losses, stemming from inadequate handling and processing practices at various stages of the distribution chain, often due to ignorance and negligence of the associated stakeholders. Prawn and shrimp stand as significant export commodities for Bangladesh, but their rapid spoilage, primarily attributed to bacterial action, enzymatic activity, and chemical reactions, results in significant post-harvest losses throughout the distribution chain. This study engaged stakeholders involved in the diverse phases of the distribution chain of prawn and other aquatic animals produced in prawn farms in Khulna, Satkhira and Bagerhat district. Its aim was to comprehend post-harvest quantitative and qualitative losses, with the ultimate goal of proposing recommendations to enhance food safety and quality for the export market.

This study revealed that prawn farmers sell small quantities from their ponds to the local auction market, but other fish they sell in bulk, so quantitative and qualitative losses in prawn are high. The study demonstrated that an increase in the number of stakeholders within the distribution chain of prawn correlates with multiple handling steps and subsequently higher losses in food quality. Inadequate handling and processing along the distribution chain led to increased deterioration in sensory, biochemical, and microbial quality of prawn.

This study recommends that a shorter distribution chain, increased awareness, appropriate handling and processing practices, and enhanced sanitary conditions can effectively mitigate the deterioration of prawn quality across various parameters. Therefore, it is imperative for the relevant government and non-government authorities to prioritize and implement appropriate measures to reduce losses, aiming to bolster the volume and value of prawn and other aquatic animals, thus enhancing food security, and boosting export earnings in Bangladesh.

PERCEPTUAL DIFFERENCES IN TWO FARMED FISH FROM BANGLADESH: LOGIT MODEL INFERENCES FOR CONSUMER PREFERENCES

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Consumer preferences of fish and fishery products are important due to several product-market characteristics such as price of the fish, its availability, affordability, freshness, flavor and texture as well as age, education, income of the consumers, health and environmental issues. Therefore, the aim of the study is to depict how consumer preferences vary with the different market segments of two popular farmed fish species in Bangladesh; pangasius (*pangasianodoan hypophthalmus*) and tilapia (*Oreochromis mossambicus*) which have a great contribution in the tremendous growth of aquaculture production in Bangladesh.

For this purpose, 600 respondents-300 of each-were interviewed through a pretested structured questionnaire from seven districts of Bangladesh between the years of 2016 and 2017. The standard dichotomous logistic regression model on the purchase decision of farmed fish (dependent variable) to all independent variables is employed to analyze factors influencing the purchase decision of farmed fish for their household consumption. It was discovered that there are notable discrepancies.

The results show that the average price of both the farmed fish is 118BDT with a minimum price of 30BDT and a maximum of 250BDT. The average price of pangasius is lower than the price of tilapia.

The results also show that several factors are important for tilapia purchase decisions including socioeconomic parameters, such as age, income, and household size. The pangasius preferences are largely important for availability, flavor and taste whereas tilapia preferences are important for freshness including its availability and flavor. The results are different while the product markets are segmented; pangasius-production & consumption zones and tilapia-production & consumption zones.

The implications of the study are actors involved in the two fish supply chain can target the different product market segments considering the findings. Additionally, the pangasius actors need to pay attention to its flavor. The government can also play a significant role in overseeing or subsidizing this sector with a view to increasing fish production and supply, and develop the nation's GDP.

Table 3. Estimated results of the logistic regression models for pangasius market segments

Variable	Production zone			Consumption zone		
	$\beta\ (\sigma_{\bar{x}})$	$exp.(\hat{\beta}_x)$	$\frac{dy}{dx}$	$\beta\ (\sigma_{\bar{x}})$	$exp.(\hat{\beta}_x)$	$\frac{dy}{dx}$
Price	$-0.004(0.002)^{***}$	0.000	130.00	$-0.043(0.064)^{*}$	-0.001	107.63
Availability	$2.343(0.987)^{**}$	0.094	0.32	$3.143(1.987)^{*}$	0.038	0.25
Bones	-2.708(0.792)	-0.146	0.23	$-0.274(1.281)^{***}$	-0.005	0.15
Flavor	0.449(0.883)	0.009	0.22			
Taste	-0.256(0.877)	-0.006	0.24	-2.047(1.473)	-0.072	0.24
Freshness	$2.147(0.928)^{**}$	0.093	0.76	$1.119(1.473)^{**}$	0.021	0.48
Age	$-0.095(0.044)^{**}$	-0.002	38.35	$0.102(0.079)^{*}$	0.002	40.64
Education	0.116(0.096)	0.003	9.68	0.143(0.238)	0.003	6.89
Income	$-0.001(0.001)^{**}$	-0.001	233.70	$-0.001(0.001)^{*}$	-0.001	337.50
HHS	-0.334(0.228)	-0.007	4.94	0.607(0.539)	0.011	5.41
Constant	$2.186(1.873)^{***}$			$-3.155(7.965)^{***}$		
N	184			116		
LR χ^2	30.06^{**}			19.05^{**}		
Pseudo R^2	0.304			0.242		

Table 4. Estimated results of the logistic regression models for tilapia market segments

Variable	Production zone			Consumption zone		
	$\beta\ (\sigma_{\bar{x}})$	$exp.(\hat{\beta}_x)$	$\frac{dy}{dx}$	$\beta\ (\sigma_{\bar{x}})$	$exp.(\hat{\beta}_x)$	$\frac{dy}{dx}$
Price	0.002(0.013)	0.000	112.63	$-1.001(0.015)^{*}$	-0.097	0.66
Availability	-0.350(0.561)	-0.028	0.46	$0.423(0.335)^{*}$	0.037	0.45
Bones	-0.298(0.539)	-0.024	0.39	$-0.737(0.585)^{***}$	-0.013	0.24
Flavor	-1.124(0.754)	-0.117	0.22	0.293(0.253)	-0.071	0.21
Taste	$0.774(0.403)^{**}$	0.061	1.31	0.679(0.268)	-0.016	1.09
Freshness	$1.585(0.564)^{***}$	0.164	0.68	$1.162(0.956)^{**}$	0.006	0.71
Age	$0.006(0.027)^{**}$	0.002	41.09	$0.972(0.033)^{*}$	0.001	36.43
Education	-0.072(0.067)	-0.006	9.76	0.997(0.088)	0.000	10.07
Income	0.001(0.002)	0.001	253.30	$-1.000(0.025)^{*}$	-0.087	560.00
HHS	$0.274(0.149)^{*}$	0.022	4.96	1.706(0.584)	0.022	4.73
Constant	$4.501(2.204)^{***}$			$8.008(21.995)^{***}$		
N	150			150		
LR χ^2	18.18^{**}			11.21^{*}		
Pseudo R^2	0.160			0.153		

EFFECTS OF DIETARY PHOSPHORUS LEVELS ON WATER QUALITY PARAMETERS IN RECIRCULATING AQUACULTURE SYSTEMS

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Fish excrete digested but excessive dietary phosphorus (P) as dissolved inorganic orthophosphate (PO_4-P). As PO_4-P is generally considered harmless to on-growing fish, recirculating aquaculture systems (RAS) typically do not apply P removal units within the recirculating loop, allowing PO_4-P to accumulate until balanced by water renewal levels (make-up water). However, if no end-of-pipe treatment is applied, high concentrations of PO_4-P in the discharge can contribute to the eutrophication of recipient water bodies. Tailoring dietary P levels to exactly match fish requirements may be a means to reduce PO_4-P discharges from RAS.

To explore this, three levels of PO_4-P concentrations in RAS were examined and achieved by using a low-P diet, a high-P diet, and the high-P diet plus the addition of Na_3PO_4. Each treatment was tested in quadruplicated pilot-scale RAS with rainbow trout (*Oncorhynchus mykiss*) for eight weeks, applying a fixed daily feed loading, fixed about of make-up water, and obtaining weekly water samples from the sumps.

The concentrations of PO_4-P in all three treatment groups quickly diverged and stabilized at three significantly different levels from week six and onwards, reflecting the loading. However, the low-P diet did not lead to near-zero PO_4-P accumulation in RAS due to compromised feed conversion ratios. Total phosphorus concentrations were likewise significantly different, while there were no effects on other physicochemical water quality parameters including nitrogen compounds, water clarity, and organic matter.

Altogether, the study shows that manipulating dietary P levels to match fish requirements can be an efficient tool to reduce the discharge of dissolved phosphorus from RAS, reducing the need for end-of-pipe treatment and rendering the production more sustainable.

EFFECTS OF CO-MANAGEMENT ON TECHNICAL EFFICIENCY AND ENVIRONMENTAL STRESSORS: AN APPLICATION TO SMALL-SCALE SHRIMP POLYCULTURE IN INDONESIA

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Shrimp polyculture is an important activity in SouthEast Asia, providing a livelihood and food for small-scale farmers. However, farmers are facing challenges due to increasing environmental problems in rivers used as the primary source of water in aquaculture pond production. To deal with these challenges, farmers have organized co-management communities, which gives them a stronger voice among river users. An illustration of such cooperative efforts is evident in Sidoarjo District, East Java, Indonesia, where the practice of co-management in aquaculture has evolved throughout the years.

This study investigates the effects of co-management on technical efficiency and farmer's resilience to environmental stressors using an input-oriented Data Envelopment Analysis (DEA) model. Environmental stressors are identified through interviews with 306 farmers in eight sub-districts in Sidoarjo, of which two are practicing co-management. The primary data collected from farmers included farm-level economic, social data, and farm characteristics, completed with farmers' opinions about five environmental stressors and an economic stressor measured on a Likert scale and additional information about co-management.

The results show that farmers in two sub-districts practicing co-management have relatively high technical efficiency after taking other drivers into account. The findings establish a correlation between technical efficiency and co-management, highlighting the apparent connection between the practice of co-management and the observed increase in technical efficiency. Moreover, the positive impact of co-management appears to be particularly significant for farms with an area under 5 hectares. In terms of farmer's resilience to environmental stressors that often occur in shrimp farming, the farmer's technical efficiency seems to be less affected by the identified environmental stressors. These results include two significant findings. First, the positive effect of co-management on technical efficiency and stress resilience suggests that farmers have an incentive to participate in collective action. Second, the results confirmed the effectiveness of co-management on the potential of collective action for solving market-failure problems.

In conclusion, co-management in this case study is an effective tool for small-scale shrimp polyculture farmers who are highly dependent on multiple-use river basins as their main water resource. Co-management could be used as a tool to improve technical efficiency and limit stress factors for farmers. It could be initiated by the farmers themselves or by policymakers and natural resource managers and could improve the livelihoods of small-scale shrimp polyculture farmers.

GROWTH PERFORMANCE, NUTRIENT COMPOSITION AND WELFARE OF AFRICAN CATFISH (*Clarias gariepinus* BURCHELL, 1822) UNDER PARTIAL REPLACEMENT OF FEED PROTEINS WITH PORK BONE MEAL

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Rising raw material prices, an increased demand for high-quality food and the desire for sustainable and regional production require new approaches and suitable protein sources. One possibility is pork bone meal (PBM) that had been earlier studied in fish feed trials with Pacific white shrimp *Litopenaeus vannamei* (C. Hernández et al., 2008) and Hybrid Catfish *Ictalurus punctatus* × *Ictalurus furcatus* (Li et al., 2018)

In this trial, the proportions of animal and vegetable protein in a commercially used catfish feed were partially reduced and replaced with pork bone meal. The proportions of PBM in the three feeds used were 0 % (control), 15 % (PBM 15) and 30 % (PBM 30). The ingredients of the feeds are given in Table 1. Attention was paid to isonitrogenous and isoenergetic in the diets. Furthermore, care was taken to ensure that the essential amino acids did not fall below critical levels.

The extent to which protein substitution with PBM affects the growth performance, nutritional composition and welfare of the African catfish *Clarias gariepinus* was investigated.

The 60 days long experiment was carried out in a randomized block design in triplicates, with 25 individuals in each fish tank at the start of the experiment. The fish were fed once a day 80 % of a commercial protocol.

No significant differences were found between the groups for either the length or the weight of the fish. Differences in mortality can be attributed to the husbandry conditions during the trial and not to the diet fed.

The results of this trial revealed that a replacement up to 30 % PBM is possible, with no negative impact onto growth performance and survival. Application of PBM instead of imported soybean meal and replacement of poultry proteins with PBM as a regional waste product increases sustainability and cost efficiency of African catfish aquaculture in northern Germany.

Literature:
C. Hernández, M.A. Olvera-Novoa, K. Aguilar-Vejar, B. González-Rodríguez, I.A. de la Parra (2008), Partial replacement of fish meal by porcine meat meal in practical diets for Pacific white shrimp (Litopenaeus vannamei)
Li, M.H., Bosworth, B.G. and Lucas, P.M. (2018), Evaluation of Porcine Meat and Bone Meal in Diets for Pond-Raised Hybrid Catfish. North Am J Aquaculture, 80: 69-73. https://doi.org/10.1002/naaq.10008

Ingredients [%]	Control	PBM 15	PBM 30
Wheat flour	30,48	30,48	28,23
Soybean meal	6	3,5	1,75
Rapeseed	10	7,5	5
Poultry meat meal	12	6	0
Feather meal	5	2,5	0
Pork bone meal	**0**	**15**	**30**
Monocalcium phosphate	0,5	0	0
Feed lime	1	0	0
Others	35,02	35,02	35,02
Total	100	100	100

Table 1 Ingredients of the individual diets

ISOLATION AND MOLECULAR DETECTION OF VIRULENCE AND ANTIMICROBIAL RESISTANCE GENES OF PATHOGENIC *Escherichia Coli* ISOLATED FROM FARM RAISED CARPS OF FISH FARMS OF KASUR AND MUZAFFARGARH

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Bacterial pathogens pose a significant challenge for fish production. *Escherichia coli* is a Gram-negative bacterium being among the leading contributors of economic loss to fish farmers worldwide. *E. coli* infects a variety of fish species making a concern for public health. In order to gain a better understanding of this issue, a study was conducted to isolate and identify *E. coli* present in *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix*, and *Ctenopharyngodon idella* sampled from fish farms of Kasur and Muzaffargarh. *E. coli* was isolated from liver, intestine, stomach and kidney of fish samples using MacConkey and Eosin Methylene blue (EMB) agar media. DNA was extracted using Genomic DNA Purification Kit and *E. coli* was detected by amplification of virulence genes viz. stx2 and eaeA and antibiotic resistance genes viz. tetA and sul1 in *E. coli* by PCR using species-specific primers. *E. coli* was recovered from 103 (41.2% prevalence) fish samples of five species. Phenotypic and morphological characterization revealed pink, smooth, and circular colonies of E. coli on MacConkey agar while dark purple and circular colonies on EMB agar media. Biochemical tests proved *E. coli* positive in catalase, indole, urease, methyl red and motility tests while negative results in Gram-staining, oxidase, citrate, Voges-Proskauer, H_2S and indole production tests. *E. coli* isolates proved to be resistant against pencillin, sulfamethoxazole and tetracycline while sensitive against ampicillin, and erythromycin, and intermediate resistance against amoxicillin and cefotaxime. Maximum prevalence of stx2 gene of *E. coli* was recorded in intestine and liver of *L. rohita* and *C. mrigala*. Phylogenetic tree analysis of our isolated *E. coli* strains revealed 97% similarity with *E. coli* strains isolated in previously studies. The results concluded that shiga toxic gene was the most significant pathogenic gene of *E. coli*. High stocking density, low water quality parameters and unchecked application of antimicrobial agents causes emergence of pathogenic bacteria in fish farms.

EFFECT OF FEEDING TIME ON THE DAILY VARIATION OF AMMONIA EXCRETION RATE IN BLACK BULLHEAD CATFISH *Ameiurus melas*

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Introduction

Evolution has selected organisms that were able to anticipate fluctuations of their environment and adapt themselves accordingly (DeCoursey, 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.

Materials and methods

Three experimental groups were constituted (ML was fed during the light phase at ZT6, MD was fed during the middle of the dark phase at ZT18, and finally, ALE, the last group had no fixed feeding time). Each per treatment was considered individually and the three corresponding sampling sand isolated in metabolic tanks. In order to measure ammonia excretion rate at a given time, 6 fish were selected per treatment and isolated in metabolic tanks. The ammonia concentration was measured in the metabolic tanks right before adding the fish, and then 4 hours after the introduction of the fish. The differences in ammonia concentration allowed us to calculate ammonia excretion rate in throughout thse 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, gill and liver samples were collected on 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 April, so the current section will only cover ammonia excretion rate.

Statistical analysis (one-way ANOVA and its post-hoc Tukey test) have showed significant differences in the daily variation of ammonia excretion rate based on the experiment groups. Cosinor confirmed the rhythmic nature of ammonia excretion in ML and MD, but fish that had no fixed feeding time did not display such rhythmicity. Acrophase of ammonia excretion occurred at ZT18 in ML (12 hours after feeding time), while the highest ammonia excretion rate was observed at ZT2 in MD, only 6 hours after feeding. This suggests that feeding time is not the lone factor controlling the dynamics of ammonia excretion.

References:

DeCoursey, P.J., 2004. The behavioral ecology and evolution of biological timing systems.

Dunlap, J.C. and Loros, J.J., 2017. Making time: conservation of biological clocks from fungi to animals. *Microbiology spectrum*, 5(3), pp.10-1128.

GUT MICROBIOME OF RAINBOW TROUT IS AFFECTED BY FEEDING PROTEIN, OIL AND CHITIN FROM BLACK SOLDIER FLIES

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Many studies have investigated the efficacy of replacing fishmeal and fish oil with black soldier flies (BSF) as a more local and sustainable resource on the growth performance of fish, although research is lacking on the effects of BSF on their gut microbiome. Furthermore, oil and chitin are commonly removed from BSF to increase the protein content and nutrient digestibility of BSF, although these extracts may improve gut health. Rainbow trout (30g) were fed for 84 and 168 days with diets based on fishmeal and a combination of fishmeal and soybean meal, respectively, to challenge the fish. Triplicate tanks of fish were fed one of seven diets that included a control, 5 and 10% full-fat BSF (FF05 and FF10), 5 and 10% defatted BSF (DF05 and DF10), 4% BSF oil (OIL) and 1% BSF chitin (CHTN). At each timepoint, the faeces and mucosa were collected, extracted, amplified and sequenced on the Illumina MiSeq platform using the V3-V4 region of the 16S rDNA. At day 84, Firmicutes were lower in the mucosa of fish fed the DF05 diet than the DF10 and OIL diets and the opposite was found for Proteobacteria (Fig. 1). Specifically, the genus *Lactococcus* was significantly less abundant in the faeces of fish fed the DF05 diet compared to the CNTRL and OIL diets. On day 168, Firmicutes were lower in the mucosa of fish fed the DF10 diet compared to the OIL and CHTN diets. Increased abundance of Firmicutes has been used as an indicator of a healthier gut microbiome, thus BSF oil and chitin may be beneficial additives in feeds for rainbow trout and they should not be removed from full-fat BSF due to these benefits.

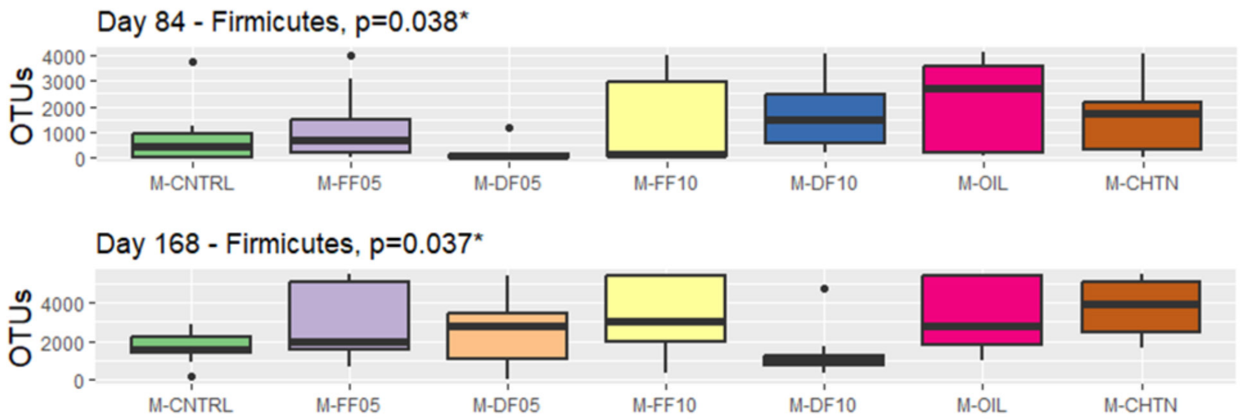

Figure 1. Boxplots of the abundance of bacterial OTUs (phyla level) in faeces (F) and mucosa (M) from the distal intestine (n=6-9) of rainbow trout at day 84 and 168.

PHYSIOLOGY AND MORPHOLOGY OF CLONAL ATLANTIC SALMON: INFLUENCE OF INCUBATION TEMPERATURE, PLOIDY AND ZYGOSITY

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Isogenic (clonal) fish lines provide a useful experimental model in aquaculture research to study fundamental effects of rearing environment and genetics on commercially important traits. Moreover, established clonal lines of interest can be maintained for generations without genetic change. This can provide an advantage over using variable and random genetic material of domesticated or wild origin prone to generational changes. Furthermore, higher individual variation in outbred fish groups makes it more difficult to interpret to what extent phenotypic outcomes are the result of genetics relative to environmental factors.

At the Matre Research Station in Norway, clonal lines of cultured Atlantic salmon (*Salmo salar*) have been established using two-generational gynogenetic techniques. These are now being used for various fundamental and applied research purposes.

In this presentation we report on experimental work with clonal diploid, triploid, and homozygous (inbred) Atlantic salmon, as well as an identical clonal diploid group incubated at a lower temperature (4°C instead of 8°C).

We used intermittent-flow respirometry to measure metabolic rates, hypoxia tolerance, the acute stress response, and subsequent stress recovery. Then we assessed the morphology of hearts and otoliths since these are known to be influenced by both environment and genotype. Finally, confirmation of clonal status, ploidy, and zygosity was achieved from microsatellite markers.

The low incubation temperature slowed down development and growth, and this group was therefore tested 9 months later than the other groups to allow for it to reach a similar size of ≈170 g. Further, the low temperature group developed a clear bimodal size distribution where one-third of the group were substantially smaller.

Group differences, causes for individual variations, and other patterns in the data will be discussed, including links between morphological and physiological traits. We conclude with our perspective on clonal Atlantic salmon in aquaculture research.

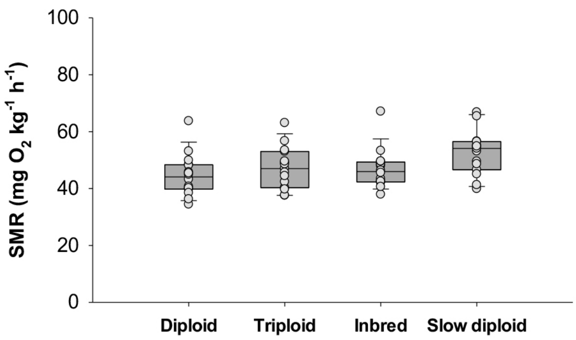

Figure 1. *Standard (resting) metabolic rates (SMR) of clonal Atlantic salmon groups: Triploids have three pairs of chromosomes instead of two pairs in diploids. "Inbred" refer to being homozygous, and "Slow diploids" were incubated at a lower temperature resulting in slower growth. N = 16.*

EDUCATIONAL MATERIAL DEVELOPMENT TO PROMOTE INTEGRATED MUTI-TROPHIC AQUACULTURE (IMTA) IN THE FRAME OF ASTRAL PROJECT

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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. To address these aspects, providing and improving access to accurate and reliable education about the marine environment is essential. 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, educators 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. Teaching youngsters certain topics about sustainable aquaculture and more complex biological processes such as IMTA can be difficult, especially when resources are scarce and information is vast, and, in many cases, too technical or in English (most used language for scientific communications). Therefore, we have developed a compilation of activities (i.e. activity guide) that intends to disseminate scientific knowledge in a pedagogical and ludic way. This material aims to be a resource for teachers while teaching children between 10 to 15 years old, to convey basic knowledge about traditional aquaculture and IMTA. The guide addresses general concepts about aquaculture and fisheries, as well as more specific production methods such as IMTA and aquaponic systems. Thirty-seven (37) activities have been created including didactical tools such as: multiple choice, open questions, join with arrows schematics, find the differences schematics, crosswords, puzzles and others, to give the teachers more alternatives to engage with students. This guide has been translated into 3 languages (English, Spanish, Portuguese) being therefore available for more than 120 countries. We hope this educational guide results in an effective tool to foster dissemination strategies which are paramount within the realm of multitrophic aquaculture projects to bridge the gap between scientific knowledge and public understanding.

The ASTRAL project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 863034. Further information: www.astral-project.eu/

COMPARATIVE GROWTH ASSESSMENT OF AFRICAN CATFISH (*Clarias gariepinus)* FED SWEET POTATO (*Ipomea batata*) LEAF MEAL AND RICE BRAN AS ALTERNATIVE FEED MEAL

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The study compared growth performance of *Clarias gariepinus* fingerlings fed sweet potato leaf meal and rice bran as alternative feed meal. Five diets, that contain 0%, 25%, 50%, 75% and 100% inclusion levels were formulated and designated as SPM_1 SPM_2 SPM_3 SPM_4 SPM_5 and RBM_1 RBM_2 RBM_3 RBM_4 RBM_5. Highest initial weight in $SPLM_3$ (50%) differ significantly ($p>0.05$) from $SPLM_1$ (0%) while RBM4 (75%) can be compared to RBM_1 (0%). Final weight and body weight gain follow similar trend with $SPLM_2$ (50%) recorded highest, which differ significantly ($p>0.05$) with other diets. Decrease of SPLM inclusion level increase fish weight. Highest SGR in $SPLM_2$ (25%) can be compared favorably with $SPLM_1$ (0%) and other diets while RBM_2 (25%) differ significantly ($p>0.05$) with RBM_1 (0%). Diet $SPLM_5$ (100%) can be compared with other diets in SGR with RBM3 (50%) that differ significantly ($p>0.05$) with only RBM1 (0%). Highest FE in $SPLM_1$ (0%) can be compared favorably with $SPLM_3$ (50%) and $SPLM_4$ (75%) in feed acceptability. RBM_5 (100%), which is highest differ significantly ($p>0.05$) with RBM_1 (0%). Apart from RBM4 (75%) other diets were accepted by the fish. It is therefore recommended that 25% inclusion level of SPM should be used in the diet of *C. gariepinus* for better performance than even 75% inclusion of rice bran, study on lower inclusion level of SPM need to be carried out, this type of study need to carried out on other cultured species of fish such as *Heterobranchus* (catfish) and even Tilapia, study on the use of other processing methods of sweet potato leaf and rice bran for feed formulation should be carried out and this type of study need to be carried out in other culture system.

FOOD AND FEEDING HABITS OF THE CICHLIDAE IN TAGWAI RESERVOIR, NIGER STATE, NIGERIA

*Ibrahim, Baba Usman ., Balogu, Dennis Odionyenfe and Yunusa, Abdu

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Four hundred and seventeen (417) samples comprising of one hundred and eighty-six *Sarotherodon galilaeus* and two hundred and thirty-one (231) *Coptodon zillii* were collected on monthly basis for four (4) months (March, 2021 to June, 2021) from the catches of local fishermen operating on Tagwai Reservoir, in Niger State, Nigeria. The fresh samples were transported in ice - chest box to the Biology Department laboratory of Ibrahim Babangida University (IBB), Lapai, Niger State, where analyses were carried out. Eight (8) different types of items that constituted plant and animal materials were found as food in the stomachs of each of *S. galilaeus* and *C. zillii*. This included detritus, sand, algae, plant material, nematode, plankton, seed and unidentified material. Plant material was highest (30.30%) followed by detritus (17.32%) then algae (16.01%) and lowest was nematode and unidentified material each with 2.16%. Feeding intensity of both species of fish was high due to low percentage of empty stomachs recorded during the period of study. 157 stomachs of *S. galilaeus* out of 186 examined had food while 181 stomachs of *C. zillii* out of 231 examined had food. *S. galilaeus* and *C. zillii* are omnivore and herbivore respectively based on their feeding habits. Diet overlap or similarity showed moderate level of association in diet and less competition for food between the two species. There is need to examine other aspects of biology, such as growth, fecundity, age of these fishes in the reservoir. This study can be used as baseline information for carrying out similar study in other water bodies.

FOOD AND FEEDING HABITS OF THE CICHLIDAE IN TAGWAI RESERVOIR, NIGER STATE, NIGERIA

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REALTIME NON-INVASIVE FISH SWIMMING SPEED MEASUREMENT BY DEPTH USING ECHO SOUNDER IN HIGH DENSITY *Seriola quinqueradiata* AQUACULTURE CAGE

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In recent years, fish welfare is emphasized in aquaculture. Swimming speed of farmed fish is one of the important indicators of fish behavior. A powerful tool to measure the fish speed is biologging, which requires attaching data-logger to fish. Therefore, there are two concerns: the fish speed may change due to drag force, and the logger may injure the fish. Furthermore, biologging logger has a limited lifetime in spite of being costly. Sample size limitation is also a problem. On the other hand, echo sounder is a tool to measure the fish speed without surgical procedures. However, it has not been widely employed as comparative evaluation of echo sounder, with existing methods in high density aquaculture cage. The purpose of this study is to evaluate the accuracy of the measuring method of fish speed using an echo sounder.

The experiment was conducted in an aquaculture cage (8m×8m×8m) of Yellowtail (*Seriola quinqueradiata*), in Kyushu Island, Japan, on May 30, 2023, for 2 hours. The fork length was 51 ± 2 cm (mean ± s.d.) and the number of fish was about 3000. The experimental echo sounder (Furuno Electric Co., Ltd., FAS-2100) was installed at 1m depth and the three stereo cameras (Furuno Electric Co., Ltd., UC-300) were installed at 3.4m, 4.8m, and 6.3m depth, respectively. Preliminary verification with the data-logger (Little Leonardo, ORI400-3MPD3GT) confirmed that the difference between the stereo camera and the data-logger was 0.01 ±0.06 m/s (mean ± s.d.). Thus, the stereo camera was used as a reference sensor. Result of the difference between echo sounder and the stereo camera was 0.02 ±0.15 m/s (mean ± s.d.) (Fig.1). This study indicates that the echo sounder is an efficient tool for measuring the vertical profile of fish speed in the aquaculture cage.

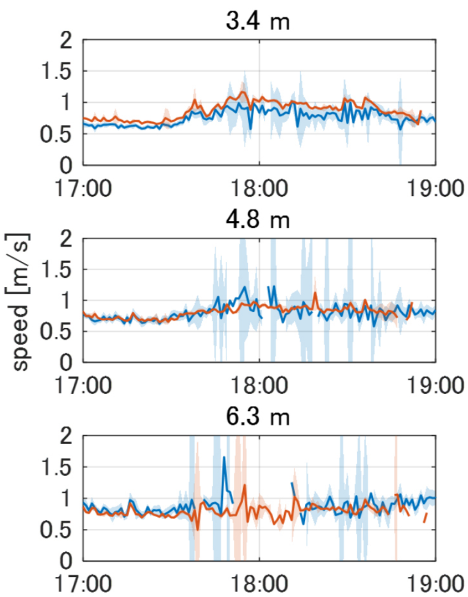

Fig.1. Time-series of fish swimming speed measured by the echo sounder (blue) and by the stereo camera (orange). The bold lines represent the means, and the highlighted parts represent 95% confidence intervals.

GROWTH PERFORMANCE OF JUVENILE SEA CUCUMBER *Holothuria scabra* IN EARTH PONDS: THE ROLE OF NURSERY SYSTEMS

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The sea cucumber *Holothuria scabra* is a highly valuable species and is experiencing declining in population due to over-exploitation, making aquaculture vital for its conservation and market supply. Hatchery production challenges, including feeding, space, and cost, make indoor nursery culture less effective. Transitioning juveniles to outdoor systems is required to optimize the hatchery-rearing phase. The objectives are to determine the impact of various nursery systems on the growth and survival of juvenile *H. scabra* in earth ponds. The study was carried out in Lombok, Indonesia, and was conducted over 84 days in various settings within earthen ponds, utilizing a completely randomized design with five replicates. The four distinct treatment sites included a reservoir (N1), a stirred pond (N2), a non-stirred pond (N3), and the main inlet sluice (N4). The results show that N4 is the most effective method for promoting the Growth Rate (0.083±0.011 g d^{-1}) and Specific Growth Rate (2.360±0.145 % d^{-1}). On the other hand, N1 had the highest survival rate, at 74.80±5.82%.373

OFFSHORE AQUACULTURE – CHALLENGES AND CHANCES
WHAT HAS TO BE CONSIDERED FOR CULTIVATION OF SPECIES FAR OFFSHORE?

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The effects of climate change on the ecosystems and society reveal the need moving away from fossil to renewable energies such as wind or solar energy. Additionally, global food security as well as healthy marine ecosystems are at serious jeopardy as marine resource production can no longer be sustained by ecosystems and fisheries production only. One approach could be the potential of low trophic aquaculture (LTA) being conducted within in a multi-use (MU)-approach combined with renewable energy installations. This could reduce food and nutrition insecurity and at the same time maximises the benefits of marine space with low additional ecological impacts.

The installation of open ocean aquaculture systems within offshore wind farm sites, which are often in distant and exposed environments, requests different approaches for the development of those structures and different protocols for permissions.

In the framework of a case study site within the OLAMUR-Project (Offshore Low-trophic Aquaculture in Multi-Use scenario Realisation, EU Project ID 101094065) we are working on the development and deployment of aquaculture system-designs in a highly exposed and offshore location within an offshore wind farm, Meerwind Süd/Ost, located approximately 14 nautical miles north of Heligoland and 45 nm off the German coast (Cuxhaven). The first months of the logistics planning, administrative organisation for permits and development of structures clearly revealed that offshore aquaculture in a MU-approach means entering new ground. Here, we would like to briefly present the obstacles this project is confronted with and that aqua farmers probably will also be facing in the future. Activities offshore concerning aquaculture, scientific or commercial, will need new regulatory frameworks and protocols. Additionally, new aquaculture structures have to be developed to be sufficiently robust to cope with harsh environmental conditions but at the same time cost-efficient to allow a financially sustainable low trophic aquaculture production.

CONTINUE OR QUIT: FISH FARMERS CARRYING COVID-19 TRAUMA IN THE NEW NORMAL PERIOD OF BANGLADESH

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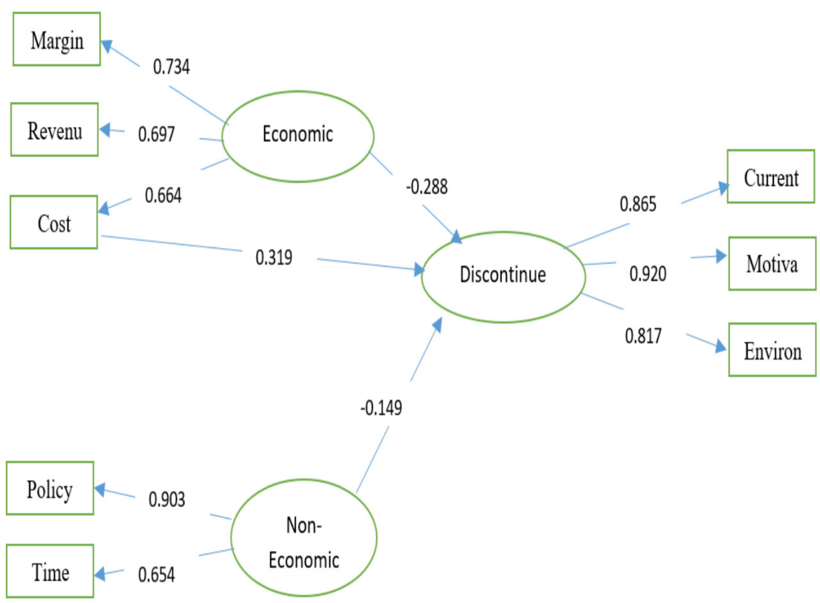
Just after the COVID-19 challenges, global inflation enhanced production costs that shrunk production capacity and margin for fish farmers in Bangladesh. The disappointment in attaining economic goals and non-economic challenges create dissatisfaction and that may lead to discontinuing farming is the research interest of the paper

The financial status was calculated and five points Likert scale was used to derive satisfaction data. The structural equation modeling was used to measure economic and non-economic satisfaction and possibilities for discontinuing farming. In comparison with covid-19 period, cost and price increased, however, the gross margin declined for the higher pace of cost than price.

The research identified that economic satisfaction had a significant influence and was negatively related to discontinuing farming and cost had a positive influence to discontinue decision. The non-economic issues were insignificant to discontinue the decision. The carp farmers were losing margin, however, they did not decide to quit farming because of unavailable better alternative business than farming, hopeful for future cost reduction and price enhancement. Besides the financial status, the physiological aspects of farmers, refer to the future sustainability of the farming is a new initiative to analyze the aquaculture sector in Bangladesh.



EFFECTS OF DIFFERENT FORMULATED FEEDS ON THE AMYLASE AND LIPASE ACTIVITY IN DIGESTIVE TRACT OF FINGERLING *Labeo rohita*

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The study was conducted to measure the activity of amylase and lipase enzymes in gastrointestinal tract of *Labeo rohita* fingerlings fed with different formulated feeds under the intensive culture system in laboratory. Acclimatized fish was stocked in the glass aquaria and 30% & 40% Crude protein feed was offered @ 5% of their body weight at dawn and dusk. The stocking density per aquarium was 15 fingerlings. The physicochemical parameters were measured and regulated if needed on daily basis as per requirements of fingerling *L. rohita*. The growth parameters viz. total length, body weight and fork length was checked on weekly basis to analyze the growth performance. The total time period of trial was 60 days, fish were slaughtered and GI tract was obtained at the end of experimental trial for analysis of digestive enzymes activity by using standard protocols. The statistical analysis of data depicts that the growth (body weight, fork length, total length) of fish showed significant variations among the trials. The fish fed 40% C.P feed have higher growth curve than the fish fed with 30% C.P feed. ANOVA showed highly significant result between these trials for weeks and replications showed non significant results. Throughout the experimental trial there was no issue of physico-chemical parameters was observed and the figures remained in the normal range. The statistical analysis showed non-significant relationship of water quality parameters with one another and the graph of correlation coefficient showed statistically non significant bars among the both trials of *L. rohita* fed 30% and 40% C.P feed. The higher activity of amylase was observed in the liver of fish as compared to the intestine of fish fed with 40% C.P feed. The amylase activity showed highly significant results between the both treatments. The higher activity was observed in the fish fed with 40% C.P feed although there was non significant relationship between the replicas. The little activity of lipase was observed in the liver in comparison to the intestine of fish fed with 40% C.P feed. Highly significant results were observed for the activity of lipase between treatments and replications.

THE EFFECT OF ACUTE ALLOSTATIC STRESS ON THE HYPOTHALAMIC-PITUITARY-INTERRENAL AXIS AND ITS IMPORTANCE ON ANIMAL WELFARE IN DIPLOID AND TRIPLOID ATLANTIC SALMON SMOLTS *Salmo salar* L.

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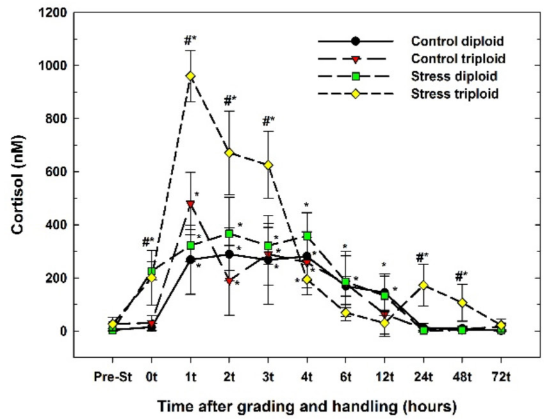
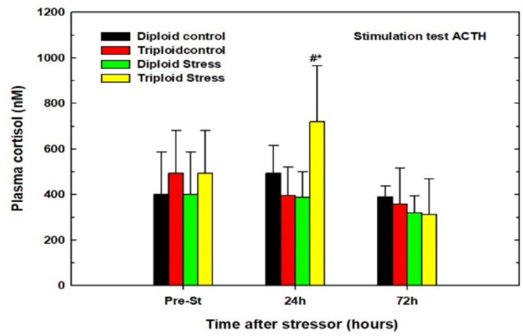
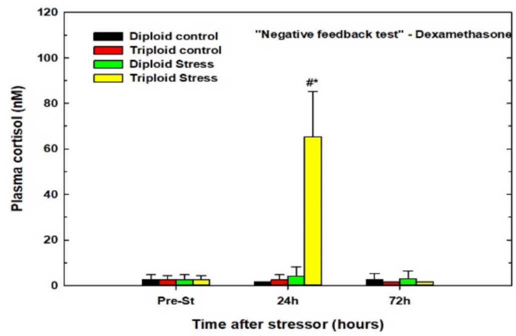
The concept of animal welfare has been given much attention in the last few years. It is beneficial to practise good fish welfare in aquaculture to achieve good fish health, low mortality, good quality and high profitability. Artificially produced triploid salmon, with an extra set of chromosomes, is sterile and produced to prevent genetic interactions with wild populations. The full biological effects of having an extra chromosome set are largely unknown, but triploids are considered more sensitive to sub-optimal environmental conditions.

This study aimed to investigate the effect of an acute allostatic load on the HPI–axis of diploid and triploid Atlantic salmon smolts (*Salmo salar* L.). Five hundred smoltified (0+) Atlantic salmons were used in the experiment. The experiment consisted of four groups (in triplicate): control diploid, control triploid, stress diploid and stress triploid. The stress groups were exposed for 20 minutes, crowding stress, and distributed to different tanks for sampling after 0, 1, 2, 3, 4, 6, 12, 24, 48 and 72 hours. Diploid and triploid had elevated plasma cortisol concentrations, but the triploid salmon showed significantly greater values than diploids. The results of this study indicate that triploid Atlantic salmon smolts exposed to an acute allostatic load result in an allostatic overload between type 1 and type 2 responses with a temporary oversensitivity to ACTH and a reduced negative feedback system, with a temporarily reduced regulatory ability of divalent ions. Although no negative tertiary stress responses like reduced growth or increased mortality were detected in this experiment, the oversensitive triploid salmon might quickly end up in an allostatic overload type 2 (chronic stress) followed by compressed welfare if subjected to a stronger or longer-lasting stressor. Therefore, the triploid salmon should be handled as gently as possible with the lowest stress load possible to prevent the animal welfare of this ploidy salmon from becoming compromised.







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ESTIMATING ECONOMIC VALUE FOR SOCIETY: THE IMPORTANCE OF INDUSTRY-SPECIFIC MATRICES IN SEAFOOD RIPPLE-ANALYSES

Iversen, A.*, Nyrud, T., Bendiksen, B.I., Robertsen, R., Steinsbø, S.

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An interesting debate arose after our report on the ripple effects of the Norwegian seafood industry (Nyrud, Iversen, Bendiksen, Robertsen, & Steinsbø, 2023). It was claimed that "The impact analysis for 2022 underestimates the financial footprint of the seafood industry". It was further claimed that "The seafood industry has a very low import share, which indicates that a large part of this consumption of goods and services will end up as Norwegian value creation". This led us to dive into the meaning of correct import shares among suppliers to an industry, for determining the full ripple effects from the industry. For a small, open economy, like the Norwegian, and an international, or global, industry like salmon aquaculture, understanding the nature and extent of trade – in both input and output – is vital for understanding the economic importance of an industry. The Norwegian seafood industry exports around 95 % of its output. At the same time, both fisheries and aquaculture import a range of input. For fishing, it is mainly vessels and fishing gear, while for aquaculture, even feed is imported. Around the seafood industry, a huge supplier industry has emerged (Blomgren et al, 2021). Wouldn´t it be reasonable to expect that this would result in most of the ripple effects to be manifest in Norway?

Methods and Results

While it is customary in Norway to use IO- analyses with IO-matrices from Statistics Norway (Brasch et al, 2020; Bruvoll et al., 2013), in line with well-proven methods for IO-analyses (Eurostat, 2008; Miller & Blair, 2009; Sanjiv et al., 2018), our study developed an IO-matrix tailored to actual trade patterns for the seafood industry. For such an IO-matrix, trade between all industries in Norway (as defined by three-digit NACE-codes) is mapped every second year (an example of the methodology is found in Brasch et al, 2020). As industries defined by three-digit NACE-codes may be diverse, our IO-matrix was based on real trade history from the seafood industry. Results show that while it is true that most of the input to the seafood industry is bought from Norwegian suppliers, the suppliers buy a large amount of their input from abroad, effectively reducing further ripple effects. So no, most of the ripple effects are not manifest in Norway. Or at least a much smaller amount than previously estimated (Johnsen et al., 2021, 2022). Previous methods used, with Standard IO, would have overestimated employment by 20.000 and net value creation by over 30 billion NOK. The biggest input factor in salmon production is feed, and this factor alone accounts for much of the estimation error from using standard matrices. While imports of aquaculture nets, fishing gear, fishing vessels et.c also contribute, this is dwarfed by the import leakage of feed. With a salmon production of 1.65 million tonnes a year, approximately 2.1 million tonnes of feed are consumed, at a total purchase price close to 40 billion NOK. For the feed companies, goods purchased accounts for approximately 85% of turnover, and 92% of the feed ingredients are imported (Aas, Ytrestøyl, & Åsgård, 2022). This translates into an import leakage of around 31 billion NOK, explaining most of the difference from earlier studies. This shows that, at least for seafood, IO-matrices not adapted to a "seafood trade pattern" will heavily overestimate ripple effects.

	Standard IO	Seafood IO
Net value creation	86.000	109 billion NOK
Employment	106.000	141 billion NOK

EFFECT OF PLASTIC AND SEAWEED SHELTERS ON THE SKIN MICROBIOME OF LUMPFISH *Cyclopterus lumpus* USED AS CLEANER FISH IN AQUACULTURE PENS

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Introduction. Lumpfish, *Cyclopterus lumpus*, is in some regions used as cleanerfish in aquaculture pens with salmon *Salmo salar* L. farming to reduce the infestation of salmon lice. In the Faroe Islands, lumpfish have for some years been used as cleanerfish by the aquaculture industry with relatively good results. However, there have in some cases been a high mortality rate for the lumpfish in the pens and a great deal of effort is currently being put towards improving their welfare. In this context, the project "Lumpfish & Akva-Nest" investigates the effect of plastic versus seaweed shelters on the welfare of lumpfish in aquaculture pens and the potential implications for their effectiveness as cleaner fish.

Methods. The project setup was located on an aquaculture facility and included six pens with salmon and lumpfish. Three of the pens had plastic shelters for the lumpfish and the other three had seaweed shelters. Sampling was performed for all sample types shortly after the lumpfish were deployed in spring 2023 and on two more occasions with about five weeks intervals. Samples for microbial sequencing were taken of the shelters in triplicates and from skin and gills of 10 lumpfish from each of the six pens. Welfare parameters of the lumpfish were also registered at each sampling. DNA extraction and library preparation was performed at Firum lab and sequencing at Novogene. Bioinformatic analyses were performed in Qiime2 and R.

Results. The analyses of the bacterial communities showed the presence of several potentially pathogenic genera. Comparisons between lumpfish in pens with seaweed and plastic shelters and the shelters themselves revealed various differences in the relative amounts of some of these bacteria (Fig 1). However, there was not a straight forward relationship between the presence of these bacteria in the shelters and the lumpfish skin.

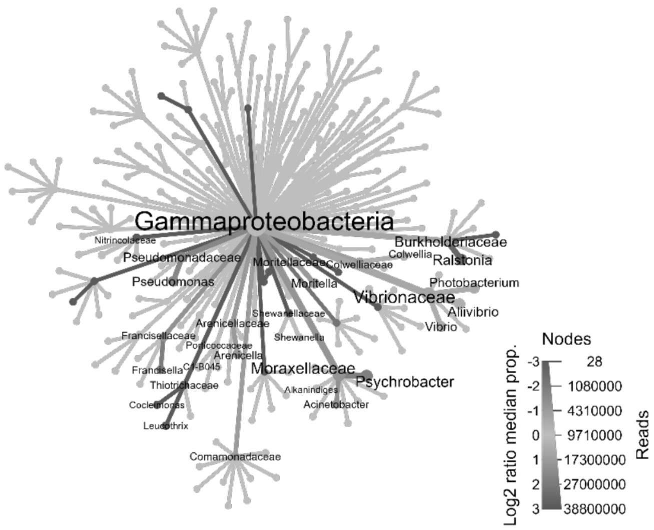

Figure 1. Heat-tree comparison of bacterial compositions in skin samples from lumpfish in pens with plastic shelters and seaweed shelters

ASSESSING AND MITIGATING GREENHOUSE GAS EMISSIONS IN BANGLADESH'S FRESHWATER AQUACULTURE: A COMPREHENSIVE STUDY ON FEED IMPACT AND SPECIES COMPARISON

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Bangladesh's aquaculture industry, a significant contributor to the global aquaculture sector, faces the environmental challenge of greenhouse gas (GHG) emissions. This study investigates GHG emissions from three prevalent freshwater aquaculture systems in Mymensingh, Bangladesh, focusing on Indian major carps (IMC), Pangasius catfish (PG), and Tilapia (T). The research aimed to quantify and compare the emissions of CO_2, CH_4, and N_2O from these systems, providing an in-depth analysis of the environmental impact of freshwater aquaculture in the region. Employing a floating chamber method for gas collection and Gas Chromatography for analysis, the study measured GHG emissions and the sampling period, spanning from February to March of 2023, aligns with the transition from the dry winter season to the pre-monsoon season in Bangladesh. The results revealed that PG systems exhibited significantly higher GHG emissions, primarily due to the extensive use of sinking feeds that lead to increased organic waste and methanogenesis. In contrast, the IMC and T systems, utilizing more efficient floating feeds, showed comparatively lower emissions. These findings are significant in the context of Bangladesh's growing aquaculture industry and its global environmental footprint. The study highlights the critical role of feed type and management practices in determining GHG emissions from aquaculture ponds. The results suggest that optimizing feed composition and management can substantially reduce the aquaculture sector's environmental impact. By providing a detailed understanding of the GHG emissions from different aquaculture systems, this research contributes valuable insights for policymakers, industry stakeholders, and environmental scientists. It underscores the need for sustainable aquaculture practices and offers a foundation for developing strategies to mitigate the environmental impact of fish farming, ensuring the sector's sustainability and its contribution to global food security.

REAL-TIME AMMONIA ESTIMATION IN RECIRCULATING AQUACULTURE SYSTEMS: A DATA ASSIMILATION APPROACH

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Recirculating aquaculture systems (RAS) are sustainable methods for fish production developed to reduce water consumption and waste discharge. To increase the fish yield in these systems, fish densities and, consequently, feed input are high, potentially leading to high levels of total ammonia nitrogen (TAN) in these systems. The un-ionized fraction (NH_3) penetrates fish cell membranes via the lipoidal segments and is considered harmful as it might cause unhealthy, suboptimal fish growth and in extreme cases, fish mortality. Therefore, the real-time detection of the un-ionized ammonia concentration is an urgent issue that needs to be addressed in aquaculture. Existing methods for estimating TAN or its fractions, NH_3 and NH_4^+, are usually either offline methods (e.g. molecular absorption spectroscopy), or methods that suffer inaccurate estimations, especially in high turbidity and metal ions (e.g. Nessler's and hypobromite oxidation). Alternatively, soft data-based methods are used, yet these methods suffer from low accuracy, large datasets, or "black box" characteristics.

In this study, we propose data assimilation (DA) as a cost-effective method for improved real-time estimation of NH_3 and NH_4^+ (and TAN) in RAS. DA combines information from both simulation models and measurements to obtain optimal estimates of variables of interest. The combination of the measurements and the simulation model is supposed to provide a higher accuracy of the state variables than both the estimation of the simulation model and the measurement. In this work, an extended Kalman filter (EKF) is used to estimate NH_3, NH_4^+, TAN, and related specifications of the RAS based on simulation estimation and a TAN measurement. A simulation model of a fish tank and a moving-bed-bio-reactor (MBBR) (bio-filter (BF)) is first composed as a set of equations that describes the dynamics of the NH_3 and NH_4^+ in RAS. EKF equations are then formulated based on the simulation model and TAN measurement. We validated our method through synthetic and laboratory case studies and demonstrated its superior estimation capability as compared to the in situ measurements or the simulation models. Additionally, BF specifications of ammonia removal rate were reliably estimated in real time. Furthermore, improved ammonia estimation led to improved current and future fish weight estimations, which can be essential for reliable RAS management. The proposed approach facilitates wider adoption of DA in challenging estimations in aquaculture.

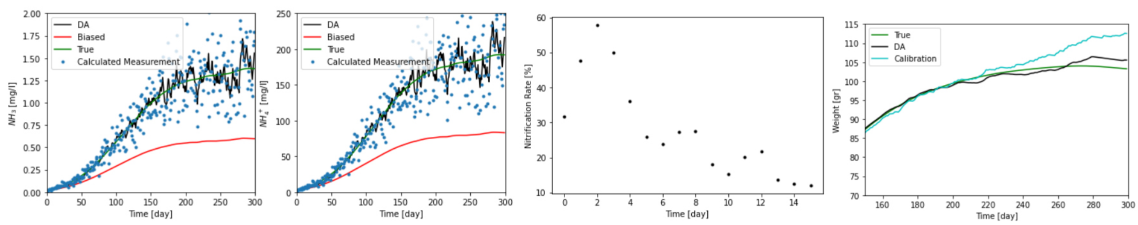

Figure 1: NH_3 and NH_4^+ throughout the fish growth cycle using DA ("DA"), biased mode ("Biased"), and true model ("True"), and the corresponding calculated measurements ("Calculated Measurement") – Case Study 1. Nitrification rate and estimated weight throughout the fish growth cycle – Case Study 2. The "Calibration" case relies on the TAN measurements solely.

WHATS IN A SEA URCHIN? - COMPARATIVE BIOCHEMICAL PROFILES AND NUTRIENT STATUS OF WILD AND ENHANCED GREEN SEA URCHIN *Strongylocentrotus droebachiensis*

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The green sea urchin *Strongylocentrotus droebachiensis* is present in large quantities along the coast of Norway although they are generally of very low quality. Sea urchin gonads are prized seafood in many parts of the world and market demand is high so there is intensive interest in harvesting and enhancement of wild sea urchins. The formulation of the enhancement feed affects growth, quality characteristics, and biochemical composition of sea urchin gonads. The gonads will also be affected by season (the time of the year they are harvested) and by the harvesting location. It is important to gain knowledge on how these parameters influence the biochemical composition of the sea urchin roe when collected from the wild as well as when it is enhanced.

In this study the nutritional composition in gonads from both wild and enhanced (fed) sea urchin was characterized. The sea urchins were harvested at 4 different locations during two seasons (summer and winter). The collected sea urchins were fed a formulated feed for a duration of 9 weeks. The effects on the biochemical analysis of the sea urchin roe depending on season, harvesting location and enhancement were compared to that of wild urchins collected at the same sampling time, from the same site. The gonadal index (GI) varied between the different harvesting sites and the seasons. All the enhanced gonads contained a favourable fatty acid composition with a higher content of the marine n-3 polyunsaturated fatty acids (PUFA) and eicosapentaenoic acid (EPA: C20: 5n-3) compared to n-6 PUFA. In enhanced sea urchins, the content of bitter AA was higher compared to wild sea urchins. Enhanced sea urchin roe appeared to have a significantly lower level of both Iodine and Arsenic compared to wild sea urchin roe in the current study. There were very low levels of Cadmium in the wild sea urchin samples, but levels were undetectable in the enhanced samples and Lead and Mercury were undetectable in all samples regardless of treatment.

Additional studies showed the composition and variation in the nutrient content of sea urchin biomass collected from the wild. These offer some insights into possible byproducts from the sea urchin industry.

The results of these studies show that sea urchin gonad enhancement is an effective way to produce seafood with a favorable nutritional profile, including a relatively high proportion of marine n-3 PUFA such as EPA. Additionally, the bioproducts of sea urchin fisheries and enhancement (or raw product that is not suitable for enhancement) has potential for commercial use.

IMPACT OF GEOLOGY, FARM MANAGEMENT STRATEGIES AND SYSTEM DESIGN ON WATER CHEMISTRY CONDITIONS EXPERIENCED BY RAINBOW TROUT *Oncorhynchus mykiss*

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The aquaculture industry is becoming increasingly aware of the importance of water chemistry on production and organism welfare when considering environmental change and technologies such as Recirculating Aquaculture Systems (RAS). Reduced growth, feed conversion ratio, and condition factor as well as increased bone remodelling, incidence of cataracts and kidney stone formation has been observed in fish exposed to elevated CO_2 synonymous with RAS. However, such impacts are not always observed suggesting that the interaction of CO_2 with other water chemistry variables is not fully understood regarding organism physiology.

Key factors which determine water chemistry include the local geology impacting the source water (Fig. 1), farm water management strategies (e.g. oxygenation, CO_2 stripping, addition of alkali, biofiltration) and system design (e.g. flow through tanks, RAS, sea cages). In the UK, the range of water chemistries experienced by rainbow trout is currently unknown. My research aims to create the first overview of trout farm water chemistry in the UK and determine any links with fish physiology, health, production etc. This has been determined by farmer surveys and collecting water samples from farm sites to measure oxygen, temperature, salinity, CO_2, total alkalinity, sodium, potassium, magnesium, calcium, chloride, nitrate, phosphate, and sulphate. Furthermore, information was gathered on local geology, farm management strategy, system design and common health problems. Data were analysed to determine which factors contribute the most to farm water chemistry and whether there were any relationships between water quality, intensity of production and fish health. There is also further scope for sampling outside of the UK, including Denmark (Fig. 1).

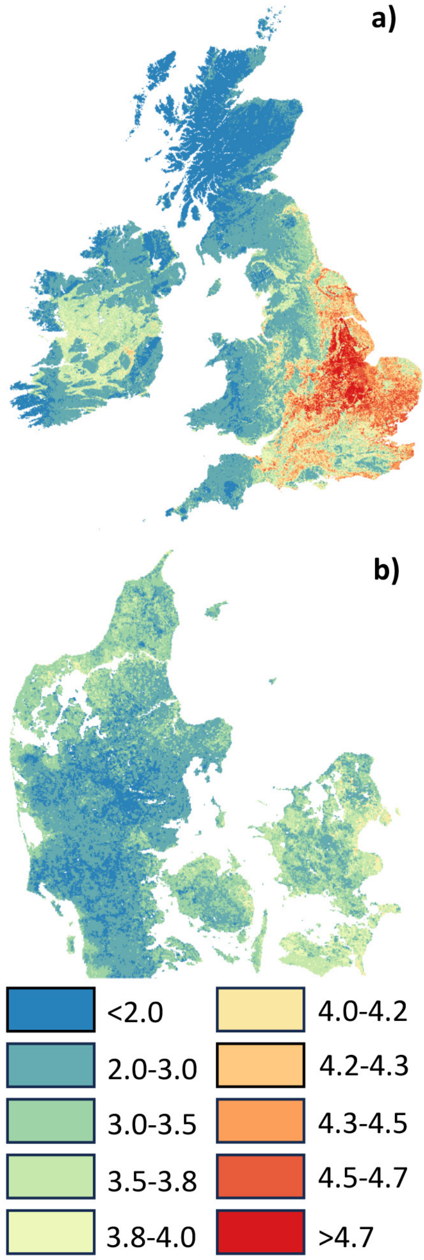

Figure 1. Maps showing the variation in groundwater calcium (mg/L) across the (a) UK and (b) Denmark (data from: Hájek *et al. 2021*)

FATTY ACID ETHYL ESTERS FOR EFFECTIVE AND SUSTAINABLE TREATMENT OF FISH PARASITIC DISEASES

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Parasitic diseases, notably those caused by protozoan and monogenean ectoparasites such as *Trichodina* sp. and *Gyrodactylus turnbulli*, present significant challenges to aquaculture, impacting fish health, growth, and productivity. The confined conditions of aquaculture facilities exacerbate the spread and impact of these parasites, leading to increased morbidity, mortality, and reduced efficiency in fish cultivation. While traditional chemical treatments offer some effectiveness, their associated risks of toxicity and environmental damage highlight the need for investigating natural alternatives to manage parasitic infections. In our previous studies, we identified anti-parasitic effect of fatty acids and fatty acid ethyl esters (FAEEs), produced from the microalga *Phaeodactylum tricornutum* and its residue material [1,2]tested against the Gram-positive Streptococcus iniae and Gram-negative Vibrio harveyi, were used to calibrate P. tricornutum culture conditions and extract preparation. Maximal antibacterial effect was obtained with a two-week-old culture, following cell breakage of the wet biomass and extraction in 100% ethanol. Fatty acid composition analysis of thin-layer chromatography fractions of the ethanolic extracts revealed that the free fatty acids liberated during cell breakage, enriched in 16:0, 16:1n7, and eicosapentaenoic acid (20:5n3. To expand the potential application of FAEEs, we investigated the effect of a broad range of FAEEs against *G. turnbulli* and *Trichodina* sp. (protozoan) and proposed the potent natural sources for their preparation.

Methods: Antiparasitic activity of FAEEs derived from fatty acids of various chain lengths and from natural sources, including: residue of *P. tricornutum*, coconut oil, and black soldier fly larvae (BSFL), was analyzed. Infection of *G. turnbulli* and *Trichodina* sp. in guppies and barramundi was examined, respectively. Analysis included *in-situ* antiparasitic testing, followed by fish toxicity assessment and *in vivo* efficacy in the infected fish.

Results: FAEE prepared from *P. tricornutum* residue has effectively treated *Trichodina* sp. infection in barramundi. Of the most effective tested FAEEs, ethyl laurate (C12) was identified as a widely available not toxic and effective treatment candidate against both the tested parasites. Overall, FAEEs demonstrated potential as a sustainable and non-toxic alternative to traditional chemical treatments, effectively lowering infection prevalence in infected fish population.

1. Jawaji, A., Zilberg, D. & Khozin-Goldberg, I. Using byproduct of fucoxanthin production by *Phaeodactylum tricornutum* for the development of a treatment against monogenean infection in fish. *J. Appl. Phycol.* (2023) doi:10.1007/s10811-023-02990-5.
2. Kim, J.-H., Didi-Cohen, S., Khozin-Goldberg, I. & Zilberg, D. Translating the diatom-grazer defense mechanism to antiparasitic treatment for monogenean infection in guppies. *Algal Res.* **58**, 102426 (2021).

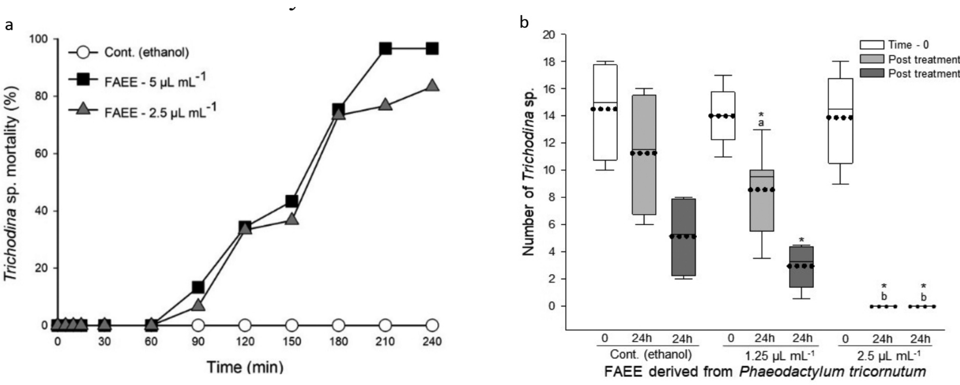

Figure 1. Antiparasitic effect of FAEE from the residue of *P. tricornutum* against *Trichodina* sp. in barramundi. Kolliphor 1% was added and sonication performed prior to analysis. (a) In-situ analysis: parasites were observed for mortality over 240 min; n = 3 wells with at least 15 observed parasites per well. (b) In vivo analysis: infected barramundi was bath-treated. Number of parasites was counted on the skin of each individual fish before and after treatment application and on the gills (G) (n = 8 fish per treatment group). *Significant difference in the number of parasites before and after treatment within treatment groups; different lowercase letters denote significant differences between treatment groups ($p < 0.001$). Ethanol served as a control. Tested concentrations of the applied solution are indicated.

PARASITIC DISEASE MONITORING IN OLIVE FLOUNDER FARMS OF JEJU ISLAND

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Olive flounder (*Paralichthys olivaceus*) is one of the most important aquaculture finfish species in South Korea. However, in recent years, several parasitic diseases occur at flounder farms, leading to significant economic loss. Therefore, comprehending the rates of disease outbreaks through a periodical monitoring program will aid in developing disease prevention strategies and enhancing olive flounder production in Korea.

Monthly monitoring of flounder parasitic diseases was conducted in ten fish farms on Jeju Island. Internal and external symptoms of the surveyed fish were observed, and tissues prone to parasite infection, such as gills, skin, fins, intestine, and bladder, were examined for parasite infection through microscopic analysis. The identification of parasites was also conducted through metagenome analysis.

According to the results collected through disease monitoring from February to November 2023, a total of 342 cases of external and internal parasite infections were recorded (Table 1). Early diagnosis of these parasitic diseases and subsequent treatment/quarantine of diseased fish are crucial to minimize mortality rates. Collectively, this study provides fundamental data for establishing a prevention strategy for parasitic diseases in olive flounder aquaculture.

Table 1 Total cases of external and internal parasite infections in the Jeju flounder farms.

	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Total
Detected ectoparasites	14	16	11	14	16	12	13	11	11	14	132
Trichodina sp.	5	7	5	6	5	4	4	6	6	5	53
scuticociliate	6	3	3	3	5	4	7	3	4	5	43
Ichthyobodo sp.	-	1	-	1	-	-	1	1	-	-	4
Amoeba sp.	-	-	-	-	2	1	-	-	1	2	6
Gill protozoa	2	2	2	3	4	3	-	1	-	-	17
Gyrodactylus sp.	1	3	1	1	-	-	1	-	-	2	9

	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Total
Detected endoparasites	11	11	14	15	13	31	21	24	32	38	210
Enteromyxum leei	6	4	3	2	4	16	11	12	16	19	93
Parvicapsula anisocaudata	2	4	5	7	4	3	4	5	7	8	49
Sinuolinea capsularis	-	-	2	3	3	8	5	7	6	5	39
Myxodavicia sp.	2	2	3	2	2	3	-	-	2	4	20
Ortholinea sp	1	1	1	1	-	1	1	-	1	2	9

ORGANIC COPEPODS AS LIVE FEED FOR MARINE FISH LARVAE – A NEW PROMISING CONCEPT

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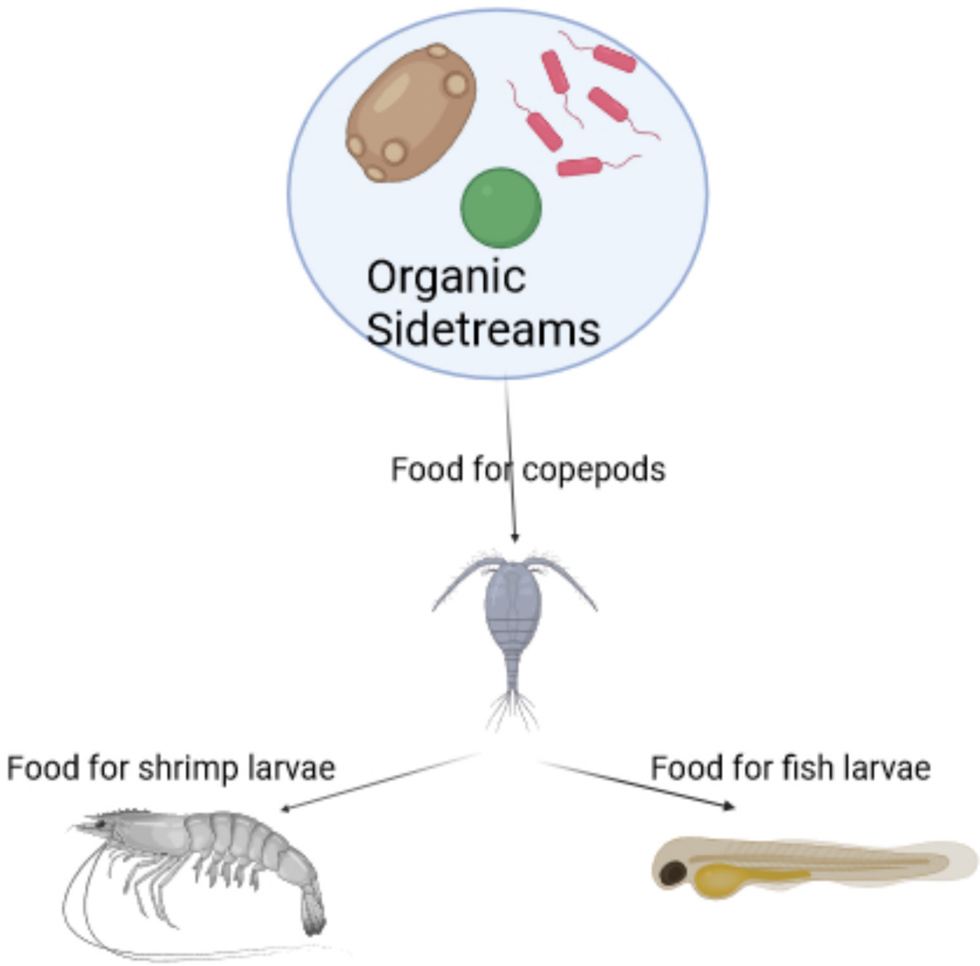
As traditional fishing methods continue to deplete wild marine fish stocks and pose environmental concerns, the need for alternative methods of fish production has become imperative. Organic aquaculture, which aims to minimize the environmental impact and promote animal welfare, has emerged as a promising solution. The project ORACLE FISH aims to provide a concept of organic marine aquaculture fish larvae, by feeding the fish larvae with copepods produced as an organic live feed. Thereby closing the loop for a completely produced organic marine fish.

Currently marine fish can be weaned to organic formulated foods after the successfully completing their larval phase. However, there are no available organic food items during the duration of the larval phase, which can vary from a week to a month, depending on the marine fish larvae.

The present live feed for marine fish larvae are rotifers and *Artemia*, that cannot be organic produced. In nature, copepods are the most prevalent live prey for marine fish larvae. Copepods are well documented as an ideal live feed for marine fish larvae and is in many ways superior when compared to rotifers and *Artemia*, both in terms of nutritional value, prey size and survival rate.



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But what if we could offer not only a new live feed but at the same time create an organic solution? This is now possible since scientist at Roskilde University, Denmark have developed a new solution to provide organic copepods as live feed. We have developed an intensive production system, a new organic food for copepods, and found the ideal copepod species for organic live feed production.

An interesting trait with the organic copepod species is that it can de novo synthesize fatty acids into rich omega n-3 and n-6 fatty acids. This ensures organic copepods, rich in essential fatty acids, which are crucial for the survival, healthy growth, and development of marine fish larvae.

This study suggests a technological solution for up scaling organic copepod production. The study validates that production after organic principles is indeed feasible, both in terms of production capacity and in terms of creating a nutritious organic live feed.

Acknowledgement
The project Oracle Fish is part of the Organic RDD 8 programme, which is coordinated by International Centre for Research in Organic Food Systems (ICROFS). It has received grants from the Green Growth and Development programme (GUDP) under the Danish Ministry of

THE NEW TECHNOLOGY HORIZON - ADVANCED GENOMICS, ARTIFICIAL INTELLIGENCE, AND PATHOGEN DIAGNOSTICS TO ASSIST FARMED SHRIMP PRODUCTION

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Shrimp aquaculture is progressively adopting innovation to increase productivity of farm systems and lower risk due to disease and environmental perturbations. Cutting-edge technologies based on artificial intelligence (AI), rapid and farm-scale disease diagnostics, elucidating role of microbiomes, and the integration of advanced genomic approaches into breeding programs, converge to address critical challenges in shrimp aquaculture and allow for the first-time precise information on the genetic performance, health and well-being of farmed shrimp. Whilst many of these innovations are not yet fully implemented into commercial practice, they have defined new areas and opportunities to resolve the hidden forms of variance that are inherent in shrimp farming, ultimately leading to informed and more proactive management decisions. This presentation will focus on several of these innovative technologies (AI/machine learning, genomic prediction, microbiomes and environmental DNA) and how by harnessing these tools we can optimize breeding, disease management, and sustainability of shrimp farming.

EARLY MUCOSAL RESPONSES IN COMMON CARP TO THE INFECTION OF *Aeromonas hydrophila*

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Common carp is an important freshwater farmed fish species worldwide. Intensive rearing in aquaculture causes increasing susceptibility to various pathogens. One of the most frequently encountered pathogens is *Aeromonas hydrophila*, which has caused huge economic losses to the common carp industry. The mucosal barrier of fish constitutes the first line of defense against the surrounding environment and various pathogens. However, despite the importance of fish mucosal immunity, systematic analysis on common carp early mucosal immune response against *A. hydrophila* is less studied. In order to better understand the genetic basis underlying the mucosal response to the bacterial infection at the global gene expression level, we examined a total of 144 transcriptome files from three primary common carp mucosal tissues at four early timepoints following the infection of *A. hydrophila*. Comparison of the transcriptome revealed 410 unigenes with significantly differential expression in all tested mucosal tissues. Through GO enrichment and KEGG pathway analysis, 28 key genes were identified, which might played critical roles in common mucosal immune response of common carp during the early stage of bacterial infection. Further WGCNA analysis showed that, besides the common response, three mucosal tissues exhibited tissue-specific gene regulatory network. Meanwhile, specific candidate hub genes in each tissue were identified. Our results will provide a fundamental basis for understanding the molecular mechanisms of teleost mucosal immunity, and might suggest strategies for developing novel teleost mucosal vaccines in aquaculture.

THE STRUCTURAL VARIATION LANDSCAPE IN THE EUROPEAN SEABASS (*Dicentrarchus labrax*) GENOME AND ITS POTENTIAL ROLE IN DISEASE RESISTANCE

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Structural variants (SVs) are typically defined as genetic polymorphisms that affect >50bp of sequence, including deletions, insertions, inversions, duplications, and translocations. While SVs are an important source of genetic variation and an important cause of inter-individual differences, they have been neglected in genetics studies compared with SNPs. Here, we defined the SV landscape in European seabass (*Dicentrarchus labrax*), a high value European aquaculture species. We used whole genome sequencing (WGS) in 90 animals to identify 21,428 high-confidence SVs using an established pipeline, with rigorous filtering and manual curation of every SV. These SVs were annotated to estimate potential effects on genes. Integrating SVs and SNP data generated previously, we imputed the SVs for 990 fish with phenotype data for viral nervous necrosis (VNN), one of the main infectious diseases in European seabass, allowing a GWAS analysis using the SVs. In GWAS, 108 (BS, binary survival) and 122 (DD, days to death) SVs exceeded genome-wide significance in a single QTL region matching previous work based on SNPs. The results will improve our understanding of the role of SVs in genetic architecture of traits relevant to aquaculture.

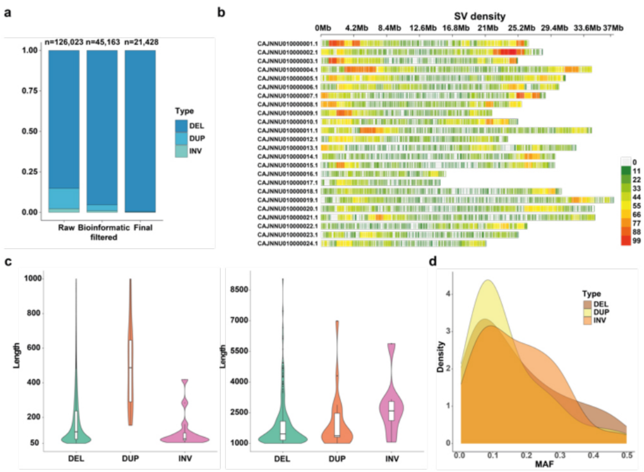

Figure 1. Landscape of SVs detected in the European Seabass. **a.** Illustration of SV counts before and after various filtering steps. **b.** SV density along different chromosomes (window size 1Mb). **c.** Violin plot for deletions (blue), duplications (orange), and inversions (pink), split into two length ranges: 50 to 1,000bp and 1,000 to 10,000 bp. **d.** SV minor allele frequency plot.

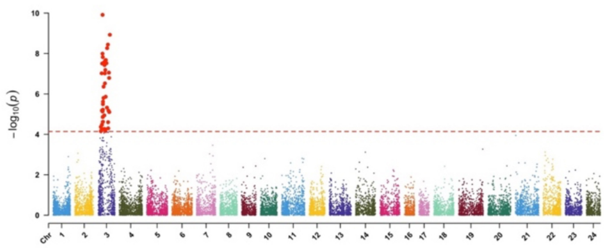

Figure 2. Manhattan plot about the GWAS with GCTA software for the trait of disease resistance. The values on the y-axis represent the −log10 of the P value and the x-axis the positions on the chromosomes. The red line is the 5% chromosome-wide significance threshold (Bonferroni correction) based on binary survival data.

RECENT TRENDS IN *Enteromyxum leei* SURVEILLANCE THROUGH SEAWATER FILTRATION IN JEJU ISLAND, KOREA

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Jeju Island contributes to over half of the total production of Olive Flounder (*Paralichthys olivaceus*) in Korean aquaculture. However, the rapid growth of olive flounder aquaculture in Jeju has also brought challenges in the form of frequent disease outbreaks, with mortality rates peaked at 47.5% of production in 2019. Recently, *Enteromyxum leei*, a myxozoan parasite causing enteromyxosis, has emerged as a major concern due to its role in causing severe mortality, especially among adult flounders, resulting in significant economic losses. To prevent the disease and establish an early alert system, we conducted monthly monitoring of the occurrence of *E. leei* in seawater at six fish farms in Jeju Island. The source and rearing seawater of fish farms were sampled, filtered through 5µm and extracted for gDNA. qPCR was conducted for quantifying *E. leei* via specific primer based on its 28S ribosomal gene.

In the source seawater, relatively higher concentrations of *E. leei* gene copies, with Cq value 28(1,193 copies/L), were observed in the Hangyeong area in June, July, and dropped to Cq value 19.5 (380,233 copies/L) in August. In Pyoseon area, absence of *E. leei* infection, indicated by Cq values above 30, from February to June. However, it drastically dropped in September to October Cq value 17.6(1,451,488 copies/L). Gujwa and Seongsan areas, *E. leei* was detected in October and November by Cq value 24.7(10,047 copies/L). In the case of rearing water, the copy number of the *E. leei* gene began to rise from June and remained elevated until October, although there were some regional variations among different farms. Furthermore, the rearing water displayed higher concentrations of *E. leei* compared to the source seawater, indicating potential proliferation of the parasites within the fish and subsequent release into the rearing water. Notably, the concentration of *E. leei* in the rearing water can fluctuate based on the presence of infected fish in the tank and the movements of cultured fish between tanks. Particularly in 2023, significantly high copy numbers of the *E. leei* gene were observed in the rearing water even during winter and spring. This could be attributed to the elevated ratio of *E. leei*-infected individuals in the tank, possibly due to *in vivo* proliferation within fish during the high temperatures of the preceding winter. Moreover, the copies of the *E. leei* gene in the source water seemed to increase earlier compared to previous years, indicating that natural infection commenced in spring, raising the likelihood of an early disease outbreak in summer.

EFFECT OF YEAST AUTOLYSATE ON GROWTH PERFORMANCE, IMMUNE PARAMETERS AND RESISTANT AGAINST *Vibrio parahaemolyticus* (AHPND) INFECION IN WHITELEG SHRIMP *Litopenaeus vannamei*

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Whiteleg shrimp farming has been constantly challenged with pathogenic Vibrio bacteria especially *Vibrio parahaemolyticus* (AHPND). Hence, the growth performance and health of shrimp are critical to farm productivity. Yeast products like yeast cell wall, autolysate, nucleotide, yeast culture and hydrolysate have been known as immunostimulants for disease prevention and growth promotion in livestock and aquatic species. The aim of this research was to investigate the effects of yeast autolysate from CBS Bio Platforms Inc, Canada on growth performance, immune parameters and disease resistance against *V. parahaemolyticus*. The study was conducted in completely randomized design (CRD) with five treatments of shrimp diets supplemented with yeast autolysate of isonitrogenous 36.47% crude protein and isolipidic 6.54% lipid. The levels of inclusion were T1-Control 0 ppm, T2-125 ppm, T3-250 ppm, T4-500 ppm, and T5-1,000 ppm. Each treatment had 6 replicates. Shrimp at initial weight of 1.36-1.39 g were stocked at 25 individuals (167 shrimp/m^3) in 240 L aquarium tank with 150L of 15 ppt saline water and fed experimental diets 3 times a day at 3-10% BW for 8 weeks. The growth parameters: final weight, weight gain (WG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR%) were recorded throughout the experiment. The immune parameters: hemocyte count, hemolymph protein, phenol oxidase activity, lysozyme activity, superoxide dismutase activity and glutathione were determined.

The results showed that 125-1,000 ppm yeast autolysate significantly increased growth, feed utilization, production and decreased feed conversion ratio (FCR) after just 2 weeks of feeding trial especially at the high dose of 250-1,000 ppm (p<0.05). At 6-8 weeks, the high inclusion rate groups (500-1,000 ppm) clearly showed better growth performance and lower FCR (p<0.05). The survival rates were not significantly different during the 8 weeks trial. Shrimp of the yeast autolysate groups (125-1,000 ppm) showed higher immune response and reduced vibrio infection in hepatopancreas under both normal and *V. parahemolyticus* immersion challenged conditions.

In conclusion, to improve growth, enhance immunity and prevent infection from *V. parahemolyticus*, it is highly recommended that 250-1,000 ppm of yeast autolysate from CBS Bio Platforms are included in the diets.

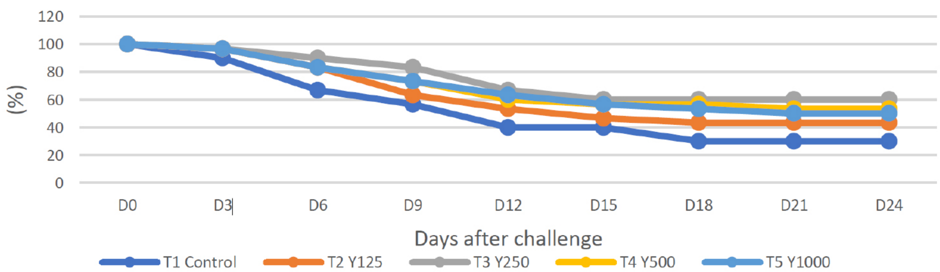

Figure 1. Survival rate of white-leg shrimp fed different levels of yeast autolysate from CBS Bio Platforms after immersion in *Vibrio parahemolyticus* at 2.7×10^7 CFU/ml.

EXPLORING THE EFFECT OF AMINO ACIDS AS POTENTIAL METAMORPHOSIS INDUCERS IN *Venerupis corrugata* LARVAE

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In bivalves, the transition from larvae to juvenile stage – settlement and metamorphosis – is a critical step, that is usually characterized by high mortality. As a result, settlement and metamorphosis represent the most challenging events in bivalve hatchery production. To overcome these constraints, hatcheries have used chemical compounds, like catecholamines (e.g. epinephrine) and neurotransmitters (e.g. □-aminobutyric acid (GABA), and serotonin), to induce settlement and metamorphosis. However, depending on their concentration and exposure time, these compounds may be toxic to the larvae and might not be easily affordable. Considering this, this study aimed to explore the role of some amino acids, namely the precursors of the catecholamines (L-tyrosine and L-phenylalanine) and serotonin (L-tryptophan) as potential metamorphosis inducers in larvae of the pullet carpet shell (*Venerupis corrugata*).

V. corrugata larvae were obtained from Oceano Fresco® and transferred to the Molluscan Experimental Station of Tavira (EEMT, IPMA, I.P), where they were kept until they were competent to settle and metamorphose (12 days). Competent larvae were placed in petri dishes (in triplicate; 100 larvae/10 ml), with filtered and UV-treated seawater, and exposed to L-tyrosine, L-phenylalanine and L-tryptophan at 10^{-5}M concentration. A negative control was set up without the addition of any amino acid, under the same experimental conditions. Larvae were exposed to the amino acids for 72h. Every 24h, larvae were monitored and evaluated for the presence of velum, foot + velum, and foot. A larva was considered to have undergone metamorphosis when it lost its velum, and it showed a completely formed and functional foot.

L-phenylalanine and L-tryptophan failed to induce metamorphosis in *V. corrugata* larvae and revealed to be toxic after 48h of exposure (35% of mortality). Meanwhile, even though L-tyrosine (figure 1) were toxic after a longer exposure (42% of mortality after 72h of exposure), it appears to be a potential inducer of metamorphosis: after 48h of exposure, all larvae had lost swimming ability, and almost 40% of the larvae had the foot completely formed, whereas in the control, 30% of the larvae still showed swimming ability and exhibited a lower percentage of foot+velum and foot. Further research is needed to fully understand the potential of amino acids as metamorphosis inducers. This knowledge may contribute to enhance bivalve hatchery practices through diet supplementation with amino acids during settlement and metamorphosis events.

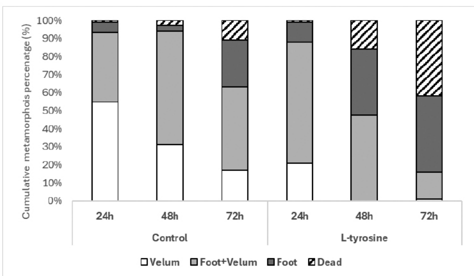

Figure 1- Cumulative percentage of *V. corrugata* larvae induce to metamorphose in response to exposure to water only (control) and to L-tyrosine (10^{-5}M).

NAVIGATING TWO DECADES: THE SHIFTING LANDSCAPE OF ON-BOTTOM CULTURE IN DENMARK

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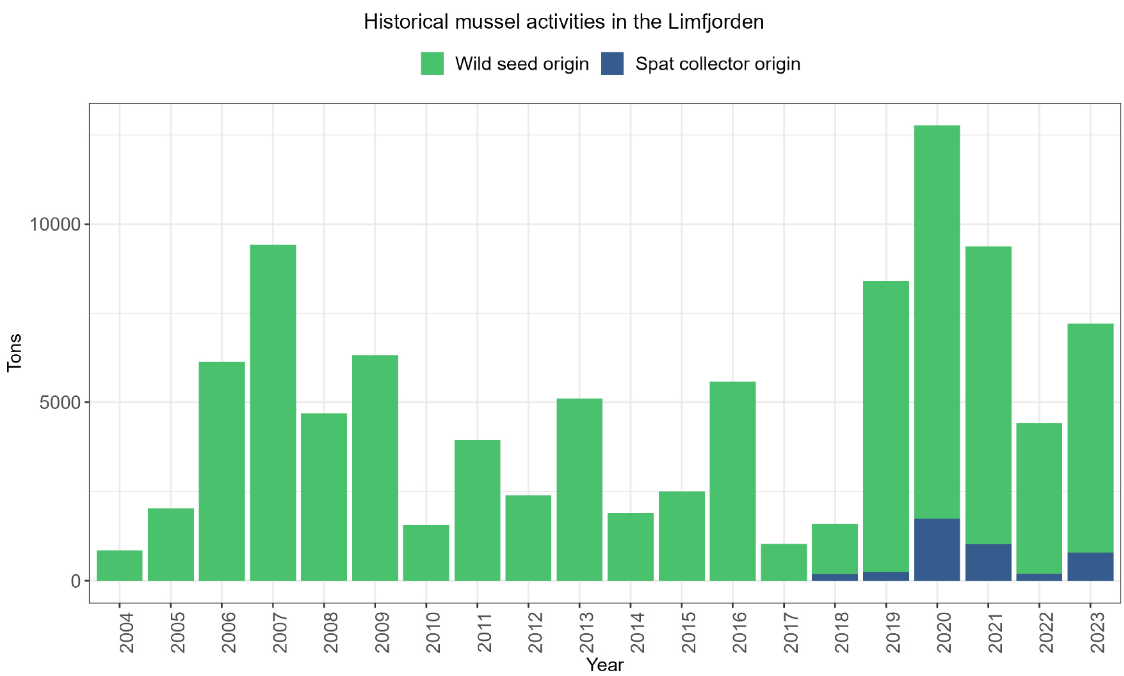
Recent trends in Europe have presumably experienced a widespread decline in wild mussel (*Mytilus edulis*) populations, as well as in fishery and aquaculture (FAO stat 2004-2021). However, in Denmark, a significant shift has occurred: mussel aquaculture production has recently surpassed traditional fisheries. While this transition toward shellfish aquaculture offers environmental benefits and introduces new mussels to the ecosystem, it also presents intricate management challenges, particularly in the realm of spatial planning.

This paper explores the evolution of on-bottom mussel farming practices, tracing their inception in 2004 and spanning the past two decades in Denmark (see Figure). It meticulously examines shifts in seed origin and relaying habits. It contrasts these developments with the concurrent decline in wild mussel populations and traditional fisheries, while also highlighting the rise of suspended mussel cultivation.

Amidst the evolving culture practices and the growing number of both bottom culture and suspended culture licenses, Denmark encountered a license moratorium in recent years. Ongoing research projects focus on assessing the cost efficiency of bottom culture, spatial planning, and mussel production carrying capacity. These endeavors aim to assist decision-makers in site selection for new mussel culture licenses. This paper also provides insights into the approach and some of the results of these research efforts.



LATERAL LINE AND SENSORY RECEPTORS IN ATLANTIC COD (*Gadus morhua*)

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The lateral system in fish differ in morphology and architecture between species. All species described till now have specialized cells associated with the lateral line, such as sensory cells and mechanoreceptors (neuromasts). These cells are used by the fish to detect i.e. water movements, pressure gradients, smell and danger. In farmed fish, deformities, infection routs and wounds in the lateral line have been described. These damages may impact the health and welfare of the farmed fish and more knowledge is required to understand how the lateral line in fish regenerates, heals, and affects fish behaviour and wellbeing. In this work we have studied the trunk and cephalic lateral line of farmed Atlantic cod (*Gadus morhua*) larvae.

Atlantic cod larvae (N=8) from the Nofima cod breeding program were euthanized in an overdose of Finquel and stained with fluorescent dye (4-(4-Diethylaminostyryl)-1-methylpyridinium iodide solved in dimethylformamide, method described by Nakae *et al*. (2012)) to visualize the lateral line neuromasts and skin sensory buds. Six fish were stained with 4-Di-2-ASP for 1 hour and two remained unstained (negative control), and subsequently photographed using fluorescence microscope (Nikon Eclipse TE2000-S). The 4-Di-2-ASP revealed canal neuromasts along the trunk- and cephalic lateral line, and in some areas the superficial neuromasts. In addition to these, the skin sensory buds were visible clustering around the neuromasts and otherwise scattered along the entire body length and head, included the fins. The fin sensory buds had highest density on the basal parts of the fins.

The sensory organ in cod skin is a complex system, including the lateral line starting in the head and ending in the tail, and sensory buds distributed in connection with and in distance to the line. High density sensory areas includes the line as well as the snout, head and fins. These areas are at risk for wound development and damages in farmed fish, especially during handling and other operational procedures in intensive production systems. A deeper understanding of the specific receptor functions will help to improve and understand fish health and welfare.

Funding:
This internal project “Lateral line” were funded by The Norwegian Research Council, NFR no.: 194050.

Reference:

NAKAE, M., ASAOKA, R., WADA, H. & SASAKI, K. 2012. Fluorescent dye staining of neuromasts in live fishes: an aid to systematic studies. Ichthyological research, 59, 286-290.

DISCOVERY ISLANDS BENTHIC REMEDIATION STUDY: SPATIAL AND TEMPORAL CHANGES IN BACTERIAL COMMUNITY COMPOSITION AND DETECTION OF ANTIMICROBIAL RESISTANCE GENES IN SEDIMENTS

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We are using a MinION™ amplicon-based sequencing method targeting the full length 16S rRNA to characterize bacterial communities that represent different stages in the benthic recovery following removal of salmon farms. We are also examining whether past antimicrobial use on these farms is associated with increased abundance and/or diversity of Antimicrobial Resistance Genes (ARGs) in the sediment microbial community.

Triplicate surface sediment samples were collected at 0m, 30m, 60m, 125m, 250m, 500m and at Reference Sites in both the subdominant and dominant current directions at 3 farms. Physical, chemical, and biological properties of the sediments were also determined.

Sites with similar physical/chemical properties share common patterns of bacterial diversity and taxa composition. Heavily impacted sites, as measured by free sulphide and high organic carbon loading, have the lowest bacterial diversity being dominated by genera within the families Sulfurovaceae and Sulfurimonadaceae.

Low levels of oxytetracycline residues were found in sediments nearest the farms. Residues of other antibiotics were not detected. The 3 methods used to characterize sediment resistomes include 1) an AMR Bait-Capture sequencing method (Shay et al., 2023), 2) Resistomap analysis (https://www.resistomap.com) for 54 genes, and 3) metagenomic sequencing. Eighteen ARGs and 1 replicon were detected in 12 of our sediment samples by Bait-Capture, including 5 tetracycline-resistance (*tet*) genes, a single phenicol-resistance (*cfr*) gene, and 5 ARGs for other antibiotics not used on salmon farms. These genes were found sporadically at both farm-impacted and reference sites. Resistomap analysis detected between 27 and 40 ARGs in 32 samples, including the 12 used for bait capture. There were few differences in the numbers and types of ARGs detected between the farm-impacted and reference sites. With respect to antimicrobials used in Canadian aquaculture our analysis detected 4 phenicol, 5 tetracycline, 7 macrolide, lincosamide, streptogramin (MLSB), and 3 sulfonamide resistance genes.

Differences between the Bait-Capture and Resistonmap results, which cannot be explained by differences in the numbers of enrichment targets, are evident in our data. We are presently using metagenomic sequencing to help understand these differences, as well as conducting a more in-depth analysis of our data which will include consideration of physical and chemical properties, including antimicrobial residues, of the sediments.

A SWEET SOLUTION TO AN AGE OLD PROBLEM: THE UPTAKE OF DISSOLVED GLUCOSE AND SUCROSE BY JUVENILE GREENSHELL MUSSELS (*Perna Canaliculus*)

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A major expense in the operation of bivalve nurseries is the culture of high-quality live microalgae feeds, with efforts to develop effective alternative feeds having limited success. Juvenile bivalves are known to be able to absorb dissolved nutrients but this ability has received minimal attention as a route for supplementary feeding. This experimental study assessed the capacity of juvenile green-lipped mussels (*Perna canaliculus*) to uptake and assimilate dissolved glucose and sucrose as a supplement to cultured microalgae. Growth and survival of the mussels was measured over three weeks. All concentrations of glucose and sucrose improved the performance of spat compared to the controls without sugar, the best performance came from a concentration of 1 mg ml^{-1} of dissolved glucose which enhanced daily spat growth 2.7 times that of the control diet.

Survival was high (i.e., > 95 %) among all treatments except for the highest experimental concentrations of glucose and sucrose, where a concentrations above 1 mg ml^{-1} resulted in mean mortalities of between 16 and 24 %. Mussel spat supplemented with dissolved sugars accumulated greater lipid and carbohydrate content per mg g^{-1} of ash-free dry weight compared to those in the control treatment, indicating they were in greater nutritional condition. This demonstration that dissolved glucose and sucrose can be readily utilised by Greenshell™ mussel spat at concentrations as low as 10 µg ml^{-1} opens up the possibility for delivering other soluble nutrients to cultured juvenile molluscs. Dissolved nutrients have the potential to improve the performance of spat in nursery systems while simultaneously reducing the reliance of cultured microalgae as a sole feed input.

Table 1. Daily growth rates from supplementation of microalgae with glucose and sucrose versus control diets without dissolved sugar

Glucose	Daily Growth (µm)		Sucrose	Daily Growth (µm)
Control	9.27		Control	18.9
Glucose 1	23.5		Sucrose 1	27.4
Glucose 2	25.5		Sucrose 2	28.1

UNVEILING THE ARMS RACE: FISH INNATE IMMUNE RESPONSES VERSUS FISH PARASITE EVASIVE STRATEGIES

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The protozoan fish parasite *Ichthyophthirius multifiliis*, causes "white spot" epidemics in a wide range of freshwater fish species worldwide. It infects fish epidermal surfaces such as fins, gills and skin and is responsible for high morbidity and mortality. *I. multifiliis* inhabits the outermost transparent epidermal layer of fish, providing a unique opportunity to visualize protective immune mechanisms involved in the host-parasite interactions. The zebrafish has in this study been used as a model organism for freshwater fish to investigate the acute immunological reactions towards *I. multifiliis* and the immune evasive behaviour of the parasite. Larvae from a double transgenic reporter line with green-fluorescent neutrophils and red-fluorescent macrophages were infected with the parasite and the host-parasite interactions were studied using *in vivo* real-time imaging (Fig. 1) and immune-relevant gene expression. Different host-parasite interactions were observed and are discussed. It was documented how neutrophils and macrophages were in some instances able to kill the parasite. The parasites created an interstitial space, in which they moved around vigorously while continuously rotating. Based on our results, we hypothesize that one immune evasive strategy of the parasite is to continuously rotate to dodge/escape attacks initiated by fish innate immune cells. Gene expression revealed that a mild acute immune response in zebrafish was induced systemically, supporting the importance of an immediate response to fight this parasite. In this study, new knowledge on host-parasite interactions was obtained and the zebrafish once again proved to be an excellent organism to investigate *in vivo* reactions.

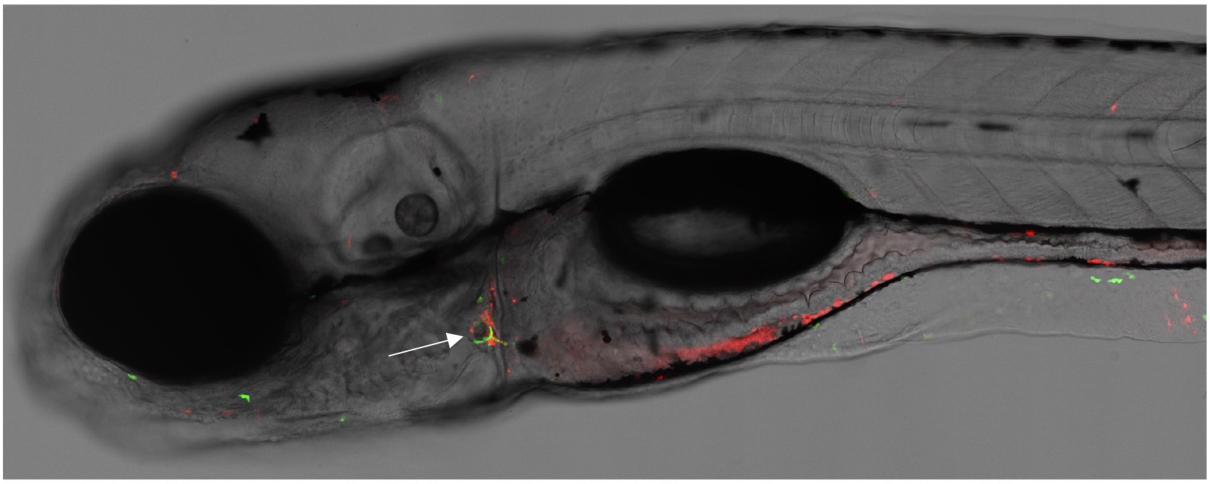

Figure 1. A double transgenic zebrafish larva with an *I. multifiliis* parasite surrounded by red fluorescent macrophages and green fluorescent neutrophils (white arrow). Image from Mathiessen et al. (2023), Frontiers in Cellular and Infection Microbiology.

A NON-INVASIVE CHIP TOOL FOR DETECTION OF DISEASE-CAUSING ORGANISMS FROM WATER SAMPLES IN DANISH FISH AQUACULTURE

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When clinical disease in fish is discovered in production facilities, the fish are usually already very sick. When possible, treatment is initiated immediately, but decreased welfare, increased mortality and economic loss is nevertheless often a consequence. Therefore, prophylaxis and early detection of pathogens could present facility managers with the possibility to intervene prior to clinical outcome and severe disease. We have developed a non-invasive Fluidigm chip tool based on environmental DNA/RNA (collectively eNA) from water samples for early detection and surveillance of 11 pathogens relevant for Danish aquaculture. For three of the diseases, controlled experiments have been conducted correlating the eNA levels in the water with the disease status in the fish. Common for these experiments were that eNA revealed an elevation of the disease-causing organisms before clinical signs appeared, enabling early intervention before fish show clinical signs of disease (Fig. 1). Our tool can therefore be used to detect pathogens in the early phase, before disease becomes severe, facilitating interventions to limit disease and increase the welfare of the fish, which is a requirement for sustainable animal production.

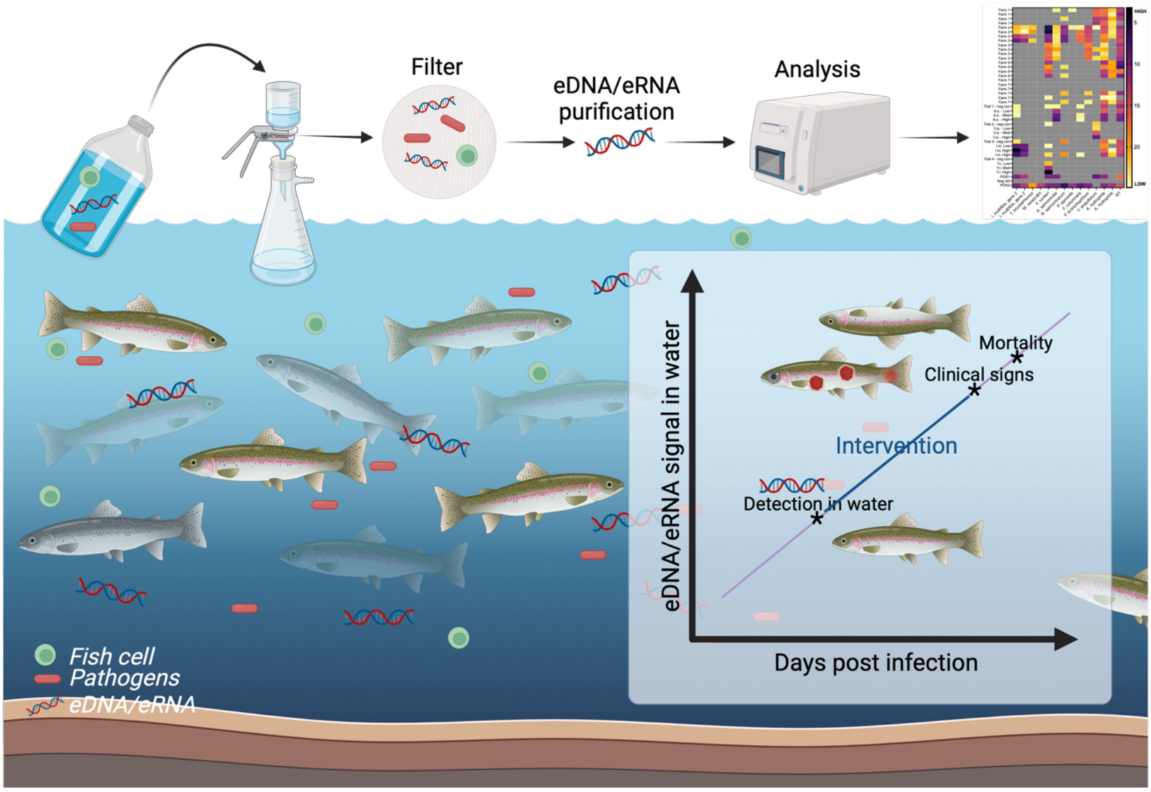

Figure 1. The image represents a fish farm with fish, pathogens and eNA in the water. eNA is purified from water samples and analysed on a Fluidigm chip, which produces data on the level of pathogen eNA present in the water. The graph shows that the pathogen can be detected in the water before clinical signs and mortality appear.

TOWARDS SUSTAINABLE OFFSHORE AQUACULTURE: A MULTI-COUNTRY POLICY REVIEW

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Aquaculture is playing a crucial role in the transition towards blue food: while the catch of wild-capture fisheries stayed stagnant for the past 30 years, the yield generated by aquaculture has increased in fourfold (FAO, 2022; Golden et al., 2021). As aquaculture intensifies, it is requiring increasingly more space in mainly coastal areas, and concern is being raised on the sustainability and environmental impact of these practices (De Silva, 2012; Price et al., 2015; Salin & Arome Ataguba, 2018). A potential solution being proposed to these spatial constraints and coastal environmental impacts is offshore aquaculture (Buck & Langan, 2017; Gentry et al., 2017). However, while research on the potential of offshore aquaculture is gaining traction, practical implementations remain largely in the pilot phase. A significant obstacle to its widespread adoption is the absence of a clear and robust policy framework (Davies et al., 2019; Lester et al., 2018). While the right ecological conditions and technological innovations are important, an unclear or lacking policy framework can discourage local aquaculture entrepreneurs from investing in offshore production (Lester et al., 2018) or lead to the unsustainable development of offshore aquaculture (Davies et al., 2019; Fujita et al., 2023).

To date, research has primarily focused on country-level assessments of offshore aquaculture policies, and a comprehensive and comparative global perspective appears to be lacking. This study aspires to address this gap by conducting a systematic multi-country review of existing policy frameworks. By examining policy documents from various regions and countries that have a history of aquaculture, we aim to identify and compare key policy strategies and instruments for offshore aquaculture innovation and governance, as well as discern the varying stages of policy development.

Our findings will provide insights into the policy landscape surrounding offshore aquaculture. By revealing trends, similarities and differences in policy approaches, we aim to facilitate the development of policy frameworks that promote the responsible and sustainable upscaling of offshore aquaculture initiatives around the world.

As this is an ongoing study, we plan to present our review framework and discuss our preliminary findings. This research will be part of my PhD dissertation on the governance of offshore aquaculture.

ENHANCING FARMERS PROFITABILITY USING SPECIALISED PRODUCTS: A STRATEGIC APPROACH TO LINE BREEDING IN NILE TILAPIA

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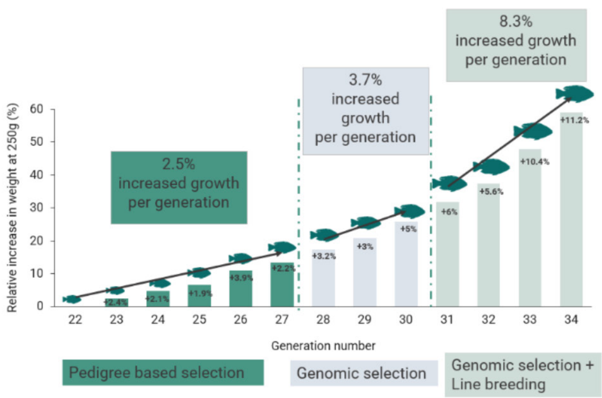
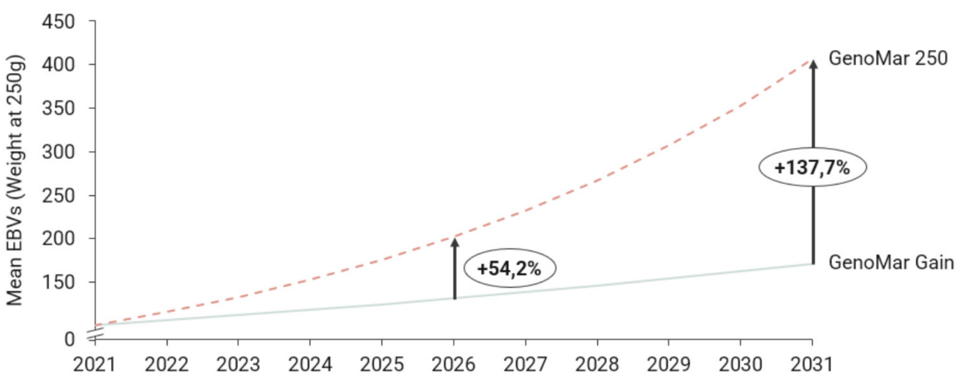
Breeding lines in tilapia breeding refer to distinct and selectively bred populations of fish that have been developed to exhibit specific traits or characteristics. Each breeding line is established with a specific breeding objective in mind, and over successive generations, the selected traits are emphasized and refined. This selective breeding process facilitates rapid genetic gain for the targeted traits and the creation of specialized lines tailored to different market preferences or production objectives. Besides these, by focusing on one trait or a set of traits within a particular line, we can minimize the impact of negative genetic correlations with other traits that might hinder overall improvement. Implementation of line breeding, together with genomic selection in GenoMar breeding nucleus has tripled our rate of genetic gain, compared to the pedigree-based selection (Figure 1). Further, simulation shows that engaging in precision breeding for 300g through a distinct line breeding program yields an additional 138% genetic gain for weight at 300g over the next ten years when compared to selecting using balanced breeding approaches (Figure 2).

Figure 1: The image showcases the significant genetic gains achieved in GenoMar broodstocks through recent innovations in genomic selection and line breeding. Over the last seven generations, the relative percentage increase in Estimated Breeding Values (EBVs) for weight at 250g in our GenoMar 250 breeding line has more than tripled, with Genomic selection introduced in Generation 28 and line breeding implemented from Generation 31.

Figure 2: Simulation illustrating the Genetic Gain for Weight at 250g in the breeding program for 10 Years under Two Distinct Scenarios. The sole distinction between the two scenarios lies in the measured trait and the generation interval. In the GenoMar Gain scenario, the focus is on selecting for the trait: Weight at 1 Kg, with a generation interval of 11 months. Conversely, the GenoMar 250 scenario involves precise selection for Weight at 250g, featuring a shorter generation interval of 7 months. The genetic correlation between Weight at 1 Kg and Weight at 250g is 0.57. In GenoMar Gain we are assuming the genetic gain of 5% every generation.





MANNAN OLIGOSACCHARIDE FROM COCONUT WASTE IMPROVES INTESTINAL HEALTH OF ATLANTIC SALMON (*Salmo salar*) PARR

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The mucosal barriers of fish are an important first line of defense against pathogenic invasion. Functional feed additives such as mannan oligosaccharide (MOS) have the potential to promote improved mucosal physiology and barrier function, which can lead to improved fish robustness, health and welfare. The Atlantic salmon is a valuable production species, with global production exceeding 2.8 million metric tonnes in 2022.

In this study, Atlantic salmon parr (ca. 11.68 ± 0.11g) were fed with either a control diet or diets comprising the same basal formula but supplemented with one of three levels of MOS (0.25%, 0.35% or 0.5% Pretego^{TM}, Greensage Prebiotics Inc) (Table 1). Fish were reared in a freshwater RAS system (16.22 ± 0.16^{o}C) for a period of 70 days, with three replicated tanks per treatment group. At the end of the trial, there were no significant differences between the treatment groups in terms of growth performance and nutrient utilization (Table 2). However, histological appraisal revealed that dietary MOS increased the length of distal intestinal villi and increased the number of goblet cells in the distal intestine and the dorsal region of the skin.

In conclusion, feeding Atlantic salmon with MOS derived from coconut by products has the potential to improve mucosal barriers. This potential will be further explored in on-going analysis which includes intestinal microbiome analysis and intestinal and skin gene expression profiling.

Acknowledgements: this research was funded by Enterprise Solutions and Greensage Prebiotics Inc.

Table 1: Dietary formulations and proximate composition(%).

Ingredients	Control	0.25Pre	0.35Pre	0.50Pre
MOS	-	0.25	0.35	0.50
Wheat	10.83	10.30	10.13	9.83
Wheat gluten	17.93	17.96	17.93	17.93
Fish oil	6.67	6.67	6.67	6.67
Fish meal	30.00	30.00	30.00	30.00
Rapeseed oil	6.39	6.39	6.39	6.39
Faba bean dehulled	7.00	7.00	7.00	7.00
Phosphate	0.56	0.56	0.56	0.56
SPC	20.00	20.00	20.00	20.00
Astaxanthin	0.01	0.01	0.01	0.01
Vitamin premix	0.19	0.19	0.19	0.19
Mineral premix	0.56	0.56	0.56	0.56
Total	100.00	100.00	100.00	100.00
Proximate compositions (g/100g)				
Moisture	8.00	8.1	8	7.9
Crude protein	50.20	50.7	51	51.1
Crude fat	18.70	18.5	18.8	19.1
Ash	5.40	5.6	5.5	5.5

-

Table 2: Growth performance metrics. Data presented as mean ± standard deviation of the mean (n = 3).

	Control	0.25Pre	0.35Pre	0.50Pre
IBW (g)	11.64 ± 0.42	11.60 ± 0.40	11.87 ± 0.14	11.60 ± 0.41
FBW (g)	32.44 ± 1.32	32.19 ± 0.44	35.02 ± 4.05	33.35 ±2.02
WG (g)	20.46 ± 1.16	20.17 ±0.75	22.48 ±3.78	21.24 ±1.90
SGR (%/day)	1.34 ± 0.09	1.33 ± 0.05	1.41 ± 0.15	1.38 ± 0.09
FCR	1.00 ± 0.06	1.03 ±0.05	0.97 ±0.10	0.98 ±0.11
SR (%)	98.10 ± 1.65	100.00 ±0.00	100.00 ±0.00	99.05 ±1.65
C.F	1.20 ±0.06	1.24 ±0.02	0.95 ± 0.02	1.22 ± 0.02

IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; SR, survival rate; C.F, condition factor.

Table 3: Histological analysis. Data presented as mean ± standard deviation of the mean (n = 9).

	Control	0.25Pre	0.35Pre	0.50Pre
MFL (µm)	329.64 ± 91.71^{a}	**523.05 ± 184.01^{b}**	435.31 ± 98.50^{ab}	447.34 ± 147.01^{ab}
IGCC (n/100 µm)	10.14 ± 2.25^{a}	**13.43 ± 2.71^{b}**	**14.20 ± 1.78^{b}**	11.32 ± 2.88^{ab}
SGCC (n/200 µm)	11.11 ± 1.84^{a}	13.78 ± 4.0^{ab**}	11.53 ± 2.66^{a}	**16.29 ± 1.67^{b}**
MVC (%)	95.11 ± 0.31	97.03 ± 0.19	96.68 ± 0.21	96.07 ± 0.22
MVL (µm)*	1.25 ± 0.31	1.44 ± 0.19	1.21 ± 0.21	1.21 ± 0.22

MFL, mucosal fold length; IGCC, intestinal goblet cell count ; SGCC, skin goblet cell count; MVC, microvilli coverage; MVL, microvilli length

DIETS OF ALESTIDAE (TELEOSTEI: CHARACIFORMES) IN TWO RIVERS (BOUMBA AND KADEI) IN EASTERN CAMEROON

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As part of a larger research program on the diversity and role of the forest in maintaining the local ichthyofauna of the Congo Basin, a study focused on the diets of Alestidae, a family of many fishes represented in the Boumba and Kadei rivers in eastern Cameroon. Indeed know the diet of savage specie fish can help to make their conservation by aquaculture processes.

106 specimens of Alestidae were captured using dormant gillnets, cast nets, hooks and creels; they were identified using specific determination keys then were labeled, photographed, measured (total and standard lengths), weighed and dissected in order to remove their stomachs which were preserved in tubes containing alcohol at 70%. These stomachs were then emptied and the contents rinsed in Petri dishes, then filtered through a sieve and the retained fractions were sorted, separated, identified, counted and weighed in order to evaluate the rate of food from the riparian forests.

A total of 106 stomachs were collected from six species of Alestidae (*Alestes macrophthalmus*, *Brycinus longipinnis*, *Brycinus macrolepidotus*, *Brycinus imberi*, *Hydrocynus vittatus* and the *Bryconaethiops spp*. complex made up of several species grouped into one due to the low abundance of each). Of 63 individuals sampled in Boumba, 89.7% had full stomachs compared to 7.7% empty stomachs while out of 43 individuals sampled in Kadei, 76.1% had full stomachs compared to 14.9% empty stomachs. The emptiness coefficients were 8.33 in A. macrophthalmus in Boumba and 50 in Kadei while in B. macrolepidotus they were zero (0) in Boumba and 9.09 in Kadei.

Table 1: Presence-absence of prey identified in the stomach contents of dissected individuals.

Sites	Prey	Fish			Macroinvertebrates									Macrophytes					Other			
	Species	Wh/Pat			AI			InsT/InvT					Cr	TP				AP			Mis	
		Sf	Fs	Fd	Co	Tr	Od	Or	Le	Hy	Is	My	De	Lf	Fl	Fr	Se	AP	Ib	Ie	Ff	Un
Boumba	*A. macrophthalmus*			+	+			+	+								+	+	+	+		+
	Bryconaethiops sp.		+				+		+									+				
	B. longipinnis								+													
	B. macrolepidotus								+	+				+		+		+				
	H. vittatus																					
Kadei	*A. macrophthalmus*							+														
	B. imberi			+	+	+	+	+	+	+	+							+	+			
	B. macrolepidotus				+	+				+		+	+		+	+		+	+		+	
	H. vittatus	+																				

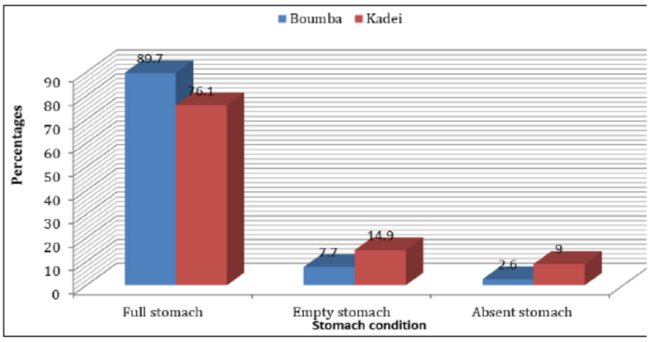

Fig 1: State of the stomachs of fish according to the sampling sites.

Four food categories have been identified in these fish: macroinvertebrates, macrophytes, prey fish and other miscellaneous particles. The frequency of occurrence (Fc) and the preponderance index (Ip), calculated, indicate that Lepidoptera are the very frequent and main prey in the diet of *B. longipinnis* at Boumba, while at Kadei it is rather Orthoptera and prey fish, respectively in *A. macrophthalmus* and *H. vittatus*. Furthermore, it has been observed that the riverside forests provide these prey (leaves, fruits and insects) found in the stomachs of certain species. Thus they mainly contribute to maintaining the balance within the fish food chain.

The study of the stomach contents of Alestidae in Boumba and Kadei reveals that this family of fish is made up of herbivores, carnivores and omnivores. It is obvious that the forests of the Congo Basin constitute an essential source of food for the ichthyofauna of the rivers they shelter. Consequently, deforestation would negatively impact certain taxa of fish such as the Alestidae which depend on them and would inevitably lead to their loss. The improving knowledge on the feeding ecology of these Alestidae fish can help to take best measure of conservation on those of the IUNC red list.

CORRELATION BETWEEN BIOLOGICAL PARAMETERS OF EUROPEAN EEL, *Anguilla anguilla*, FROM THE NERETVA DELTA, CROATIA

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Total length (TL), body weight (W) and the gonadosomatic index (GSI), the hepatosomatic index (HSI), and condition factor (CF) are important to evaluate the physiological condition of eel. While GSI is an important indicator of reproductive activity, the HSI reflects liver participation in vitellogenesis. The aim of this study was to evaluate these biological parameters of ell from Neretva Delta, Croatia, as a potential site for broodstock selection.

In February 2024, 14 eels in different silvering stages were chosen from fisherman catch in the mouth of river Neretva and analyzed. For each fish TL and W were measured. After dissection, gonads and the liver were taken and weighed. Fulton's condition factor was calculated using the formula: $CF = W/TL^3 \times 100$. GSI was calculated using the formula $GSI = \text{gonad weight}/W \times 100$. HSI was calculated using the formula $HSI = \text{liver weight}/W \times 100$. The data was checked for normality using Shapiro-Wilk test. Homogeneity of variances was checked using Fligner-Killeen test. Normality of errors was checked using Shapiro-Wilk test. Pearson correlation coefficient was calculated to measure linear correlation of CF, GSI and HSI with TL and W. Linear regression was used to analyze the relationship of GSI and HSI with TL and W. To check for extreme values, Cook's distances was plotted.

W values ranged from 59 to 1060 with a mean value of 343.86 ± 374.80. TL values ranged from 38 to 83.30 with a mean value of 52.66 ± 15.97. CF values ranged from 0.11 to 0.22 with a mean value of 0.15 ± 0.04. HSI values ranged from 0.59 to 1.58 with a mean value of 1.19 ± 0.27. GSI values ranged from 0.13 to 2.63 with a mean value of 1.26 ± 0.71 (Table 1).

A very strong positive correlation was recorded between CF and TL (r= 0.86), between CF and W (r=0.87). Strong positive correlation was recorded between GSI and TL (r=0.71; Fig.1.), while the correlation between HSI and TL was very weak negative (r=-0.05). A positive correlation was recorded also between GSI and W (r=0.61), while between HSI and W almost were no correlation (r= 0.03).

Table 1. Range, mean value and standard deviation (SD) of TL, HSI, and GSI of the eels caught in February of 2024 in the mouth of river Neretva

	Min	Max	Mean	SD
W	59	1060	343.86	374.80
TL	38.00	83.30	52.66	15.97
CF	0.11	0.22	0.15	0.04
HSI	0.59	1.58	1.19	0.27
GSI	0.13	2.63	1.26	0.71

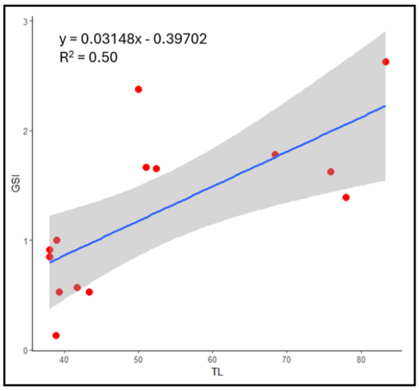

Figure 1. Linear regression of GSI and TL

CRISPR/CAS9-MEDIATED KNOCKOUT OF IL6 IMPAIRED DEFENSE SYSTEM AGAINST *E. piscicida* IN ZEBRAFISH

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Interleukin 6 (Il-6) is a multifunctional cytokine that plays a crucial role in the immune response and inflammation. It is a member of the interleukin family of cytokines, which are signaling proteins secreted by immune cells and certain other cell types that regulate a wide range of biological activities.

In this study, we generated the *il6* knockout zebrafish using CRISPR/Cas9 system and investigated its absence and how the absence of *il6* affects the immune system. The *il6* gene of zebrafish is found a chromosome 19 and its transcript consists of 5 exons. The full-length cDNA sequence of zebrafish *il6* consists of 696 base-pair (bp) of ORF flanked by 5' and 3' sequences. Developmental expression analysis showed that *il6* was expressed from early developmental stages. In tissue distribution in adult zebrafish, the highest expression was detected in the ovary, followed by the testis and spleen. To generate the mutants, a we targeted the sgRNA binding site within exon 2, resulting in a 7 bp deletion in the target area and the introduction of an early stop codon. The downstream gene analysis in 7 days embryos of wild type (WT) and *il6*$^{(-/-)}$ zebrafish revealed that there is no significant difference in *stat3* expression. The expression of *socs3*, *mpx*, and *mpeg* was upregulated and the expression of *tnf-*α*1*, *il-1*β was down regulated in *il6*$^{(-/-)}$ mutant compared to WT zebrafish. To assess the functional consequences, we performed an *Edwardsiella piscicida* (*E. piscicida*) immersion challenge using WT and *il6* ablated zebrafish. We examined mortality of WT and *il6*$^{(-/-)}$ zebrafish caused from *E. piscicida* infection at different concentrations. The *il6* ablated fish displayed increased susceptibility to bacterial infection and exhibited a higher mortality rate than WT fish, irrespective of the bacterial concentration. Furthermore, downstream gene expression analysis highlighted distinctive patterns in the absence of *il6* during bacterial infection.

In summary, our study reveals the critical role of *il6* in antibacterial defenses, shedding light on its importance of *il6* in the immune responses against infection.

THE GENETIC VARIATION OF HATCHERY PRODUCED JUVENILES OF ATLANTIC SALMON (*Salmo salar*) RELEASED INTO POLISH RIVERS

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Native Atlantic salmon in Poland became extinct in the mid-20th century. Due to this, Poland has started to restore its populations within the program "Stocking of Polish Marine Areas". It is focused on the stocking of the tributaries of the Vistula, Drawa River, and main Pomeranian rivers with fry and smolts obtained in the aquaculture conditions. The aim of the present study was to evaluate the genetic variation in the salmon broodstocks and its populations occurring in the rivers.

Three salmon broodstocks: Rutki (RUT), Aquamar (AQU), Żelkówko (ZEL) and its two river populations: Słupia (SLU) and Parsęta (PAR) were investigated. Eleven microsatellite loci were amplified and genotyped. The values of the genetic variation indicators were calculated.

Among the stocks the total number of alleles detected across all loci ranged 46-81 (Table 1). The average observed heterozygosity across all stocks (AC) and loci was 0.59; expected heterozygosity was 0.58. The populations remained in Hardy-Weinberg equilibrium. The average value of Garza-Williamson *M* index for all populations was low. The inbreeding values per generation ranged from minus 0.08% to 0.08%. Genetic distance was high between the Lithuanian stock (RUT and PAR) and that of Latvian origin (AQU, ZEL and SLU) (Table 2). Several admixture traces were recorded in all stocks with the smallest evidence in RUT (Figure 1).

The genetic diversity in the investigated stocks/ populations is slightly lower than that usually recorded in the Baltic Sea salmon populations, probably due to genetic isolation, founder/bottleneck effect, and genetic drift. The actual level of inbred or outbred in the Polish salmon is not high enough to be a problem in the production of its stocking material. However, monitoring of inbreeding and other indicators of salmon genetic variation would be recommended.

TABLE 1. Genetic properties of the investigated stocks. Total number of alleles (*N*), allelic diversity (*AD*), observed (H_o) and expected (H_e) heterozygosity, the inbreeding coefficient (F_{IS}), value of Garza-Williamson index (*M*).

Indicator	RUT	PAR	AQU	ZEL	SLU	AC
N	46	54	63	69	81	97
AD	4.18	4.91	5.73	6.27	7.36	8.82
H_o	0.59	0.50	0.67	0.55	0.65	0.59
H_e	0.56	0.45	0.63	0.59	0.67	0.58
F_{IS}	-0.07	-0.08	-0.07	0.08	0.04	-0.02
M	0.52	0.58	0.61	0.58	0.59	0.58

TABLE 2. Genetic differences between stocks based on $\delta\mu^2$ and F_{ST} values

		F_{ST}				
		RUT	PAR	AQU	ZEL	SLU
$\delta\mu^2$	RUT	x	0.28	0.16	0.20	0.16
	PAR	2.60	x	0.17	0.15	0.15
	AQU	3.46	4.97	x	0.03	0.02
	ZEL	4.39	5.69	0.61	x	0.03
	SLU	2.31	2.52	0.93	1.10	x

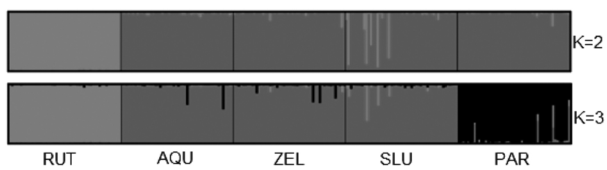

FIGURE 1. Clustering of salmon from analyzed stocks/populations with putative K = 2 and K=3.

FOOD DYNAMICS OF COMMON CARP IN AQUACULTURE PONDS: FROM AVAILABLE RESOURCES TO ACTUAL CONSUMPTION

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Introduction

Pond inland aquaculture in Europe predominantly focuses on the production of common carp (*Cyprinus carpio*) under semi-intensive management, where carp feed on both natural prey and supplemental feed (mostly cereals). Common carp are known to be omnivorous, consuming a diverse array of food items. This study aims to provide a detailed analysis of the dietary preferences of common carp that enhance our understanding of their feeding behaviour in aquaculture ponds. The insights into the seasonal dietary preferences of common carp might provide knowledge for potential revisions of feeding practices in European ponds.

Material and Methods

The study was conducted in three experimental ponds in South Bohemia, Czechia (surface area: 0.16 ha; average depth: 80 cm). Each pond was stocked with 2-year-old common carp (mean total length: 267.9 ± 15.2 mm; mean weight: 337.2 ± 56.6 g) at a density of 938 individuals ha^{-1} and biomass of 316 kg ha^{-1}. Fish were fed cereals starting in May. Monthly sampling was performed to monitor fish growth and to analyse digestive tract contents using the non-lethal method of gut flushing. Environmental parameters and food items (zooplankton and zoobenthos) were also monitored and sampled monthly.

Results and Discussion

The total biomass gain of common carp over the growing season (April–September) was 1,420 kg ha^{-1}, with a food conversion ratio of 2.83. The feeding behaviour of common carp changed with food availability. Initially, carp consumed zoobenthos (April–June), with cereals playing a minor role. From mid-summer (June/July) onwards, the diet included increased amounts of both cereals and zooplankton (especially cladocerans and copepods). Common carp, interestingly, displayed strong preferences for small cladocerans (*Bosmina* spp.) and copepods. Large filter-feeding daphnids are expected to not only be effective transformation links in the pond system but also to be the most nutritious food item for common carp and, therefore, to be preferably consumed in large amounts. Nonetheless, the preference of common carp for daphnids was ambiguous.

Acknowledgement

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DOES FARMED ATLANTIC SALMON GET FRIGHTENED OF WILD FISH AROUND SEA CAGES?

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Farming of salmonids in open sea cages can attract wild fish species (Banno, et al., 2022; Dempster, et al., 2009; Uglem, et al., 2014). The attraction can be caused by excess fish feed or the construction itself (Uglem, et al., 2014). Presence of sea cages may impact the migrating behavior of wild fish species, and large aggregations of wild fish may increase the risk of transferring diseases or parasites between farmed and wild fish (Uglem, et al., 2014). Previous studies have focused on the potential impact of fish aggregations around salmon farms in terms of environmental challenges and the wild fish itself. However, there are currently limited knowledge on how salmon are influenced by the presence of wild fish. For example, smolt of wild Atlantic salmon have been found in the stomach of Atlantic cod (*Gadus morhua*) and saithe (*Pollachius virens*), suggesting these fish species prey on salmon when they are migrating through the fjord (Hvidsten, Lund, 1988). It is not known whether salmon contained in a closed environment will respond to any wild fish approaching the sea cage. Farmed Atlantic salmon were anecdotally observed altering its behavior on a random video recording in a commercial sea cage when a large cod approached the net. Such behavioral alterations may impact the welfare of salmon if it occurs on a regular basis when wild fish approach the sea cage. In the present case study, we investigated the immediate behavioral response of farmed Atlantic salmon when Atlantic mackerel, saithe and cod approached the sea cage with salmon.

This study took place at a commercial farming site in Møre and Romsdal when wild fish were expected to be present in the area. A ROV were first used to identify high densities of wild fish (saithe or mackerel) in the vicinity of a particular sea cage. Secondly, a 360-degree camera were placed in the same area inside the sea cage. This made it possible to assess the swimming behavior of salmon at the same time as wild fish outside the cage approached salmon inside the cage. Videos were collected from one cage containing large salmon (≈ 2.5 kg) and three cages containing small salmon (< 1 kg). More than 700 minutes of video were collected and were analyzed for any escape response (defined as a clear change in swimming speed or swimming direction) among salmon when wild fish instantly appeared close to salmon.

No escape response was observed. In total, Mackerel instantly appeared close to large and small salmon, 10 and 48 times respectively. Saithe instantly appeared close to large and small salmon, 59 and 69 times respectively. This indicates that salmon are not regularly frightened by mackerel or saithe, but more research on other potentially larger wild fish is required.

Banno, K., Kaland, H., Crescitelli, A.M., Tuene, S.A., Aas, G.H., Gansel, L.C., 2022. A novel approach for wild fish monitoring at aquaculture sites: wild fish presence analysis using computer vision. Aquac. Environ. Interact. 14, 97-112.https://doi.org/10.3354/aei00432

Dempster, T., Uglem, I., Sanchez-Jerez, P., Fernandez-Jover, D., Bayle-Sempere, J.T., Nilsen, R., Bjørn, P.-A., 2009. Coastal salmon farms attract large and persistent aggregations of wild fish: an ecosystem effect. Mar. Ecol. Prog. Ser. 385, 1-14.https://doi.org/10.3354/meps08050

Hvidsten, N.A., Lund, R.A., 1988. Predation on hatchery-reared and wild smolts of Atlantic salmon, *Salmo salar L.*, in the estuary of River Orkla, Norway. J. Fish Biol. 33, 121-126.https://doi.org/10.1111/j.1095-8649.1988.tb05453.x

Uglem, I., Karlsen, Ø., Sanchez-Jerez, P., Sæther, B.S., 2014. Impacts of wild fishes attracted to open-cage salmonid farms in Norway. Aquac. Environ. Interact. 6, 91-103.https://doi.org/10.3354/aei00112

THE ADDITION OF HYDROCHLORIC ACID INCREASES USEFULNESS OF AQUAFEEDS FOR THE INTENSIVE FEEDING OF CYPRINIFORM FISH

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The low biological quality of cypriniform fish grown in RAS is a frequently reported problem. The distinctive nature of food digestion in these agastric (stomachless) organisms limits their ability to effectively utilize phosphorus from modern commercial aquafeeds, which are successfully used in gastric fish. Therefore, intensive feeding of cypriform fish with commercial feed often results in phosphorus (P) deficiency, followed by the appearance of body deformities or poor disease resistance. Improving the availability of P in fish diets can increase the quality of fish, reduce production costs and waste, helping to mitigate environmental pollution. The use of acids as feed additives seems a simple way to achieve this goal.

The study was conducted within the National Inland Fisheries Research Institute Project Z-005.

TABLE 1. Effect of the addition of 1.5% HCl to dry commercial aquafeeds on the performance of different cypriniform fish in the RAS

Species	Growth rate of body weight	Growth rate of total length	Condition factor	Feed conversion ratio	Incidence of body deformities	Immunity
barbel	↑	↑	0	↓	0	n/a
chub	↑	↑	0	↓	0	n/a
common carp	↑	↑	↓	↓	↓	↓
common dace	0	0	↓	0	0	n/a
crucian carp	0	0	↓	0	↓	n/a
rudd	0	0	0	0	↓	n/a
tench	↑	↑	↓	↓	↓	↑
vimba bream	0	0	0	0	0	↑

↑ - increase, ↓ - decrease, 0 – no significant effect.

HOW DO DIFFERENT PERIODS OF ADMINISTRATION OF DIETARY PREBIOTIC SUPPLEMENTS AFFECT THE IMMUNE CELL ACTIVITY OF VIMBA BREAM JUVENILES?

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Feeding fish with diets supplemented with polysaccharides is increasingly important part of routine preventive procedures in aquaculture. However, there are few publications addressing the issue of side effects caused by long-term use of immunostimulants, and the results obtained are often contradictory. In our study, we determined the effects of three commercial prebiotics dedicated to fish (Leiber® Beta-S, Biolex® MB40 (Leiber), CeFi® Pro (Leiber)) and Orafti GR inulin (Beneo-Orafti SA, Hortimex) on the immune response of juvenile vimba bream (*Vimba vimba*) when administered for 55 days. Our study showed that such long-term administration of all prebiotics tested weakened innate cellular immunity, as evidenced by a significant decrease in respiratory burst activity of head kidney phagocytes (RBA) (Table 1).

In another study, we used Leiber® Beta-S to determine after what period of administration immunosuppression occurs. Vimba bream juveniles received feed supplemented with this prebiotic (BS group) for 2, 4 or 8 weeks. Two-week treatment had a beneficial effect on the activity of fish head kidney phagocytes (PKA). Longer administration of glucans resulted in a gradual decrease in cell activity as compared to the control group (Table 2). After 4 weeks, RBA and PKA in the BS group reached levels similar to those in the control group. After 8 weeks of treatment, RBA was significantly lower in the BS group compared to the control group. Our results confirm that short-term oral administration of glucans to fish has the expected immunostimulating effect, but its excessive prolongation may have the opposite effect. This finding seems important in animal nutrition, where glucans are increasingly used as a permanent component of the diet.

TABLE 1. The head kidney immune cells activity (stimulation index, SI) in vimba fed for 55 days Leiber® Beta-S (BS), Biolex® MB40 (MB), CeFi® Pro (CE), inulin Orafti GR (IN) supplemented feed or commercial diet (Control); * different as compared to the Control group at $p<0.05$, Dunnet's test

Parameter	Control	BS	MB	CE	IN
RBA	2.12 ± 0.15	1.48 ± 0.21*	1.36 ± 0.04*	1.44 ± 0.16*	1.50 ± 0.14*
PKA	1.21 ± 0.06	1.24 ± 0.15	1.12 ± 0.17	1.32 ± 0.17	1.70 ± 0.35

TABLE 2. The head kidney immune cells activity (stimulation index, SI) in juvenile vimba bream fed commercial dry diet (Control) or the same diet supplemented with Leiber® Beta-S (BS); * different at $p<0.05$, Student's *t*-test

Parameter	Control	BS
	After 2 weeks	
RBA	1.269 ± 0.065	1.566 ± 0.129*
PKA	1.310 ± 0.105	1.570 ± 0.107*
	After 4 weeks	
RBA	1.516 ± 0.153	1.527 ± 0.429
PKA	1.500 ± 0.115	1.431 ± 0.316
	After 8 weeks	
RBA	1.803 ± 0.149	1.482 ± 0.091*
PKA	1.434 ± 0.093	1.293 ± 0.166

The study was conducted within the National Inland Fisheries Research Institute Project Z-011.

A DETERMINATION OF OPTIMAL SODIUM BICARBONATE CONCENTRATIONS IN RECIRCULATING AQUACULTURE SYSTEMS FOR ATLANTIC SALMON (*Salmo Salar*)

Noah S. Kantsiper and Ayush R. Ghosh

Background: The welfare of housed fish is paramount to the practice of aquaculture. It has been well established that the production of carbon dioxide and feeding practices contribute to the natural decline in pH of aquatic tanks. In order to combat this, many aquaculture facilities utilize a buffer, oftentimes sodium bicarbonate, to make the housed water more alkaline and therefore mitigate any changes in the pH. However, a streamlined approach to calculating the ideal quantity of sodium bicarbonate to add has not been established in modern aquaculture facilities. Because of this, many aquaculture facilities experience a volatile pH in their tanks which serves as a detriment to aquatic life. Therefore, it is necessary to determine a system which calculates the ideal sodium bicarbonate concentration to achieve optimal alkalinity thereby maintaining a constant and healthy pH.

Objective: To develop a prototype to calculate the ideal concentration of sodium bicarbonate that must be added to maintain an ideal pH in recirculating aquaculture systems, while maintaining fish health.

Methods: By conducting a meta-analysis of papers and graphs on sodium bicarbonate buffering, we developed a prototype which should ensure that pH and alkalinity of the system stay within a safe range upon daily addition of sodium bicarbonate. Many of the equations were drawn from chemical relationships within the carbonic acid equilibrium as outlined in Snoeyink and Jenkins 1980; Stumm and Morgan 1981.

Results: Upon the conclusion of the development of the device, it is able to effectively and efficiently output a reasonable amount of sodium bicarbonate additions in grams from the current pH and alkalinity values, system volume, and goal pH set by the user.

Conclusion: The development of a device to calculate the amount of sodium bicarbonate to add to a recirculating aquaculture system is viable; however, it requires further testing to prove that the device is reliable.

ZiD-AI: A MOBILE AI TOOLS FOR THE AUTOMATIC MORPHOMETRIC AND BEHAVIORAL ANALYSIS OF ADULT ZEBRAFISH (*Danio rerio*)

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AI technology has been proven as a valuable tool to collect behavioral data and individual size measurements of the reared stocks in finfish aquaculture, thus avoiding invasive methods involving fish sampling and handling (e.g., behavioral analysis[1]). Similar integrated systems have been developed for the embryos, larvae, and adults of zebrafish (*Danio rerio*), a valuable model species in biological research (e.g., developmental biology, ecotoxicology, genetics, fish biology[2,3]) In this study I developed an object detection and analysis model (*Zid-AI*), based on narrow AI technology which minimizes the needs for pricy equipment and can be done in any mobile phone camera, to automatically monitor the size and behavior of the individual zebrafish in the holding aquaria.

A total of 3607 zebrafish images from various free sources were annotated and augmented to form a diverse dataset of 13999 images. The YOLO V9 architecture was used to build the object detection model. For software testing, an experimental setup of 10 small tanks was used, each containing 3 fish (of small, medium and adult size). The size of the fish was estimated (mean total length, TL, of multiple measurements) by the Zid-AI software and the use of two ArUco markers placed in the tank. The accuracy of the Zid-AI output was estimated by comparing the results with the real fish TL (following anesthetization and digital photography).

The model's precision-recall value (% of true positive fish identification) ranged from 83.0% to 87.6% (Figure 1). The trials on the accuracy of TL estimation are in progress. The Zid-AI system shows significant progress in non-invasive zebrafish monitoring, achieving reliable fish detection. However, visual occlusion and lens distortion pose challenges. Future work should focus on refining length measurement accuracy and expanding the dataset for more robust results. Testing in diverse environments and integrating real-time monitoring will further enhance its utility in zebrafish research.

References

Fan, Y.L., Hsu, F.R., Wang, Y. and Liao, L.D., 2023. Unlocking the potential of zebrafish research with artificial intelligence: Advancements in tracking, processing, and visualization. *Medical & Biological Engineering & Computing*, *61*, 2797-2814.

Li, H., Liu, Y., Chen, Q., Jin, L. and Peng, R., 2023. Research progress of zebrafish model in aquatic ecotoxicology. *Water*, *15*, 1735.

Nguyen, Thanh Khoa, Madeline Petrikas, Brooke E. Chambers, and Rebecca A. Wingert., 2023. Principles of zebrafish nephron segment development. *Journal of Developmental Biology* 11, 14.

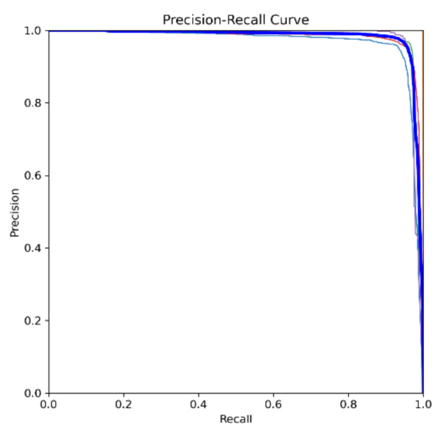

Figure 1. Precision-Recall curve. X-Axis: Recall (True Positive Rate), Y-Axis: Precision (Positive Predictive Value)

PREVALENCE OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) IN SEAWATER-CULTURED RAINBOW TROUT *Oncorhynchus Mykiss* AND AMAGO TROUT *Oncorhynchus Masou Ishikawai* IN JAPAN

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In Japan, Rainbow trout *Oncorhynchus mykiss* and Amago trout *O. masou ishikawai* have mainly been cultured in freshwater but recently seawater trout farming has been attracting more interest due to the larger harvest sizes in sea cages. Researches have been conducted to examine the biological characteristics such as growth, survival and production associated with the development of seawater culture in trout, there is limited information on the problems with viral and bacterial diseases.

Infectious hematopoietic necrosis virus (IHNV) is one of the most serious viral pathogens of salmonid fish. Originally enzootic in western North America, IHNV has spread following movement of salmonid fish and eggs to European and Asian countries, and is subdivided into five major genogroups: upper (U), middle (M), lower (L), Europe (E), and Japanese rainbow trout (J). The J genogroup is closely related to the U genogroup and is endemic in Asian salmon fish-farming regions. IHNV isolates in Japan were classified into J genogroup: three known Shizuoka (JS), Nagano (JN), and North Kanto (Jnk) lineages (Namba *et al*., 2021). Since the 1970s, IHNV has caused severe damage to freshwater trout farms in Japan, but the risk to seawater cultured fish is unclear.

In the present study, therefore, we analyzed the prevalence of IHNV in Rainbow trout and Amago trout obtained from 11 seawater farms in Japan from 2019 through 2023. As a result, the prevalence was 0-8% per year, and detected IHNV were classified into JS and JN lineages. To estimate the risk of IHNV infection in sea cages, we are currently analyzing the salinity tolerance of IHNV isolates and the pathogenicity of those in saltwater-reared rainbow trout.

Table 1 Presence of fish positive for IHNV

Isolataoin		Host			
Year	Farm**	Species	Average BW (g)	Number of fish	Lineage
2019	F-1	*O. mykiss*	364	0/9*	
	F-2	*O. mykiss*	388	0/5	
	K-1	*O. mykiss*	560	0/5	
	K-2	*O. mykiss*	520	1/5	JN
	K-3	*O. mykiss*	360	0/5	
2020	F-1	*O. mykiss*	417	0/7	
	F-2	*O. mykiss*	873	0/4	
2021	F-1	*O. mykiss*	770	0/7	
	F-2	*O. mykiss*	1,355	0/4	
	K-1	*O. mykiss*	683	2/5	JS
	K-2	*O. mykiss*	603	0/5	
	K-4	*O. mykiss*	1,043	0/5	
2022	K-2	*O. mykiss*	798	0/5	
	F-2	*O. mykiss*	848	0/5	
	H-1	*O. mykiss*	690	0/5	
	A-1	*O. mykiss*	1,458	0/9	
2023	K-5-1	*O. mykiss*	671	0/5	
	K-5-2	*O. mykiss*	753	0/5	
	K-5-3	*O. mykiss*	778	0/5	
	F-2	*O. mykiss*	1,151	0/5	
	H-1	*O. mykiss*	286	0/5	
	A-1	*O. mykiss*	441	0/6	
	A-2	*O. mykiss*	859	0/8	
	t-1(N.D)	*O. masou*	219	1/5	JN

*Farms are indicated as random letters and names withheld to protect privacy
**Number of IHNV positive samples/total number of samples.

Namba et al. (2021): Fish Pathology, 56(3), 35-42. https://doi.org/10.3147/jsfp.56.35

EFFECT OF KRILL MEAL ON SEA LICE INFESTATION AND SKIN MUCOSAL HEALTH IN ATLANTIC SALMON

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Sea lice challenges in the salmon industry highlight the urgent need for fish-sensitive alternative control measures, like functional nutrition, to minimize handling and promote fish health and welfare. The present study investigated the efficacy of krill meal (KM, 8% and 12%), towards reducing sea lice infestation and enhancing skin mucosal health in Atlantic salmon smolts (average weight: 170g). Following an 8-week feeding period on test diets (two doses of KM and a control diet mimicking conventional feed), the fish underwent a 2-week sea lice infestation challenge while maintaining the test diets, reaching an average weight of 350g. After 8-week pre challenge feeding, fish fed with 8% KM diet exhibited a thicker skin epithelial layer (72.3 μ) compared to the 12% KM (51.3 μ) and control groups (43.8 μ). Additionally, skin mucosal health parameters, including cell size (208 μ), cell density (25.2%), and defense activity (1.19), were significantly enhanced with 8% KM compared to control (cell size: 173.5 μ, cell density: 16.4% and defence activity:0.93) and 12% KM (cell size:162.3 μ, cell density: 17% and defence activity: 1.04). Further after the 2-week sea lice challenge the 8% KM-fed fish had statistically significantly the lowest number of sea lice (median of 6.5 per fish) in comparison to 12% KM and control groups (median of 9 per fish). In addition, 8% KM group exhibited a significant reduction in skin mucus cell size while maintaining a high abundance of mucous cells indicating a rapid production and turnover of skin mucosal layer for effectively “washing off” lice. This study, for the first time, highlights the potential of KM as a part of functional nutrition strategy for mitigating sea lice challenges in Atlantic salmon. However, further studies are warranted to explore the biological pathways and mechanisms underlying the observed results.

BENEFITS OF DIETARY KRILL MEAL TOWARDS BETTER UTILIZATION OF NUTRIENTS AND RESPONSE TO OXIDATIVE STRESS IN GILTHEAD SEABREAM *Sparus aurata* JUVENILES

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Krill meal (KM) emerges as a promising alternative to fish meal (FM) in aquafeeds, providing a rich source of protein, amino acids, phospholipids, omega-3 fatty acids, and bioactive compounds. This study aimed to investigate the effects of partially replacing FM with increasing levels of KM (3, 5 and 7% of the diet) on growth performance, nutrient utilization, and antioxidant defenses in juvenile gilthead sea bream (*Sparus aurata*) exposed to a crowding stress challenge. The dietary inclusion of 7% KM could effectively replace up to 7% FM in the diet, resulting in a tendency to improve feed conversion ratio (FCR) and nutrient efficiency ratios compared to the control FM diet. Under stress conditions, a significant interaction between diet and time was observed in fish blood omega- 3 index (OI3). At 24h after the stress challenge, all dietary treatments except 3% KM presented a significant increase in OI3. At 7d post-stress, fish fed the Control diet led to a significant reduction in O3I down to the basal levels. On the contrary, those fish fed 5% KM and 7% KM diets kept increased O3I at the end of the stress challenge (Table). Fish fed 5% KM and 7%KM also showed a lower increase of *cat* and *sod* gene expression 24h after stress, which was inversely correlated with fish blood OI3. Therefore, these results show that KM modulates red blood cells fatty acid profile by increasing fish OI3 after crowding stress as well as potentially function as an antioxidant modulator in fish feeds for mitigating stressful conditions. Thus, KM could be a viable candidate for FM replacement in aquafeeds, aiming to expand the basket of raw materials with functional properties to be used in aquafeed formulation by demonstrating its efficacy in improving nutrient utilization, as well as exhibiting benefits in mitigating oxidative stress in fish.

TABLE: Blood omega-3 index (% total fatty acids) of gilthead sea bream (*Sparus aurata*) fed the experimental diets over the stress challenge.

Diet	Time			Two-Way ANOVA		
	0h	24h	7 days	Diet	Time	Diet x Time
	18.76 ± 3.40^{1}	22.56 ± 3.76^{2}	19.92 ± 4.59^{1a}	$p <$ 0.05	$p <$ 0.05	*n.s*
	17.88 ± 2.52^{1}	20.46 ± 4.18^{12}	22.14 ± 3.93^{2ab}			
	19.15 ± 2.97^{1}	22.31 ± 3.85^{2}	23.85 ± 2.67^{2b}			
	19.52 ± 3.48^{1}	23.72 ± 3.26^{2}	23.12 ± 2.56^{2ab}			

Different numbers denote significant differences ($p < 0.05$) between experimental sampling points (Two-way ANOVA: Diet x Time x Diet*Time; Tukey post-hoc test); Different letters denote significant differences ($p < 0.05$) between experimental diets (Two-way ANOVA: Diet x Time x Diet*Time; Tukey post-hoc test); KM3- 3% krill meal ; KM5- 5% krill meal; KM7- 7% krill meal. ns: not significant

FUNCTIONAL AMINO ACIDS FOR PRECISION AND SUSTAINABLE AQUAFEED FORMULATION IN ASIAN SEABASS (*Lates calcarifer*): GROWTH AND HEALTH

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In pursuing more sustainable and efficient aquaculture, this study explores the concept of functional amino acids and their benefits in fish nutrition above and beyond the traditional approaches of nutritionally dispensable or indispensable amino acids (AA). Functional AA are defined as those that participate and regulate key metabolic pathways to improve growth and health. Moreover, functional AAs supplementation is crucial for precision formulation in a challenge-oriented nutrition strategy. This study aimed to evaluate the dose-response effects of supplementing functional AA for Asian Seabass (*Lates calcarifer*) on growth and health. Parameters included midgut histo-morphology, digestive enzymes activity, liver and serum antioxidant capacity, blood biochemistry, immune and growth-related genes expression. The mix of functional AA used in this study was obtained from extensive hydrolysis of poultry keratin (KFAA), commercial name Kera-Stim®50. Five isonutrient diets, a control, and four diets supplemented with different KFAA levels: 0.25% (KFAA0.25), 0.50% (KFAA0.50), 0.75% (KFAA0.75) and 1.0% (KFAA1.0) were used in the experiment. Fish were fed to apparent satiation twice a day for eight weeks. Samples were collected at the end of the feeding trial after 24 h of fasting.

Overall, fish fed the KFAA0.75 and KFAA1.0 diets resulted in significant ($P < 0.05$) improvement on most parameters evaluated. A regression analysis indicated an ideal inclusion of 0.876% for feed conversion and 0.752 for protein efficiency ratio. The main highlights were the midgut histo-morphology including the mucosal fold height, width, thickness, and goblet cell, were better developed in fish fed the KFAA1.0 diet. The expression of growth-related genes including growth hormone (GH), GH receptor (GHR), insulin-like growth factor I (IGF-I), and IGF-II were greater in fish fed KFAA0.75. Antioxidant markers and innate immune enzymes were also evaluated and increased in fish fed KFAA0.75 and KFAA1.0 citing lysozyme, myeloperoxidase, catalase, and glutathione peroxidase. Digestive enzymes activity, (amylase, protease, and lipase) were boosted by the supplementation of ≥KFAA0.5%. Unlike, whole body fatty acids profiles were greater for polyunsaturated (C18:3 n6 and C18:3n3) in fish fed KFAA0.25. These findings provide a scientific basis for applying FAAs as a sustainable solution for a more efficient and profitable aquaculture, especially for marine carnivores such as Asian seabass.

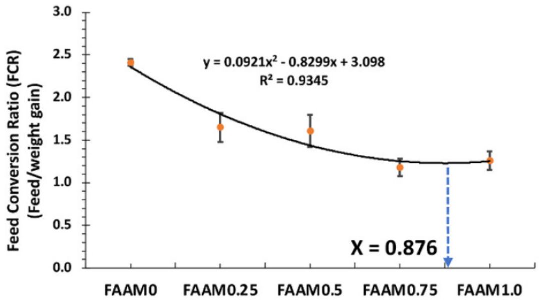

Fig. 1. Dose-response regression for FCR fish-fed functional amino acids mix.

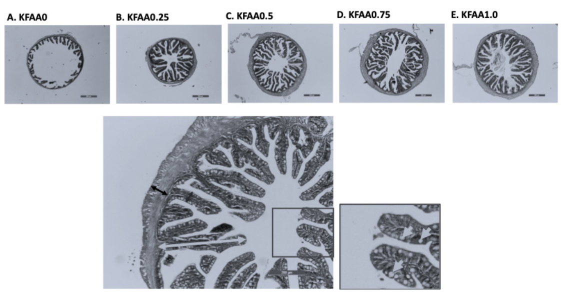

Fig. 2. Morphology of the intestine (magnification × 100) of Asian seabass.

YEAST HYDROLYSATE IMPROVED THE PERFORMANCE, SURVIVAL RATE AND IMMUNITY OF WHITELEG SHRIMP *Litopenaeus vannamei*

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Feeding strategies are needed for reducing the impact of microbial diseases on the production performance of whiteleg shrimp (*Litopenaeus vannamei*). The present experiment studied the effect of dietary yeast hydrolysate (YH) on the performance, survival rate and immunity of whiteleg shrimp in three conditions: a) without an intentional challenge factor, b) with *Vibrio parahaemolyticus* (*Vp*) challenge, and c) with white spot syndrome virus (WSSW) challenge.

The study was started in 27 aquariums of 120 liters with 15 ppt saline water, and 30 juvenile shrimp with a mean initial weight of 1.54 g. Commercial-type shrimp feed was amended with YH (Progut® Extra, Hankkija Oy, Finland) at 0.0, 0.5, and 1.0 kg/ton for the dietary treatments T1-T3, respectively (9 replicate aquariums/diet). Feed was given at 3-5% of body weight 3 times a day. Uneaten feed was siphoned out 1 hour after feeding, dried and weighed. Shrimp weight, feed intake, feed conversion ratio (FCR) and mortality were recorded every 2 weeks. Six replicate aquariums/diet were allocated to the 8-week performance test without a challenge factor. At the 4-week time point, shrimp from the remaining 3 replicate aquariums/diet were allocated into 100-litre challenge aquariums, 10 shrimp/aquarium and 3 aquariums/diet for both challenges. For the *Vp* challenge test, the highly virulent EMS/AHPND strain was used as an immersion treatment. Shrimp mortality was monitored for 21 days post-challenge. For the WSSV challenge test, the virus was introduced by subcutaneous injection to each shrimp. Six hours post-infection haemolymph was collected for confirming the infection, and mortality was monitored for 7 days post-challenge. After each test, samples of haemolymph, hepatopancreas, and intestinal contents were collected for analyses of microbiological and immunological parameters. The study was conducted in a completely randomized 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.

In the performance test without intentional challenge, dietary YH improved shrimp weight gain between weeks 4 and 6, and FCR for periods 0-4 weeks, 0-6 weeks, and 0-8 weeks. The 8-week survival rate was over 80% for all treatments (NS). Density of *Vibrio* (background challenge with low virulence) in hepatopancreas and intestine was lower for T2 and T3 than T1. Haemolymph protein concentration and the activities of lysozyme and superoxide dismutase were higher for treatments T2 and T3 than T1. In the *Vp* challenge test, the 21-day post-challenge survival rate was 66.7%, 73.3%, and 83.3% for T1-T3, respectively ($p<0.05$). Dietary YH decreased *Vibrio* counts in hepatopancreas and intestine. Hemocyte count, haemolymph protein concentration, and activities of phenoloxidase and lysozyme were higher for T2 and T3 than T1. In the WSSV challenge test, the 7-day post-challenge survival rate was 23.3%, 66.7%, and 80.0% for T1-T3, respectively ($p<0.05$). Haemolymph protein, and activities of phenoloxidase, lysozyme and superoxide dismutase were higher for T2 and T3 than T1. Moreover, dietary YH decreased the virus counts in haemolymph.

According to the results, dietary YH improved the performance, immunity, and antioxidative capacity of whiteleg shrimp in the unchallenged condition, and increased shrimp survival when challenged with virulent strains of *Vp* or WSSV. In conclusion, dietary YH amendment may be a suitable strategy for improving the production performance and disease resistance of whiteleg shrimp.

THE ROLE OF THE BIOFILTER IN STRUCTURING THE WATER AND FISH MICROBIOMES IN RECIRCULATING AQUACULTURE SYSTEMS

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Although the release of biofilm particles into the water by biofilters is acknowledged to influence the suspended water microbiota in recirculating aquaculture system (RAS), several studies have described significant differences between the biofilm communities on biofilters and those in the water (Schreier et al. 2010; Bakke et al. 2017, Vadstein et al. 2018). The bacteria present suspended in the RAS water may interact with fish mucosal surfaces (Bugten et al. 2022), pointing to the necessity of maintaining optimal microbial water quality to mitigate the proliferation of opportunistic and pathogenic bacteria. Despite recognizing the importance of these interactions, the role of the biofilter's biofilm microbiota in structuring the microbiota suspended in the water and associated with the fish is not well comprehended. Consequently, this study aimed to assess the influence of the biofilter's biofilm microbiota on the microbiota associated with the water and fish.

Two identical laboratory-scaled RAS were operated under the same conditions, differing only in the biofilm carriers utilized in their biofilters. These carriers were obtained from two commercial RAS situated in different geographical regions of Norway (Trøndelag and Sunnhordland) and represented distinct biofilm communities. After 30 days of RAS operation, the biofilm carriers were exchanged between the systems and operated for another 10 days. The water, fish, and biofilter microbiota were characterized by Illumina sequencing of 16S rDNA amplicons. Before swapping the biofilters, we found that the water microbiota differed significantly between the two RAS, indicating that the biofilm microbiota of the biofilters influenced the water microbiota. After swapping the biofilters, the water microbiota became more similar to the newly introduced biofilter's microbiota, thus supporting the finding that the biofilter microbiota influenced the microbiota suspended in the water (Figure 1). Notably, the skin and gut microbiota of the fry differed significantly from both biofilter and water microbiota. A noticeable difference in skin microbiota was observed between the two RAS after the initial 30 days of operation, suggesting a potential influence of biofilters' biofilm microbiota mediated by the water microbiota. Thus, we demonstrated that the biofilter is an important determinant for system's microbiota in RAS.

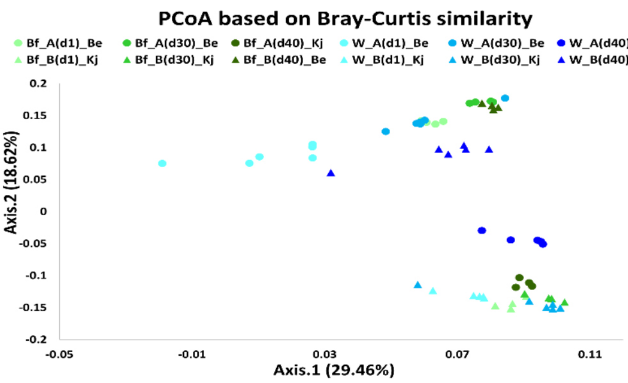

Figure 1: PCoA ordination based on Bray-Curtis similarities for microbiota of Biofilter (Bf) and Water (W) samples, A: RAS A, B: RAS B, d1: Day 1, d30: Day 30, d40: Day 40, Be and Kj: biofilm carriers originating from Trøndelag and Sunnhordland located in the

MITIGATING THE IMPACT OF WINTER TEMPERATURES ON STRIPED CATFISH (*Pangasianodon hypophthalmus*) USING FUNCTIONAL FEED ADDITIVES

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The drop in temperature during the winter months can lead to a decrease in growth, induce metabolic stress, and increase mortality incidences. The present study evaluated the potential of functional feed additives in mitigating the negative impacts of winter-rearing conditions in striped catfish (*Pangasianodon hypophthalmus*). Four test diets were evaluated over a twelve-week feed trial: (1) zinc-acetate (Zn-acetate), (2) selenomethionine (Se-Met), (3) β-glucan, and (4) control with no added feed additive. The survival rate in the β-glucan (95.00%) and Zn-acetate (93.30%) dietary groups was higher than the control (78.30%) and Se-Met (85.00%). Fish fed with the β-glucan had the highest weight gain (20.75% increase), specific growth rate (13.75% increase), and lower feed conversion ratio (9.64 % decrease). However, feed additives did not influence the fatty acid profiles or whole-body proximate composition. Although, Zn-acetate and Se-Met had higher body ash content. Serum cortisol and glucose levels were lower in β-glucan and Zn-acetate than in other treatment groups. All feed additives resulted in higher superoxide dismutase, glutathione peroxidase, and catalase activity in the liver and muscle. This study found that β-glucan and zinc-acetate can improve cold stress resistance and offer a sustainable strategy for catfish rearing over winter.

KNOWLEDGE, ATTITUDE AND PRACTICE ON SAFE PRAWN PRODUCTION AMONG FARMERS IN BANGLADESH

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Despite increasing international demand and an increasing production, Bangladesh lags behind in prawn exports due to its inability to uphold minimum international safety and quality standards. This deficiency undermines its competitiveness in the global market, hindering potential economic gains. To thrive in the lucrative prawn export sector, Bangladesh must prioritize stringent adherence to internationally recognized safety and quality protocols. By doing so, it can enhance its reputation as a reliable supplier, meet the expectations of discerning consumers worldwide, and unlock the full potential of its prawn industry on the global stage. With farmes being the central stage in the value chain to improve safety and quality, this study investigates the Knowledge, Attitude and Practices (KAP) of safer prawn production among prawn producers in Bangladesh. Primary data included one hundred and eight respondents that were randomly selected from the Khulna district to fulfill the objectives. The KAP index using the Likert scale was calculated based on collected data to assess the level of KAP and the Partial Least Square Structural Equation Model (PLS-SEM) was used to identify the factors affecting and the interrelationship among KAP towards safe prawn production.

Results reveal that although the majority (76%) exhibit a positive attitude towards safety measures, about two-thirds of the farmers display a moderate to poor level of knowledge on safe prawn production. A substantial portion (43%) of them were unable to fully implement best practices for prawn production. The study further found that farmers' knowledge has a significant effect on the relationship between attitude and practices, where attitude positively impacts practices toward safe prawn production (Figure 1). The hypothesized model for safe prawn production knowledge, attitude, and practices had a good fit and was acceptable. Education, farm size, and training were identified as influential factors in shaping farmers' knowledge levels, while age, experience, and training significantly influenced their attitudes. Moreover, a strong positive link between knowledge, attitudes, and practices emphasizes the importance of fostering a comprehensive understanding of safe prawn production.

To ensure safe prawn production at the farm level, this study recommends developing and implementing targeted education and education programs, supporting young farmers, promoting extension services, providing financial incentives, implementing monitoring and certification systems and providing government incentives for safe production among the farmers.

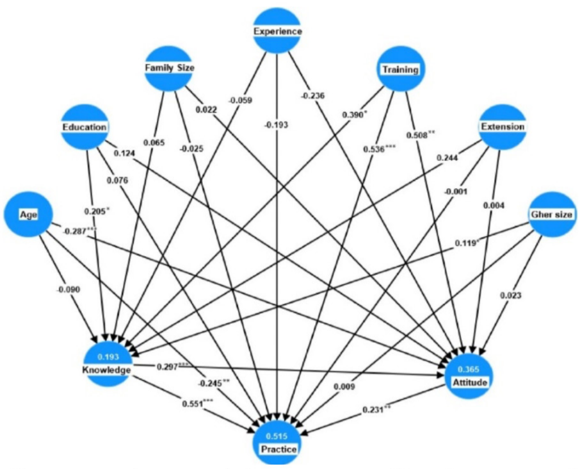

Figure 1: Factors affecting and relationship of knowledge, attitude and practice of prawn farmers using PLS-SEM (Note: * =p < 0.1, **=p < 0.05 and *** =p < 0.01.)

AN OVERVIESW OF SHRMIP AND PRAWN SECTOR IN BANGLADESH: WAY TO ENTRY INTO INDUSTRY 4.0

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The shrimp industry is playing significant role to the economy of Bangladesh for the last few decades. The aim of this study is to review the last forty (40) years trend, present status and propose a blockchain based framework to entry into fourth industrial revolution for enhancing export potentiality. In doing so, the study gathers different information from both secondary and primary data sources. A significant production trend and yield with the area expansion were found in shrimp industry. This has been possible due to the adoption of new production and management technologies, and need based policy formulation. Despite substantial progress achieved with shrimp production during the last four decades, growth of shrimp export remains minimal in Bangladesh. Traditional paper- based record-keeping methods for the shrimp supply chain are disparate and, therefore, cannot provide effcient traceability capacity and holistic view of the supply chain. Focusing on the export market, we propose a distributed and accumulative score-based certification approach that will grade packaged shrimps according to the completeness and accuracy of the authenticated data entered during different stages. In this framework, from the post-larva purchasing to the final packaging stage, relevant data for every stage will be entered by the associated actors via mobile/web app or Internet of Things devices to the blockchain network. Such distributed approach of certification will enhance not only food safety but also the quality and compliance to best practices.

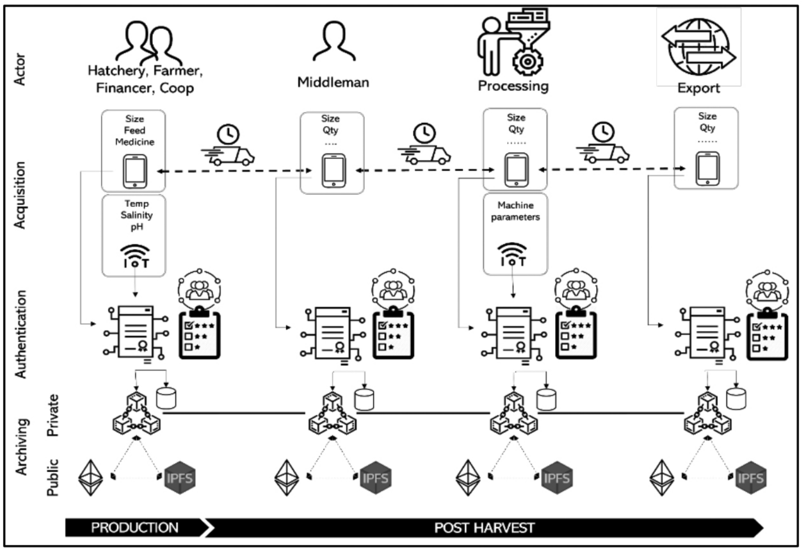

Figure 1: A blockchain based shrimp value chain.

DOES PROBIOTIC INCREASE TECHNICAL EFFICIENCY IN FRESHWATER PRAWN FARMING? EMPIRICAL EVIDENCE FROM BANGLADESH

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Prawn is a freshwater species and is produced in rivers and estuaries. Due to the increasing and intensified production, bacterial diseases are now a major threat to prawn production. To reduce bacterial diseases, eco-friendly technology using probiotics has been developed to reduce disease-related mortality rates and increase farm productivity. This study assesses the technical efficiency of freshwater prawn farms and what effects using probiotics has on farm-level efficiencies. To achieve this, a stochastic frontier model was computed, incorporating the influence of the technical inefficiency effects. A sample covering 318 farms was collected in Bangladesh using a structured questionnaire. The mean technical efficiency score was 0.55, which implies that if all farmers were producing like the most efficient farmers the production could be 0.45 without increasing the input use. The inefficiency model shows that using probiotics increases farmers' technical efficiency and the comparative analysis showed that the mean technical efficiency score for probiotic users (0.78) is significantly higher than for non-users (0.36) at $p < 0.05$. The findings highlight that the adoption of probiotics offers a promising solution to enhance disease resilience and increase the overall technical efficiency of prawn production.

Figure 1. Benefits of probiotic use in freshwater prawn

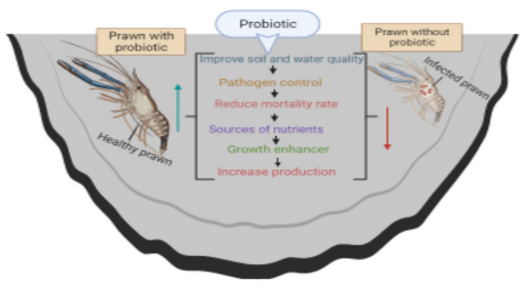

Table 1. The maximum likelihood estimates of the production frontier.

Stochastic production frontier	Parameters	Estimate	Std. Error
Constant	β_0	5.194***	0.6064
***Ln*Seed**	β_1	0.0834(*)	0.0475
***Ln*Feed**	β_2	0.0718*	0.0339
***Ln*Labour**	β_3	0.0654	0.0447
***Ln*Othercosts**	β_4	0.0346*	0.0174
***Ln*Fixedcosts**	β_5	0.0208	0.0148
Variance parameter			
SigmaSq	σ^2	0.1081***	0.0120
Gamma	γ	0.9719***	0.0161
$\gamma/[\gamma+(1-\gamma)\pi/(\pi-2)]$	γ^*	0.952	
Log-likelihood value		-15.88	
Return to Scale		0.28	
Mean Technical Efficiency		0.55	

Note: (*), *, **, and * denote statistical significance at the 10%, 5%, 1%, and 0.1% levels, respectively.**

PRAWN PRODUCTION DYNAMICS: MAXIMIZING PROFITABILITY, EFFICIENCY AND POLICY CONSIDERATIONS FOR FARMERS

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The expansion of prawn farming in Bangladesh has been driven by factors such as favorable environmental conditions and demand both domestically and internationally. Farmers have adopted various production systems to maximize profitability. This study focusing on the profitability and technical efficiencies (TEs) associated with three different methods of prawn cultivation: monoculture (solely prawn), PS (prawn and shrimp), and polyculture (prawn, shrimp, and fish combined) of 452 prawn-dominant farms in the coastal areas of Khulna and Bagerhat, Bangladesh. The findings reveal that polyculture farming systems tend to be more profitable compared to monoculture and PS systems. Conversely, monoculture farming demonstrates higher efficiency levels compared to polyculture and PS methods. Statistical analysis, including the Kruskal-Wallis test, highlights significant differences among the three production systems. Additionally, the Mann-Whitney Wilcox test indicates notable differences between monoculture and polyculture, as well as between PS and polyculture, while no significant variance is observed between monoculture and PS in terms of technical efficiencies. The findings of the study have introduced a conundrum regarding farmers' profitability and the choice of prawn production systems. Given that farmers' primary objective is profit maximization, the polyculture system emerges as more favorable for them. However, from an export perspective, the monoculture system appears to be more suitable. Monoculture farming often yields prawns of consistent quality and size, which aligns well with the preferences of international markets. This dilemma underscores the complex interplay between farmers' profit motives and the demands of export markets. While polyculture systems may optimize profits at the farm level, monoculture systems may offer advantages in accessing lucrative export markets due to product consistency and market demand. Policymakers and stakeholders in the prawn farming sector need to carefully consider these economic dynamics when formulating strategies to support farmers and promote the sector's growth and competitiveness both domestically and internationally.

RESILIENCE CAPACITY AND SUSTAINABILITY OF PRAWN FARMING IN BANGLADESH

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Promoting environmental friendly prawn farming is essential for maintaining natural resources, creating a healthy environment, and guaranteeing the food security of millions of people worldwide. This study aimed to evaluate the resilience capacity and sustainability of prawn farming in climate-vulnerable areas of Bangladesh. A total of 200 prawn farmers were randomly selected from Bagerhat and Khulna districts and data were gathered through direct interviews. The SAFA (Sustainability Assessment of Food and Agriculture) framework and the USAID resilience capacity model were employed to analyze the farmers' sustainability and resilience capacity, respectively. It is found that the resilience capacity across eight dimensions was notably insufficient. Particularly, the majority struggle in handling marketing risk, political uncertainties, and environmental hazards. Merely 15% of farms shows moderate capability in managing these risks, encompassing political and environmental challenges. The comprehensive resilience index value stood at 0.430, indicating a notably low score. This signifies farmers' weaknesses in cooperation, business strategy, and diversity fundamentals, reflecting a limited ability to safeguard their farms against salinity and unforeseen threats. Nevertheless, farmers exhibit strength in power dynamics fundamentals. In four sustainability metrics, local farmers score well in terms of social well-being, with 80% performing at best and good levels. However, there are challenges in other areas like environmental integrity, economic resilience, and governance. For instance, their performance in long-term profitability in prawn farming, sustainable business practices, environmental conservation, water management, and economic resilience is moderate at best. Moreover, the absence of written records for business purposes and limited awareness about crucial environmental factors like natural forest conservation and endangered species pose significant concerns. Therefore, resilience capability, environmental integrity, and governance need to be improved for the long-term sustainability of prawn farming.

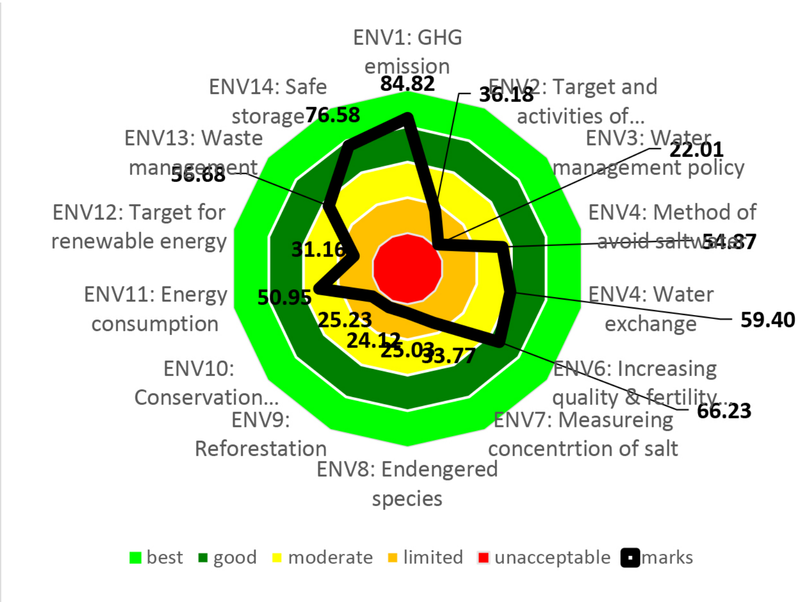

Figure 1: SAFA radar chart for the environmental integrity dimensions

EFFICACYOFNOVELIMMUNOSTIMULANTSAGAINSTINFECTIOUSHEMATOPOIETIC NECROSIS VIRUS (IHNV) INFECTION IN RAINBOW TROUT *Oncorhynchus mykiss*

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The rainbow trout *Oncorhynchus mykiss* is one of the most suitable fish for cultivation in cold waters and accounts for 20% of the freshwater aquaculture products of Japan. However, since viral and bacterial diseases are causing serious problems in trout cultures, many producers are searching for optimal communicable disease control methods to minimize the potential risk of disease. The use of novel immunostimulant may be one approach for fish culturists to reduce losses in their facilities due to disease. The present study was designed to investigate the efficacy of four immunostimulants prepared from either 2 types of lactic acid bacteria (LABa and b), yeast, or plant extract against infectious hematopoietic necrosis virus (IHNV) that cause serious infections in rainbow trout farms of Japan.

Fish (about 3g) were fed commercial diets supplemented with each immunostimulant for 11 days, and then challenged by bath exposure with IHNV NT-1304. As a results, the cumulative mortality of LABb and yeast groups infected with IHNV was decreased than that of control group, and IHNV were isolated from all dead fish in each group (Fig. 1). These results indicate that LABb and yeast supplementations enhance resistance against IHNV infection in rainbow trout and is effective in reducing losses in trout cultures due to virus diseases. We are currently conducting experiments on the optimal concentrations and effective mechanisms of the two immunostimulants that have shown efficacy in this study.

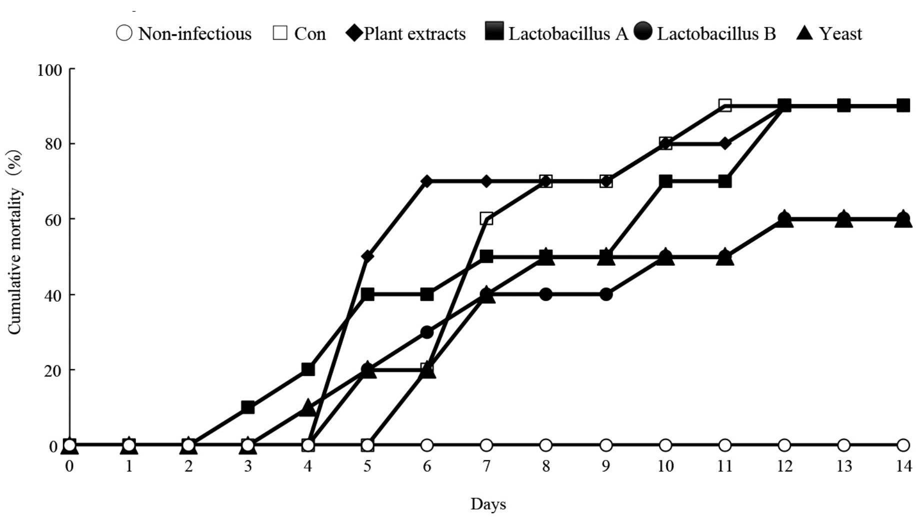

Fig.1 Changes in cumulative mortality of rainbow trout followed by bath exposure to IHNV.

GENOME-WIDE ASSOCIATION STUDY (GWAS) FOR DETECTING GENETIC VARIANTS ASSOCIATED WITH GROWTH TRAITS IN OLIVE FLOUNDER (*Paralichthys olivaceus*)

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The olive flounder (*Paralichthys olivaceus*) is a commercially important fish species in East Asian countries, with most productions coming from South Korea. Selective breeding has been applied for about 20 years to improve growth performance of olive flounder, which is one of the most important traits in aquaculture breeding programs and productivity. This study aimed to detect genetic factors associated with growth traits (body weight and total length) through Genome-Wide Association Study (GWAS), incorporating mixed linear models, Bayesian model and simple regression model.

National Institute of Fisheries Science in Korea initiated family-based breeding programs to enhance growth traits of olive flounder, and selection has been conducted for 8 generations since then. Growth traits (body weight and total length) were measured at 18 months in the 8th generation, consisting of 217 families, and the phenotypic distribution of growth at 18 months were analyzed.

To detect single nucleotide polymorphisms (SNPs) associated with growth traits, the individual genotypes of 3,223 fish measured for body weight and total length at 18 months were analyzed using a 120K SNP chip specific to olive flounder, which is a customized Affymetrix Axiom Genotyping Array. As a result, 69 SNPs associated with body weight and 85 SNPs associated with total length were identified using mixed linear model, and the detected SNPs using Bayesian model were shown. The lambda values were found to be 0.947 and 0.964 in the mixed linear model. For both body weight and total length traits, due to overestimation, the simple regression model was shown to be inappropriate for identifying genetic variants associated with growth traits of olive flounder. Our results suggest that genetic variants associated with growth traits can be discussed in the context of their potential role in underpinning genetic variation in growth traits, and these detected SNPs may contribute to promoting genomic selection in breeding programs of olive flounder.

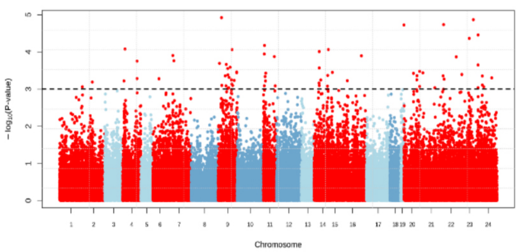

Figure 1. Manhattan plot for body weight at 18 months of olive flounder using mixed linear model of GWAS.

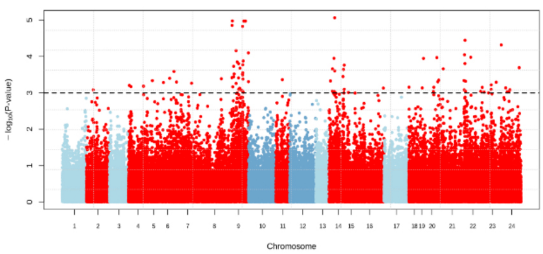

Figure 2. Manhattan plot for total length at 18 months of olive flounder using mixed linear model of GWAS.

GENOME-WIDE ASSOCIATION STUDY (GWAS) AND GENOMIC PREDICTION TOWARD EFFICIENT SELECTION BREEDING OF RESISTANCE TO *Enteromyxum leei* IN OLIVE FLOUNDER, *Paralichthys olivaceus*

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Enteromyxum leei (E.leei) infestation, belonging to the microscopic metazoans known as myxozoans, adversely affects fish health by suppressing appetite, hampering food metabolism, and causing growth impairment, ultimately leading to emaciation and cachexia. Additionally, it causes high mortalities and significant financial losses within South Korean aquaculture industries. In this study, our primary objective was to identify single nucleotide polymorphisms (SNPs) associated with resistance to *E. leei* in olive flounder using GWAS.

Initially, we obtained intestine samples from 752 olive flounders sourced from a farm naturally infected with *E. leei*. We utilized quantitative real-time PCR to quantify the infection levels, classifying them as high, medium, or low based on absolute copies using genomic DNA from the segment of mid-intestine. Furthermore, we established two indices using absolute copies and spores per cell. We performed SNP genotyping using the Affymetrix® Axiom® myDesign™ Array, leading to the discovery of 627 samples and 59,509 high-quality SNPs.

According to the GWAS conducted by the Gaston and ASReml R packages, several significant SNPs were mapped to chromosome 7. Functional annotations revealed that genes encoding nucleobindin-2-like and SET binding factor 2 were associated with regulating food intake and energy homeostasis, as well as cellular communication and the signaling network.

The heritability was estimated for two indices (absolute copies and spores per cell) range from 0.228 to 0.435. Additionally, we utilized a cross-validation approach to carry out genomic prediction employing various models, with the goal of estimating the prediction accuracy. Results revealed that Random forest(RF) (0.490~0.521) has higher genomic prediction accuracy than in GBLUP (0.472~0.479).

Collectively, this study has the potential to increase the availability of *E.leei* resistant broodstocks, thereby reducing parasitic infection rates in South Korea aquaculture industry.
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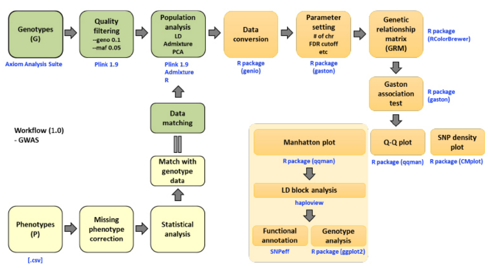

Figure 1. GWAS workflow for *E.leei* resistance trait

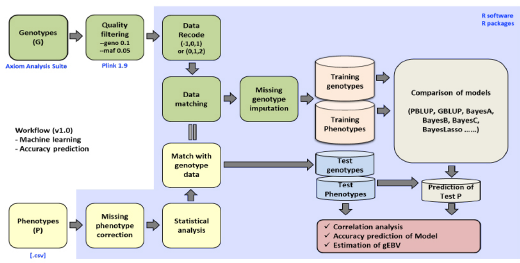

Figure 2. Machine learning & Accuracy prediction workflow for *E.leei* resistance trait

GENOME-WIDE ASSOCIATION AND GENOMIC PREDICTION FOR RESISTANCE TO *Edwardsiella piscicida* IN A SOUTH KOREAN OLIVE FLOUNDER POPULATION

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Olive flounder (*Paralichthys olivaceus*) is one of the major mariculture fish species in the aquaculture industry of South Korea. In recent years, flounder farms have been facing huge economic losses due to several diseases. In particular, *E. piscicida* is an important pathogen that causes high mortality in a variety of farmed fish, and flounder is also susceptible to it. Currently, management programs such as vaccination and improvement of aquaculture facilities are being implemented in Korea, however, genomic breeding programs to develop *E. piscicida*-resistant flounder have not been conducted.

In this study, we performed a genome-wide association study (GWAS) and prediction model construction to identify significant SNPs and prediction accuracy associated with *E. piscicida* resistance in flounder. 810 healthy flounders were subjected to an *E. piscicida* challenge experiment, and genomic DNA was extracted from excised fin tissue and utilized for high-density SNP chip analysis. After quality control, a total of 49,363 SNP markers from 577 flounders remained from an initial set of 57,754 markers. Through GWAS, we identified at least one significant SNP from each defined trait: day post-challenge (DPC_date), and stage of mortality (STAGE). The heritability of flounder resistance against *E. piscicida* was around 0.13-0.20. Among the prediction methods tested, GBLUP and BayesB achieved the highest prediction accuracy using a 3-fold cross-validation approach. Collectively, we suggested that these findings provide an approach to genomic breeding programs in flounder for the resistance to *E. piscicida*.

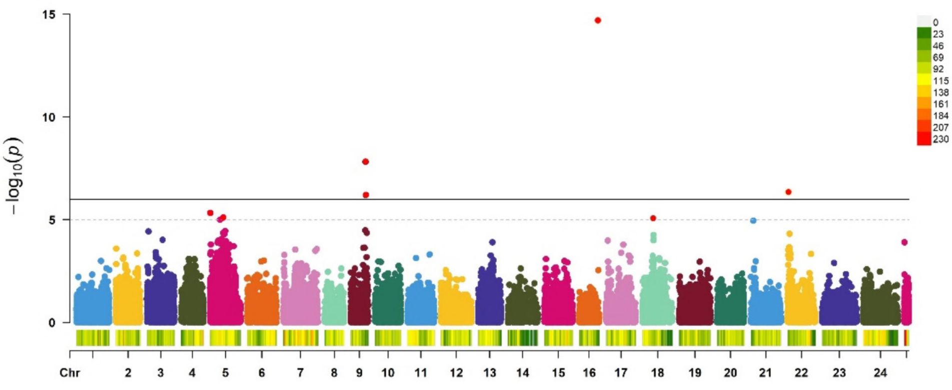

Figure 1. Manhattan plot for DPC_date of olive flounder from genome-wide association analysis.

NITROGEN DYNAMICS IN CULTURED OLIVE FLOUNDER, *Paralichthys olivaceus*, USING DIVERSE DEVELOPED ADSORBENTS, INCLUDING DEVELOPED CHITOSAN-HYDROGEL AND ALGINATE-COATED ZEOLITE

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Total ammonia nitrogen (TAN) and its oxidized forms, nitrite and nitrate, pose severe threats to fish growth. This study investigates the efficacy of adsorption technologies for removing nitrogen contaminants, comparing them with a conventional method, the moving bed biofilm reactor (MBBR). Commercial zeolite proved ineffective in seawater due to high salinity. However, crosslinked hydrogels such as chitosan-based hydrogel (Cht-HG) and chitosan-based hydrogel modified with p-hydroxybenzoic acid (M-Cht-HG) demonstrated notable efficacy in controlling nitrite concentrations. Notably, M-Cht-HG achieved a 383% reduction in TAN concentration within 24 h. These hydrogels were also applied as coatings on commercial zeolite particles, resulting in an alginate-hydrogel-metal-coated zeolite (Al-H-M-Z), which outperformed the conventional MBBR in TAN and nitrite removal under seawater conditions. Specifically, Al-H-M-Z exhibited 2.14- and 1.11-fold greater removal efficiencies for TAN and nitrite, respectively, than MBBR. Regarding activation rates, MBBR required 18 h to prepare for TAN and nitrite removal, whereas Al-H-M-Z required only 12 h to remove nitrogenous byproducts through adsorption. Furthermore, Al-H-M-Z exhibited an advantage for long-term application potential, maintaining concentrations below 0.1 mg L^{-1} for TAN and nitrite over 7 d.

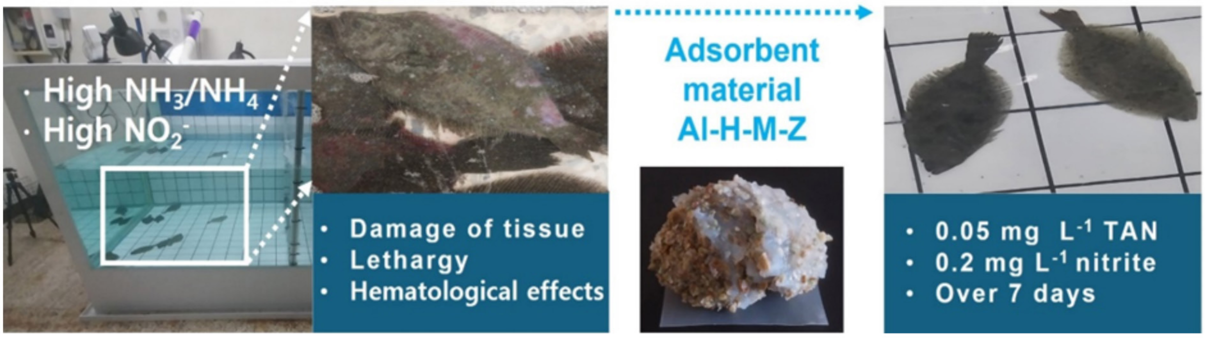

FIG. 1. Alginate-hydrogel-metal-coated zeolite (Al-H-M-Z) outperformed in olive flounder aquaculture

PRODUCING VIABLE LARVAE OF AFRICAN CATFISH *Clarias gariepinus* FROM IN VITRO MATURED AND OVULATED OOCYTES

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In vitro maturation (IVM) of oocytes is a reproductive technology that enables mature eggs to be produced ex vivo, without the need for prior hormonal treatment of females. This approach is becoming a useful tool in aquaculture, helping further understanding of oogenesis, refining spawning practices, and providing alternative methods of egg production.

In this study, we describe an IVM protocol for African catfish ovarian follicles, which results in fully fertilizable and developmentally competent eggs. The effects of 17α,20β-dihydroxy-4-pregnen-3-one (DHP) and human chorionic gonadotropin (hCG) were evaluated based on their ability to promote ooplasm translucency and germinal vesicle breakdown (GVBD. Among the tested groups, only follicles exposed to DHP underwent GVBD, with the highest rates (>80%) observed within 10 to 12 hours of incubation. Furthermore, adding hCG before or during incubations with DHP did not influence the GVBD outcome. Although high concentrations of DHP (1 µg/ml) were alone sufficient to induce complete nuclear and cytoplasmic maturation, only 4 ± 3% of these oocytes ovulated in culture. To promote ovulation, we incorporated prostaglandins (PGs) as an additional step following DHP. Exposure to both $PGF_{2\alpha}$ and PGE_2 led to increased rupture of the follicular layer, most notably in groups with 5 µg/ml $PGF_{2\alpha}$ (71 ± 8%). Furthermore, the effects of culture media pH and supplementation—serum albumin (BSA) and fetal bovine serum (FBS)—were considered. Compared to results in media with pH of 7.5, significantly fewer follicles matured and ovulated in a more alkaline environment (pH 8.5). *In vitro* matured and ovulated oocytes obtained in non-supplemented media (pH 7.5) maintained their developmental competence and were successfully fertilized. The hatching rate was 39%, after which the survival rate of larvae was 8% at 72 hours post fertilization (hpf).

Funding: National Research, Development and Innovation Office of Hungary (NKIFH_K138425)

LACK OF VGLL3A DELAYS ONSET OF MALE MATURATION IN ATLANTIC SALMON

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Early male maturation is one of the major problems in farming of Atlantic salmon (*Salmo salar*), as premature puberty in males is associated with increased disease susceptibility and osmoregulatory problems, leading to higher mortalities, production losses and reduced animal welfare. Previous studies have identified a major effect locus for age at maturation in the vgll3 gene on chromosome 25, with alleles responsible for early and late maturation. However, it is not clear how vgll3a controls the onset of puberty in salmon. We therefore decided to generate a vgll3a loss-of-function mutant using CRISPR/Cas9 to study the role of vgll3 in onset of male maturation in salmon in vivo.

We have followed the F1 generation of vgll3 mutants until the fish were 2 years old. Males were subjected to two different environmental conditions known to stimulate the onset of puberty in salmon: i) 16 degrees, constant light, freshwater, for 6 weeks about one year post hatching, or ii) 13 degrees and constant light in freshwater from start feeding until 2 years post hatching. Individual growth was recorded every second month after start feeding and the maturation status was determined by ultrasound, steroid hormone concentrations and dissection.

Our results showed that wild type and heterozygous males matured as expected (61-100% maturation), while the knockout males showed significantly lower maturation rates (below 30%).

Atlantic salmon males lacking vgll3a show a delayed entry into maturation. Further studies will reveal if the use of fish lacking vgll3a could be a potential solution to reducing the problem with early male maturation in Atlantic salmon farming.

INNOAQUA – SUSTAINABLE AQUACULTURE PRACTICES FOR INNOVATIVE SEAFOOD PRODUCTS: SALMON AND MICROALGAE LAND-BASED IMTA

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The Farm-to-Fork Strategy of the European Green Deal acknowledges the potential of algae to become an important source of alternative low-carbon footprint protein and contribute to improving the sustainability and competitiveness of the aquaculture sector. Nonetheless, the European algae industry is still in an early phase lagging behind the overall increase seen at a global level, mostly driven by Asia. Within this context, the EU project INNOAQUA aims to pave the path towards the upcoming sustainable and diversified EU in-land aquaculture industry by demonstrating and mainstreaming innovative algae-based foods and solutions, based on sustainability, circularity, and digitalization concepts. The INNOAQUA project is centred around the demonstration of the integration of fish and algae cultivation in coupled, land-based RAS/IMTA (Recirculating Aquaculture Systems/Integrated MultiTrophic Aquaculture) systems, aiming to minimize energy and nutrient losses and maximise resource efficiency by closing the nutrient loop, at two locations: 1) microalgae and salmon in Norway, and 2) seaweed and sole in Portugal. Next, new biorefining approaches are developed to optimise the extraction yield of targeted valuable compounds from the microalgae, seaweed and fish processing side-products that will be used in the formulation of innovative seafood products.

Earlier results, obtained in the projects iFishIENCi (EU 818036) and SLAM-DUNK (RCN 326861), showed that microalgae can successfully be grown on effluent water from the production of various fish in RAS using both biofilm and tubular photobioreactors (Fig. 1).

Full nutrient uptake can be achieved as well as high microalgae productivities, by altering the process design and conditions. We have demonstrated this for both freshwater and marine species (fish and microalgae). In these aforementioned projects, we received RAS effluent water to grow the microalgae in our labs. In INNOAQUA, we have brought the microalgae photobioreactor to the fish production site and integrated both production systems. We are currently working on further optimising the results, through operational and technological enhancements, including tailor-made digital solutions.

Total project eligible costs for INNOAQUA are 7.3 million euros, 6 million of this is funded by the European Union under grant agreement number 101084383.

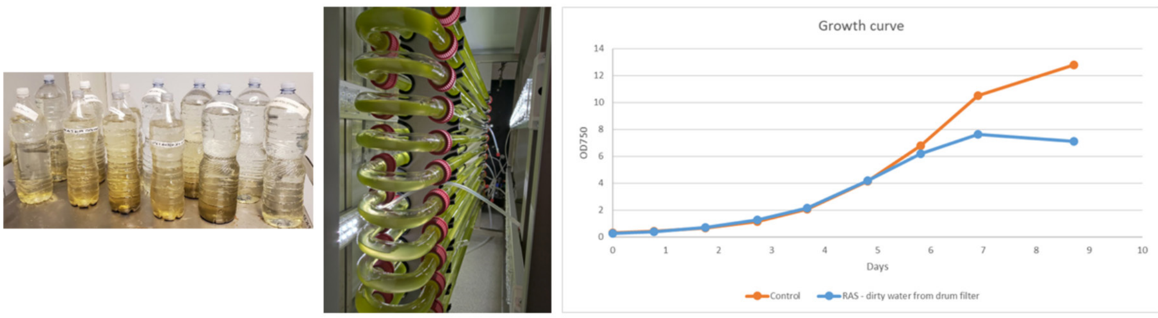

Figure 1: From left to right: RAS effluent water collected from drum filter from Barramundi produced in seawater. *Microchloropsis gaditana* grown on RAS effluent water. Microalgae growth curves based on optical density (OD750) of *M. gaditana* on control medium (orange) and RAS effluent water (blue).

GENOME-WIDE ASSOCIATION MAPPING OF HOST GENETIC POLYMORPHISMS ON VACCINE-INDUCED SCUTICOCILIATOSIS DISEASE RESISTANCE IN OLIVE FLOUNDER *Paralichthys olivaceus*

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Scuticociliatosis, a catastrophic parasitic disease instigated by the scuticociliate, *Miamiensis avidus*, in olive flounder aquaculture leads to significant morbidity and mortality. Vaccination stands as a pivotal and reliable strategy in disease management, supplanting conventional therapeutic methods fraught with limitations. Concurrently, efforts in selective breeding to bolster disease resistance in olive flounder stocks are ongoing, contingent upon challenge tests with unvaccinated cohorts. However, the genetic predisposition to the infection may be influenced by the vaccination status, prompting an exploration into the genetic diversity underlying resistance. Our study pioneers this investigation, scrutinizing the genetic basis of resistance to scuticociliatosis post-vaccination in olive flounder.

A cohort of 474 fish from 141 full-sib families underwent formalin-killed vaccination followed by an intraperitoneal challenge with *M. avidus*. Genotyping of 474 fish was performed using a custom-made high-density 70K single nucleotide polymorphism (SNP) array designed for olive flounder. Substantial genetic variation in resistance to scuticociliatosis post-vaccination was observed, with an estimated heritability of around 0.10. Genome-wide Association (GWAS) analysis revealed sixteen significantly associated SNP variants across chromosomes 1, 7, 11, 12, and 13, explaining a considerable phenotypic variance. Sixteen candidate genes linked to scuticociliatosis resistance post-vaccination were discerned and enriched with gene ontology terms concerning cell migration, morphogenesis, extracellular and cell membrane constituents, cell adhesion, and innate immune response.

Our findings provide critical insights into the genetic mechanisms underpinning vaccine-induced resistance to scuticociliatosis, with implications for enhancing selective breeding initiatives in olive flounder.

DESIGN OF AN IMPLANTABLE BIOSENSOR FOR REAL-TIME *IN VIVO* MEASUREMENTS IN AQUATIC ORGANISMS

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We present a design of a chip that could be integrated on an *in vivo* biosensor for real-time monitoring of biomarkers in the blood of aquatic animals, specifically fish and shellfish. Real-time measurements of biomarkers in the bloodstream would offer a great improvement in assessing physiological functioning.

The design focusses on the cytokine Interleukin-6 (IL-6), which serves as a relevant parameter to assess the immunological status in fish and shellfish. But, besides its role as cytokine in disease prevention, IL-6 is also expressed in muscle tissue and functions as an energy allocator, making it specifically interesting for the purpose of modelling the energy economy of individual organisms (e.g., as a digital twin). In literature, reported concentrations of IL-6 in fish blood are in the range of 2-78 pg mL^{-1}. Circulatory IL-6 concentrations increase manyfold when the immune system is triggered and can reach levels over 200 pg mL^{-1} as we measured in LPS-challenged Atlantic salmon.

The University of Twente, department of Integrated Optical Systems, has developed a label-free sensor chip that is able to sense concentrations of humane IL-6 (Bio-legend 570802) down to 300 pM (or 6.0 ng mL^{-1}). For its applicability to detect IL-6 in fish blood, its sensitivity needs to be enhanced. Furthermore, the required tunable laser source is not implementable on a chip. Our proposed solution utilizes a Mach-Zehnder Interferometer (MZI) with coherent phase read out instead of a microring resonator (MRR). A MZI allows for a fixed-wavelength, enabling the implementation of all components on a chip and reducing costs, while having similar or even smaller detection limits. Furthermore, we propose to develop a functionalisable antifouling layer, based on diblock polymer brushes, building on earlier work from the Laboratory of Organic Chemistry (Wageningen University & Research). These polymer brushes can be directly grown from the sensor surface (Al_2O_3), eliminating the SiO_2 buffer layer. This reduces the waveguide losses and makes the system more sensitive.

The current design targets IL-6 but the chip can easily be adapted to measure other biomarkers, provided there exists a suitable receptor and adequate concentrations, such as cortisol. The chip could be integrated on a sensor. Such an implantable sensor, to continuously monitor biomarkers in aquatic organisms, would provide insights into the immunological status, energy metabolism and health and welfare of aquatic organisms in general and therefore may become a valuable tool for researchers and practitioners in aquaculture and in the field.

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INCLUSION OF GREENHOUSE GAS AND NUTRIENT EMISSIONS FROM TROPICAL AQUACULTURE PONDS IN THE ENVIRONMENTAL FOOTPRINT OF AQUACULTURE

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Tropical pond-based aquaculture fulfils an essential role in the global food and food system as the main production system for popular species such as carps, catfish and shrimp. Aquaculture products are recognized as a relatively low impact animal protein source. Various actors in the seafood industry are embracing the adoption of LCA. However due to a lack of standardization and guidelines there is a risk of divergent LCA's with differing results potentially leading to confusion for consumers.

The interest of the industry has led to several studies as well as the development of LCA tools for aquaculture production. As standardization and guidance from the PEFCR and FAO LEAP are lacking this has led to development of the underlying methods through engagement with industry, NGO's, academia, and government organizations. One of the main issues has been the direct and indirect greenhouse gas (GHG) emissions from tropical aquaculture ponds, the main GHGs from tropical aquaculture ponds are Nitrous oxide and methane. The inclusion of these GHG emissions is necessary to be included for consistency and completeness as the GHG emissions associated with terrestrial livestock products is commonly included. In this presentation we present the current state of the emission modelling approach and identify the primary gaps for further research.

Because of the complex interactions of feed, excretion and the food web in aquaculture ponds it is not feasible to use a fate-based model based on the digestion of the animal as proposed in the first version for public consultation of the PEFCR for marine fish when dealing with tropical pond systems. Therefore, a mass balance approach was applied for emissions of nutrients to water.

Direct N2O emissions within the aquaculture pomds were based on the volatilization of surplus nitrogen in the water as by-products of nitrification and denitrification processes within the pond, using the conversion rate of surplus N to N2O: 0.71%. For indirect emissions of N2O due to nitrogen released as dissolved inorganics or particulate matter into the environment a conversion factor of 1% was used. Methane emissions could have a significant contribution to the total footprint of tropical aquaculture and have been been included based on the IPCC wetlands guidance.

The current state of the emission methodology provides an indication that the contribution of the direct GHG emissions lies in the order of 10-30% of the total footprint of tropical aquaculture production. The current methodology can capture the effects of basic farm management practices such as feed protein content, FCR and productivity allowing producers to improve their footprint. However, many management practices that could influence the GHG emissions cannot be reflected in this emission model. Further research is needed to provide insight into all the factors that influence GHG emissions. This requires more direct measurements of the flows of GHGs and the relation of these emissions to pond management.

DEVELOPMENT OF LCA TOOLS FOR AQUACULTURE PRODUCTION SYSTEMS

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Life Cycle Assessment (LCA) is essential in the assessment of aquaculture production systems offering a comprehensive framework to evaluate the environmental impact. While LCA is a powerful method to assess the environmental footprint of aquaculture production systems it requires a high degree of specialized knowledge of LCA, its standards and guidelines. LCA software typically provides very open canvasses that allow LCA practitioners to model any system in any way that is applicable to their study. This is useful for LCA practitioners however, for industry stakeholders this freedom is impractical and without detailed knowledge of LCA leaves too many options for the user leading to inconsistencies. By providing tools to the aquaculture production sectors non-experts can be supported empowering them to take full advantage of LCA.

To support this Blonk Sustainability has worked with various stakeholders to develop tools for LCA assessments of aquaculture production systems for industry stakeholders. These tools cover various aquaculture production systems from tropical species such as Shrimp and Tilapia and cold-water species such as Atlantic salmon from feed to processing and distribution.

The robustness and consequently the applicability of LCA results that are generated through these tools are strongly dependent on various factors. We identify three main pillars: consistency, data quality, and completeness. By employing standardized tools consistency in underlying methods can be guaranteed. These methods include emission modelling, background process selection, and inclusion of relevant processes. This is especially relevant for sectors where standardization is not present. In these cases, the tool allows experts to take decisions ensuring well substantiated and documented methods which can be used consistently by industry stakeholders. The downside of these pre-defined systems is that when the use case does not connect to the parameters in the tools this use case cannot be performed with the tool. The second pillar, data quality is guaranteed in various ways. The primary data requirements are pre-defined and where required default data of sufficient quality is pre-defined. This simultaneously ensures the third pillar of completeness, as the tools enforce that data is complete before generating a result. A challenge here remains the fact that the data entered by the user remains an unknown in terms of quality.

The development of LCA tools for industry stakeholders is performed using a rigorous method. This rigor is necessary to ensure consistency, quality, and completeness. The tools developed for aquaculture production system empower stakeholders to measure and reduce their environmental impact without intervention of LCA experts. Enabling these businesses to change into more sustainable business based on solid LCA methods.

SUSTAINABLE AQUAFEED INGREDIENTS? THE DEVIL IS IN THE DETAIL.

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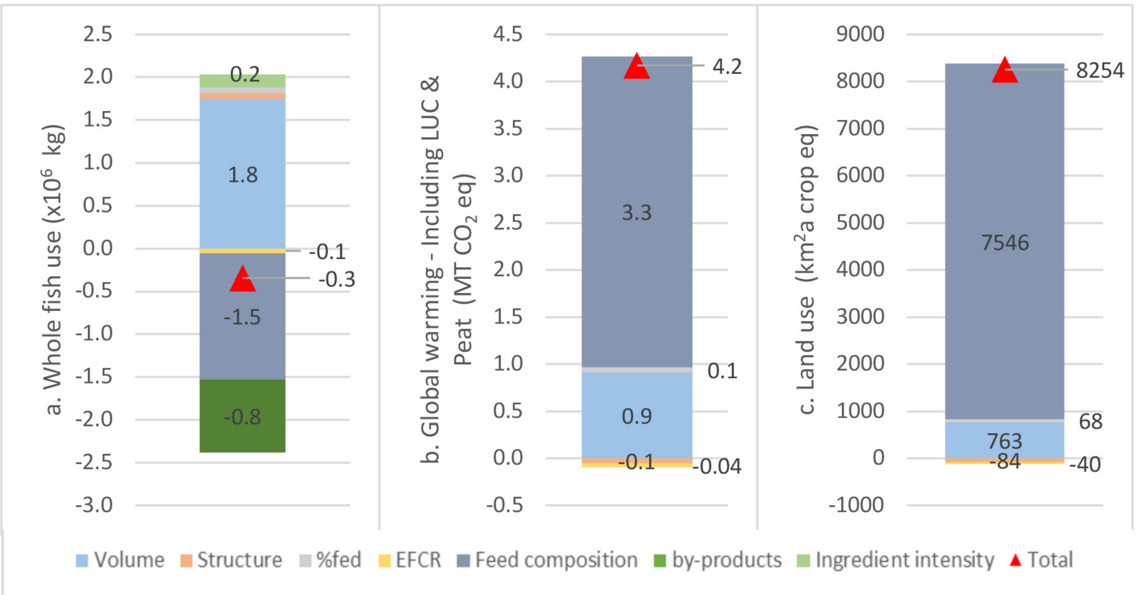
Aquaculture is essential to meet the increasing demand for nutritious seafood. The European (European Economic Area (EEA) + United Kingdom (UK)) aquaculture industry is highly dependent on aquafeed input, which represents the majority of the environmental impact and production cost. Aquafeed formulations consist mostly of a combination of marine and plant-based ingredients. Driven by economic and sustainability incentives, there has been a shift from marine ingredients, towards plant-based ingredients, and smaller inclusions of (fish) by-products and novel feed ingredients.

We applied an Index Decomposition Analysis (IDA) IDA to assess the changing environmental impact of European aquaculture from 2000 to 2020. Five finfish species, Atlantic salmon, rainbow trout, gilthead seabream, European seabass and common carp included as these species represent most of the European aquaculture production.

We find a substantial increase in global warming potential (320%), land use (595%), water consumption (256%), marine eutrophication (624%) and freshwater eutrophication (436%), while fish use was reduced by only 13%. Most of the increase in global warming potential, land use water consumption, marine and freshwater eutrophication was the result of marine ingredient substitution through changing feed composition. The reduction in fish use was mostly attributed to the substitution of, and improved by-product utilization for marine ingredients.

The substitution of marine ingredients with plant-based ingredients to date has not been an environmentally sustainable transition. Although it has reduced marine resources overall, the pressure has disproportionally shifted towards terrestrial systems. A shift mostly attributable to the use of two terrestrial plant based ingredients, soy protein concentrate and rapeseed oil as a replacement of fishmeal and fish oil.



THE IMPACT OF MINERAL SUPPLEMENTATION LEVEL AND SOURCE ON ATLANTIC SALMON PERFORMANCE, WELFARE, AND QUALITY IN COMMERCIAL PRODUCTION ALONG THE NORWEGIAN COASTLINE

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Dietary microminerals like iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and selenium (Se) are essential nutrients playing critical roles in multiple physiological processes in fish (Baeverfjord et al., 2019; Prabhu, Schrama and Kaushik, 2016). Balancing these minerals in aquafeeds is vital for optimal growth, health, and immune function, particularly in challenging commercial fish farming conditions (Sommerset et al., 2023). As EU legislation imposes strict limits on mineral supplementation, knowledge of mineral availability from various sources is vital. In commercial salmon feeds, inorganic minerals are commonly used, yet their bioavailability and physiological impacts vary significantly (Maage and Sveier, 1998; Standal et al., 1999). Studies across different fish species highlight the impact of variable mineral supplementation levels and sources (Buyinza et al., 2023; Kokkali et al., 2023; Nguyen et al., 2019; Xu et al., 2021) but most of these studies are performed under controlled conditions. Our study sought to investigate the impact of essential micromineral supplementation levels and source in commercial production settings along the Norwegian coastline, considering regional variations and challenges while assessing the mineral needs of farmed Atlantic salmon.

The study utilized four research farming licenses from the project "EINVU: Nutritional innovations – key to the big welfare challenges," across three regions along the Norwegian coastline. Atlantic salmon from approx. 500g body weight at the trial start to slaughter (approx. 4.5 kg), reared under commercial conditions (including e.g., lice and other therapeutic treatments), were fed 4 different diets containing either organic or inorganic minerals at two premix levels (Se: 0.7ppm, Cu: 22ppm, Mn: 69-81ppm, Zn: 166-199ppm and Fe: 248-318ppm). There were 2 replicate cages per treatment and trials were repeated in 3 different locations (North, Mid and West) and 2 stocking seasons (spring and autumn). General performance, whole body and tissue (filet, skin, liver, gills, milt, and kidney) mineralisation, biometrics, fish welfare and skin histology were studied.

Our study revealed a significant correlation between mineral level and fish performance parameters, final product quality, tissue mineralization, and skin histology. Decreasing dietary Fe levels by 22% and Mn levels by 15% showed no reduction in whole-body Fe or Mn levels in fish. Moreover, this reduction led to increased uptake of other dietary minerals, evidenced by elevated whole-body Cu and Mn, and fillet Cu and Fe, linked also with reduced fillet gaping. Moreover, supplementing Se and Zn at levels higher than legal limits boosted tissue concentrations without reaching saturation. Organic minerals were found to positively correlate with tissue mineralization in whole-body, fillet, and head kidney, with higher Cu levels in fillet linked to decreased fillet gaping. Additionally, the use of organic minerals positively impacted salmon's fin score and slaughter yield. Finally, salmon survival, growth, welfare, and fillet quality, as well as tissue mineralization, were significantly influenced by the rearing location; emphasizing the importance of tailored mineral supplementation to meet their specific needs and environmental stressors, ultimately optimizing health and performance.

TRANSCRIPTOMIC STUDIES OF RAINBOW TROUT STRAINS WITH DIFFERENT SUSCEPTIBILITY TO VIRAL HEMORRHAGIC SEPTICEMIA VIRUS PROVIDE INSIGHT INTO IMMUNOLOGICAL PATHWAYS LINKED TO RESISTANCE

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Viral haemorrhagic septicaemia virus (VHSV) is a highly contagious pathogen affecting salmonid fish populations. Recent results suggest that host genetics play a key role in the susceptibility to the virus, where different in innate immune responses underlie VHSV resistance in rainbow trout strains. The aim of the present study was to investigate gene expression signatures in a VHSV resistant local German strain (R7) of rainbow trout under VHSV-infection and compare them to expression signatures in a highly susceptible commercial strain (R9).

For this, tissues from gut, fins, gills, spleen and kidney were collected from n=3 susceptible and resistant adult rainbow trout (n=3) at the second and fourth day post infection with VHSV. An RNA-seq was performed from kidney samples at 2dpi. In addition, the gene expression of 27 selected genes was determined in all collected samples by RT-qPCR. The results were compared to expression of the genes in primary cell cultures from fins, scales and kidney of fish from the same strains and infected with native and inactivated virus.

The investigations revealed that especially the genes involved in the interferon response of rainbow trout show clear differences in the level of gene expression between the two origins. Especially on the second day after infection with VHSV, a large number of genes, particularly in the spleen and kidneys, differed significantly from each other ($p < 0.05$). Particularly striking were the gene expressions of pro-inflammatory cytokines as well as type I and II interferons. While gene expression increased steadily in tissues from the resistant origin over the observation period, there was a rapid increase (day 2) followed by a decrease in gene expression (day 4) in samples from the susceptible origin. The *in vitro* experiments confirmed *in vivo* results, that in cells derived from the R9 strain, the VHSV induces a stronger immune response.

Our results could not confirm that an increased antiviral response is the main factor behind higher resistance. Furthermore, it can be concluded that trout from the susceptible R9 origin died because of a dysregulated inflammatory response and a cytokine storm.

Acknowledgements:
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CONTRACTING SALMONID HEART CELL CULTURES AS A TOOL FOR MEASURING THE EFFECTS OF ENVIRONMENTAL POLLUTANTS *IN VITRO*

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Cardiovascular disorders are a growing threat to the survival of salmonids in their natural environment. Alongside pathogens and stressors, acute or chronic toxic exposure to oil spills and nano- and micrometre-sized plastics poses a significant risk to aquatic life. Currently, there are limited studies on cell cultures that can be used to assess exposure to environmental stressors. The objective of this study was to examine the impact of environmental stressors, specifically varying concentrations of crude oil and nanoplastic particles, on heart cells. The toxicity was assessed both *in vivo,* using larvae of Atlantic salmon, brown trout, and rainbow trout, and *in vitro*, using heart cell cultures at different developmental stages.

A novel method was used to cultivate cardiac primary cultures of salmonids, which contracted permanently after two to three weeks. The cultures were subsequently treated with either plastics or crude oil. The number of heartbeats in salmonid larvae and contractions in the cultures was recorded, and samples for gene expression analysis were taken. The cultures treated with nanoplastics or crude oil were sampled immediately after exposure and on days 1, 3, 5, and 7 post-exposure. Atlantic salmon samples were used to measure 10 genes involved in cytokine signalling as well as oxidative stress and pro-inflammatory responses by RT-qPCR.

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 a change in the number of contractions after exposure. On gene expression level we could not detect any signs of oxidative stress or inflammation in the heart or in heart cell cultures after the exposure.

Contracting salmonid heart cell cultures can be a valuable tool for monitoring host-environment interactions involving cardiac cells of fish species used in aquaculture.

Acknowledgements:
This project was funded by the Federal Ministry of Education and Research in Germany.

DEVELOPMENT AND APPLICATION OF A FAST AND SENSITIVE AMPLICON-BASED METHOD FOR WHOLE GENOME SEQUENCING AND CHARACTERISATION OF ISAV-HPR0 FROM FIELD SAMPLES IN FARMED ATLANTIC SALMON

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The virulent Infectious Salmon Anaemia Virus (ISAV-HPRΔ) is an Orthomyxovirus that causes Infectious Salmon Anaemia (ISA), an important disease of farmed Atlantic salmon (*Salmon salar*). It caused a major epidemic in the Faroe Islands in the early 2000. The nonvirulent ISAV-HPR0 variant causes a transient epithelial infection localised to the gills and skin and has been identified as the progenitor of all virulent ISAV-HPRΔ variants. ISAV-HPR0 has been shown to circulate in freshwater RAS smolt farms and marine farms and is potentially introduced from the environment, thus highlighting the paramount importance of biosecurity in smolt farms. A previous study based on the partial sequencing of ISAV-HPR0 segment 6 has suggested that ISAV-HPR0, once introduced, can persist over time in the smolt farms. Mounting evidence also suggests that ISAV-HPR0 mutation in these farms can cause disease outbreaks when introduced in marine sites. To date, there has not been a successful cultivation of ISAV-HPR0. Our study utilises an in-house developed, fast, and efficient ISAV whole genome sequencing method to understand the transmission and evolutionary dynamics of ISAV-HPR0 between land-based smolt farms and marine sites in the Faroe Islands.

Approximately 100 HPR0 positive samples collected over time (2007 to 2024) and space (8 smolt and 23 marine farms) were subjected to whole genome sequencing. Twenty-five samples were sequenced on MiSeq, and all other samples will be sequenced on the Nanopore GridION. In addition, HPR0-positive aerosol samples collected from certain smolt stations close to the sea will be included.

The Illumina MiSeq sequencing yielded high-quality complete genome sequences from all the field samples. Preliminary phylogenetic analysis of the complete genomes shows that identical ISAV-HPR0 variants persist in the smolt farms as “house strain” over several years. We also show the recurrence of an old HPR0 variant in a smolt farm after total disinfection. Preliminary analysis also suggests the introduction of ISAV-HRP0 into the smolt farms from the external environment. The comprehensive whole genome data is expected to provide insights into the transmission dynamics and genetic variability of ISAV-HPR0, including how naïve RAS smolt farms, which are Closed Containment Systems (CCS) without prior detection, are increasingly testing positive.

This study was funded by the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 956481.

ENHANCING SUSTAINABILITY AND EFFICIENCY IN AQUACULTURE THROUGH KROMA A/S SILAGEMASTER SYSTEM.

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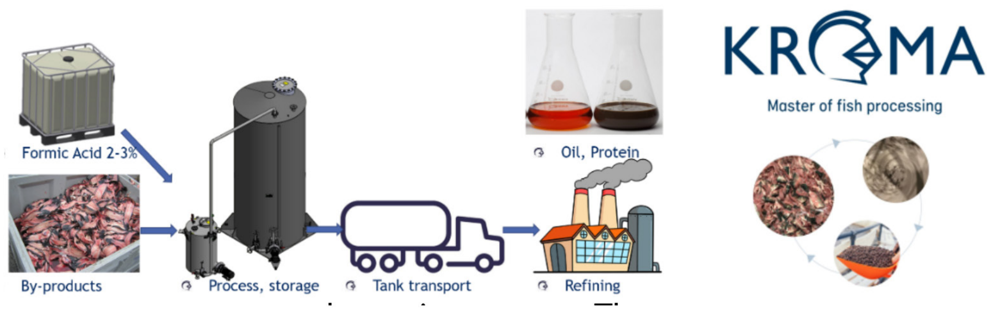
Aquaculture faces complex challenges that require a global perspective for comprehensive solutions. One major issue is the management of dead fish and fish waste, which, if mishandled, can lead to logistical problems and severely increase environmental concerns such as rapid bacteria growth. Another challenge lies in ensuring convenient access to the system for monitoring purposes. To address these challenges, a holistic approach is required.

Kroma A/S offers a pioneering solution by efficiently collecting waste from fish processing plants, also automating its transport into the advanced SILAGEMASTER system, as well as to monitor and control the system itself. This innovative technology, capable of processing dead fish, significantly reduces manual labor and waste disposal time during post-production cleaning.

The strategically placed SILAGEMASTER system, both inside and outside fish processing plants, offers flexibility in integration. With a user-friendly touch screen panel on the system or through the Monitor access Control service on the customers' mobile devices, a single operator can oversee the entire process. The system transforms fish waste and dead fish into valuable silage products, creating an additional revenue stream for aquaculture facilities.

Efficient handling of by-products is essential for sustainable resource management, minimizing the environmental footprint associated with waste. Silage processing stabilizes by-products for efficient storage and adds economic value. Moreover, the Monitor Control service ensures easy access to adjust the innovative system from anywhere in the world.

Kroma A/S SILAGEMASTER system empowers individual fish processing plants with a comprehensive waste management strategy, promoting a zero-waste policy. This innovative solution not only enhances sustainability but also turns waste into a profitable initiative, creating a harmonious balance between primary product processing and by-product utilization. Additionally, the Monitor Access Control service ensures seamless adjustment and oversight of the entire system.



APPLYING AN ECOSYSTEM-BASED-MANAGEMENT FRAMEWORK TO THE MANAGEMENT OF HUNGARIAN CARP FISHPOND SYSTEMS: AN UPDATE ON DECISION SUPPORT USING ECOPATH & ECOLOGICAL NETWORK ANALYSIS

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Amidst an increasing emphasis on environmental sustainability in agricultural policy, there is a growing recognition of the need for aquaculture to contribute positively or neutrally to ecosystem services and biodiversity. This includes support for low-intensity aquaculture methods such as traditional carp pond farming. Additionally, there is a focus on promoting polyculture techniques, which are purported to enhance biodiversity and ecological resilience. While production and economic outputs are easily quantifiable, monitoring and assessing ecosystem health in terms of biodiversity and functioning pose significant challenges. The objective of this study is to develop a decision support framework (DSF) for assessing the environmental performance of carp ponds in Hungary, using an ecosystem-based management approach.

Data from previously conducted biodiversity and environmental surveys in experimental carp ponds of varying management practices were used to parametrise Ecopath models. Models were created for 6 different management scenarios based on food webs of sampled ponds. Scenarios differed by the intensity of production (low/standard/high; based on initial stocking of *Cyprinus carpio*) and the inclusion of common polyculture species, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* (monoculture/polyculture). Ecological network analyses (ENA) were performed on each model, resulting in a set of 5 ecosystem function indices, chosen for their relevance to pond systems: Robustness index (R), Connectance index (CI), Detritivory/Herbivory index (D/H), Finn's Cycling Index (FCI), and Common Carp Yield.

Results from the ENAs showed little difference between standard and high intensity management scenarios. However, low-intensity management scenarios showed lower levels of D/H, CI and FCI, resulting in ecosystems with short trophic chains that are reliant on new production rather than the cycling of biomass within the pond. Polyculture scenarios resulted in higher D/H than monoculture scenarios, indicating that primary production is insufficient to support more herbivores. In the case of a low-intensity polyculture scenario, R decreased, as greater herbivory in the system led to a depletion of both detritus and trophically-important primary producer species.

This has important implications for the management of pond systems, especially with regard to the policy goals of EU aquaculture. In this case, standard management scenarios resulted in better ecosystem health indicators than low-intensity and polyculture scenarios. The results confirm that assessments must take a whole-ecosystem approach (including socio-ecological interactions like management) to ensure sustainability of aquaculture. Although there is necessity to test on a larger scale, this approach remains applicable to aquaculture sustainability policy and regulatory needs.

EFFECTS OF *PmSTAT* AND *PmSOCS2* KNOCKDOWN ON WHITE SPOT SYNDROME VIRUS INFECTION IN *Penaeus monodon*

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White spot syndrome virus (WSSV) is a lethal pathogen that causes 100% mortality within 3-7 days after infection. Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway play a role during WSSV infection. Previous work has shown that *PmDOME* and *PmSTAT* silencing in black tiger shrimp (*Penaeus monodon*) reduced WSSV copy numbers and delayed the cumulative mortality. In this work, the next-generation sequencing (NGS) was performed using STAT-silenced shrimp, WSSV-infected STAT-silenced shrimp and WSSV-infected shrimp. Transcriptome data showed that a total of differential expression genes (DEGs) of WSSV infection was 675, while 138 up-regulated genes were from STAT-silencing + WSSV. Ten genes were randomly selected and their expression levels were verified by qRT-PCR. In addition, the roles of *Pm*SOCS2, a JAK/STAT inhibitor, were also investigated. Silencing of *PmSOCS2* significantly affected the expression of *ProPO2*, *ALFPm3*, *Penaeidin 3*, *CrustinPm1* and *CrustinPm7*. This work emphasizes the importance of the JAK/STAT signaling pathway during WSSV infection.

TECHNOLOGICAL APPROACHES TO GROW-OUT: COMPARATIVE STUDY OF PIKEPERCH (*Sander lucioperca*) CULTURE IN THREE DIFFERENT REARING SYSTEMS DURING VEGETATIONAL SEASON

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Introduction

European inland aquaculture needs to diversify production (Baekelandt et al., 2018). Pikeperch (*Sander lucioperca*) was chosen due to its high value, fast growth, and flesh quality as a prime candidate and embedded into freshwater inland aquaculture. Thanks to its qualities production of pikeperch in aquaculture is constantly increasing. In case of west Europe such as Belgium. Netherlands and France, the missing pond network is forcing the farmers to produce pikeperch in closed recirculating aquaculture systems (RAS). In Central and East Europe countries overall share of aquaculture production up to 80-88% (Vavrečka et al., 2019) originates from ponds. The existing pond infrastructure in the combination with new technological equipment offers the opportunity to increase the production capacity of the ponds. IPRs (In-Pond Raceways) provide better water quality, are easy to install with lower input investment to existing ponds and also provide better water circulation and aeration while using airlift as a powering medium. IPRs was so far used for pikeperch culture only in two published studies regarding broodstock management (Ljubobratović et al., 2019) and grow-out phase of juveniles (Nagy et al., 2022)it is of interest to evaluate the intensive pond-connected system for pikeperch rearing. Therefore, the present study evaluated the grow-out performance in recirculation aquaculture system (RAS.

Material and methods

In this study juveniles of pikeperch were introduced into experimental RAS, IPRs and POND. Into each experimental group, in total 1500 juveniles were stocked. Fish were cultured in each system for 24weeks in monoculture (RAS and IPRs) and polyculture (POND) with cyprinid stocking. After the duration of the experiment pikeperch juveniles were harvested and data were collected. Production parameters concerning growth and feed utilization were calculated (only in RAS and IPRs). To provide a comprehensive overview of pikeperch physiological state, blood and tissue sampling were carried out together with fin erosion grading. From collected samples of blood and tissue biochemical parameters of the blood were assessed together with somatic indexes.

Results and discussion

Fish from RAS displayed the highest growth FTL (Final Total Length), FBW (Final Body Weight), CF (Condition Factor), SGR (Specific Growth Rate) and significantly lowest FCR (Food Conversion Ratio) compared to other groups. Physiological status of the fish regarding biochemical profile of plasma and somatic indexes was in the correlation with fast growth and efficient feed utilization. Increased hepatosomatic index in RAS based fish together with high levels of ammonia (NH_3) in blood suggests increased metabolism rate. High feed intake also manifested on significantly increased fat deposits in RAS juveniles. Increased level of albumins may suggest beginning of RAS based fish liver malfunction. In according to (Policar et al., 2016)frequency of fin erosion, skeletal and gill covers deformities, proximal composition of fish body with special emphasis on fatty acid contents in liver and muscle, oxidative stress and antioxidant response in gill, liver, muscle and intestine were analyzed and compared in exclusively RAS- and POND-cultured juveniles and juveniles produced with the combination of POND and RAS aquaculture in pikeperch (Sander lucioperca L. more frequent erosion of caudal fin was found in RAS based individuals. Group raised in IPRs achieved slower but satisfactory growth. FCR was found significantly higher in IPRs group. Until today no study covering feeding in IPRS is published therefore it represents the possibility of increase of culture efficiency via feeding management optimization. Biochemical profile of plasma uncovered significantly increased levels of glucose directly connected to a stressful harvest of otherwise unbothered fish in IPRs. Fish raised in IPRs displayed erosion on both pectoral fins probably because of presence of the fish in a more turbid environment on the bottom of the IPRs tanks. Pikeperch juveniles in POND group didn't cope well with transition to their natural environment in pond polyculture with cyprinid fish. Pikeperch individuals in POND group displayed significantly lowest SR amongst all groups. Biochemical plasma profiling suggested starvation via increased levels of ALP

(Continued on next page)

(alanine aminotransferase) and non-existing fat reserves. POND raised juveniles also displayed significantly longest gut. Low survival rate was probably also caused by excessive algae bloom, oxygen deficiency and turbidity. In summary fish in RAS displayed most intensive growth and most successful feed utilization. IPRs was proven as an appt way of pikeperch juveniles production during vegetational season. Despite slower growth of the fish in IPRs reduced production cost per juvenile makes up for the difference.

Acknowledgements
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References

Baekelandt, S., Redivo, B., Mandiki, S. N. M., Bournonville, T., Houndji, A., Bernard, B., El Kertaoui, N., Schmitz, M., Fontaine, P., Gardeur, J. N., Ledoré, Y., & Kestemont, P. (2018). Multifactorial analyses revealed optimal aquaculture modalities improving husbandry fitness without clear effect on stress and immune status of pikeperch *Sander lucioperca*. *General and Comparative Endocrinology*, *258*, 194–204. https://doi.org/10.1016/j.ygcen.2017.08.010

Ljubobratović, U., Péter, G., Sándor, Z., Kugyela, N., & Rónyai, A. (2019). The effect of hormonal preparation (gonadotropins vs. gonadoliberins) on pre-seasonally obtained eggs and larvae quality in pikeperch (Sander lucioperca L.). *Aquaculture International*, *27*(4), 1009–1024. https://doi.org/10.1007/s10499-019-00368-4

Nagy, Z., Ardó, L., Demény, F., Gál, D., Sándor, Z. J., & Ljubobratović, U. (2022). Case study on the aptness of in-pond raceways for pikeperch, Sander lucioperca, grow-out. *Aquaculture Reports*, *27*(May). https://doi.org/10.1016/j.aqrep.2022.101356

Policar, T., Blecha, M., Křišťan, J., Mráz, J., Velíšek, J., Stará, A., Stejskal, V., Malinovskyi, O., Svačina, P., & Samarin, A. M. (2016). Comparison of production efficiency and quality of differently cultured pikeperch (*Sander lucioperca* L.) juveniles as a valuable product for ongrowing culture. *Aquaculture International*, *24*(6), 1607–1626. https://doi.org/10.1007/s10499-016-0050-9

Vavrečka, A., Chaloupková, P., & Kalous, L. (2019). Differences in live fish marketing of traditional pond aquaculture and intensive aquaculture in Czechia. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *67*(1), 189–196. https://doi.org/10.11118/actaun201967010189

CIRCULAR FEED INGREDIENTS AND ENHANCEMENT OF NUTRIENT DIGESTIBILITY IN *Cyprinus carpio* POND AQUACULTURE

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Introduction

Common carp (*Cyprinus carpio*) contributed 8.6% (4.2 million tonnes, live weight) to total inland finfish aquaculture in 2020, ranking it fourth after grass carp, silver carp, and Nile tilapia (FAO 2022). More than 90% of common carp production from Europe comes from Russian Federation, Poland, the Czech Republic, Ukraine, Hungary, Belarus, Serbia, Germany and France (Marković et al. 2016)this system has a whole range of options, from those based on traditional rearing methods with use of plants from the region where the fish are reared to production based on the use of high-quality concentrated feeds. In this paper, we conduct a comparative analysis of the effect of using cereal grains as opposed to compound feed (pelleted and extruded. In European carp fishponds, three different diet scenarios exist: an abundance of zooplankton compared to cereals in April-May, a balanced amount of zooplankton and cereals in June-July, and a deficiency of zooplankton compared to cereals in August-September. Experimental simulations of the 3 dietary scenarios demonstrated that when the carp's diet consisted of either a low or high amount of zooplankton, it resulted in nutritional imbalances and high digestive and metabolic losses (nitrogen (N) and phosphorus (P)) compared to scenarios where the diet was somewhat balanced (Roy et al. 2022)temperate shallow-lake ecosystems like central European fishponds – combining animal nutrition and plankton ecology group model principles. In the traditional yet predominant pond farming in central Europe, carp stocks start the vegetative season on a ketogenic diet (high in natural food.

This research is therefore, aimed at evaluating locally available feed ingredients (circular) through digestibility trials. This will serve as the basis for selecting the best (highly digestible) ingredients for formulating balanced and cost-effective diets for *C. carpio* in ponds. Additionally, more circular feed ingredients in aquafeeds means more phytates in aquafeeds. Therefore, pond-associated microbes were also isolated and evaluated for their ability to hydrolyse phytate and other nutrients.

Results

Digestibility

The apparent digestibility coefficient of protein (nitrogen) (ADC-N), phosphorus (ADC-P), nitrogen free extracts (NFE+fibre), and energy of the 12 ingredients (circular) are presented in Table 1. The ADC-N of all ingredients tested were above ~75 % except for malt sprout waste and pea, whereas ADC-P of all ingredients were above 25 % except for lupine sprout, rapeseed expeller, and sunflower expeller.

Table 1: ADCs of the tested ingredients

	Apparent digestibility coefficients (ADC) (%)			
Ingredient	Protein	Phos-phorus	NFE	Energy
Corn DDGS	83.86	65.59	39.75	62.69
Rapeseed expeller	86.99	5.41	20.48	52.81
Sunflower expeller	89.61	14.20	3.48	43.46
Lupine-crushed	97.07	29.48	5.95	45.08
Lupine-sprouted	83.26	21.96	33.18	54.70
Malt flour waste	74.97	50.46	4.41	39.61
Malt sprout waste	67.99	33.34	31.09	32.91
Pea	68.31	48.74	42.64	52.34
Noodles-pasta	98.76	80.83	93.59	99.00
EMC-discards	93.17	72.14	91.64	97.01
Unsold bread	97.98	74.22	97.35	99.00
Sugar beet discards	92.04	62.10	1.00	27.38

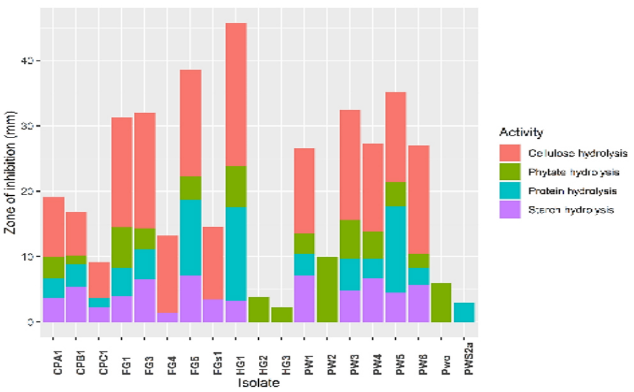

Figure 1: Nutrient hydrolysing capacity of the isolates

(Continued on next page)

Characteristics of the isolates

Nineteen (19) out of the 25 isolates had the ability to hydrolyze at least one of the substrates. All the four substrates were hydrolyzed by 11 isolates with HG1 showing the highest hydrolysis (Figure 2).

Discussion

Aside from lupine and pea, all the ingredients investigated in this study are 'waste' from local bakery, brewery, oilseed processing, and other industries. This study shows the potential of valorising waste into fish meat and lipid for humans. Ingredients such as noodles-pasta, unsold bread, sugar beet discards, and extruded morning cereals (EMC-discards) have high protein sparing potential with low N and P content (data not shown), thus are best suited for beginning-of-season diets to spare zooplankton (reduced grazing pressure from carp as a result of satiation). The other ingredients are best for formulating complete balanced diets for the end-of-season (when zooplankton are depleted) (Roy et al. 2022)temperate shallow-lake ecosystems like central European fishponds – combining animal nutrition and plankton ecology group model principles. In the traditional yet predominant pond farming in central Europe, carp stocks start the vegetative season on a ketogenic diet (high in natural food.

Plant feed ingredients are high in phytate-P but fish are unable to digest phytate-P effectively (Kumar et al. 2012). Isolates such as PW2 especially is best suited for the external hydrolyses of phytate in feed ingredients. Other potential isolates for bioprocessing of feed ingredients include HG1, FG1, FG3, FG5, PW1, PW3, PW4, PW5, PW6. Further safety studies are however, recommended.

A NOVEL FEEDING STRATEGY BASED ON MUSCLE FIBRE RECRUITMENT TO MAXIMIZE THE PERFORMANCE OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Fish growth is a consequence of hyperplasia and hypertrophy. In our previous study, based on the muscle fiber recruitment, we identified four growth phases (PH) in rainbow trout: hyperplasia (PH1: 2.2-15 g), hypertrophy (PH2: 15-50 g), hyperplasia (PH3: 50-150 g), and hypertrophy (PH4: 150-350 g). Based on these growth phases, this study aimed to determine the least-cost feeding strategy for rainbow trout to maximize growth by investigating its influence on muscle fiber recruitment and related myogenic gene expression.

In a 28-week feeding trial, 1400 fish (5.8 g) were distributed into seven treatments in triplicates (70 fish/tank), fed three diets (42% crude protein and 20% lipid) namely FM: fishmeal; PP: plant-based protein; and AF: additive mixture of krill meal, taurine, and organic selenium, via switching the diets at different phases (Figure 1). Treatments were T1 (FM: all phases), T2 (FM: PH1, PH3; PP: PH2, PH4), T3 (PP: PH1, PH3; FM: PH2, PH4), T4 (PP: all phases), T5 (FM: PH1, PH3; AF: PH2, PH4), T6 (AF: PH1, PH3; FM: PH2, PH4), and T7 (AF: all phases).

Results showed that both FM (T1) and AF (T7) individually or in any combination (T5 and T6) showed higher ($p<0.05$) weight gain than other groups in the last phase (Phase 4). A significant ($p < 0.05$) reduction in muscle fiber density across various treatments with the progress of the feeding trial. Dietary switching notably influenced muscle fiber density and recruitment patterns, with T7 followed by T5 showing the highest density and recruitment of smaller fibers. Myogenic gene expression varied significantly ($p < 0.05$) across treatments, with MyoD2, Myf5, MyoG, and MRF4 showing phase-dependent regulation. MEF2 family genes also showed notable changes, particularly in T2, T4, and T6. Growth inhibitors MSTN1 and MSTN2 exhibited differential expression patterns influenced by dietary switching, with T1, T2, and T4 showing higher expression.

Conclusively, during hyperplasia, the high-quality protein-demanding phase, AF performs better than FM. Additionally, dietary switching, particularly using FM during hyperplasia followed by AF during hypertrophy, can enhance muscle fiber recruitment.

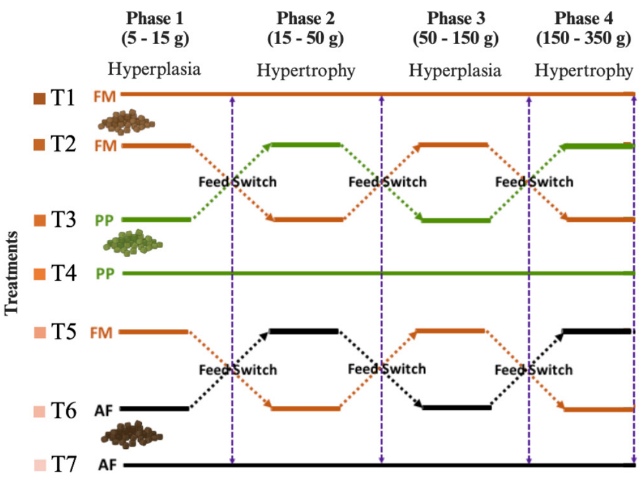

Figure 1: Overview of the feeding protocol

A NUTRITIONAL AND GENETIC APPROACHES ENHANCING THE FEED EFFICIENCY OF SOY PROTEIN BASED DIETS IN RAINBOW TROUT

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Aquaculture Research Institute at the University of Idaho in collaboration with USDA has genetically selected several lines of rainbow trout (*Oncorhynchus mykiss*) that show higher growth rates when fed all plant protein diet (PPD) than non-selected lines of trout fed a fishmeal-based diet. So far, no commercial breeding programs have yet started to improve feed utilization efficiency in fish, mainly because of the difficulty in accurately measuring individual feed intake of fish reared in groups.

This study aimed at identifying trout families with better efficiency in using soybean-based diets through two experiments. In the first experiment, 15 families (CX-118, CX-125, CX-134, CX-135, CX-137, CX-138, CX-141, CX, 143, CX, 144, CX-145, CX-146, CX-147, CX-148, CX-149, and CX-152) of rainbow trout (80 fish/family) with an average initial weight of 32.7±9.4 g (±SD) were PIT tagged and randomly distributed in four tanks (450 L). Fish were reared in this environment for four months and went through two periods of feed deprivation (FD) and two periods of refeeding (RF). During the RF periods fish were fed at satiation with a soy-based extruded diet. Fish performance was classed as FD-, FD+, RF- and RF+ for fish exhibiting loss (FD) and gain (RF) of weight relatively lower (-) and higher (+) than the population mean (FD-/RF-, FD+/RF+, FD-/RF+ and FD+/RF-). These four groups were distributed in 800-L tanks (~45 fish/tank) with three replicates to measure the Feed Conversion Ratio (FCR). In the second experiment, another group of 1200 rainbow trout belonging to the same 15 families were individually PIT tagged and distributed into two groups of 40 fish/tank and fed two diets. Diet 1 was supplemented with lyophilized powder of spirulina whole cells and Diet 2 was supplemented with U-^{15}N labeled spirulina. After 18 days of feeding, 0.4 mm diameter punches were used to take muscle samples from each fish to analyze for ^{15}N isotopes.

Results for the first experiment showed families CX-148, CX-146, and CX-125 with significantly higher weight gain and families CX-138, CX-135, and CX-152 with lower weight loss during RF and FD periods, respectively ($P<0.05$). FCR and final weight were slightly improved in the best group (FD-/RF+) but results were not significant. Results for the second experiment showed a similar trend for weight gain ($P>0.05$). Also, families CX-148, CX-146, CX-147, CX-141, and CX-135 showed the highest ^{15}N isotope in muscle.

Overall, the results of the present study, so far, have demonstrated that there are substantial genetic-based variations among different families of rainbow trout in utilizing soybean-based diets. The selected individual fish from both experiments will be used for the breeding program.

DIETARY INCLUSION OF WHOLE INSECT LARVAL MEAL AND ORGANIC ACID (SODIUM BUTYRATE) IMPROVE THE SOYBEAN MEAL UTILIZATION IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Soybean meal (SBM) is a core ingredient in aquafeed. However, high SBM inclusion in salmonids feed induces enteritis (SBMIE) ultimately reduces nutrient utilization, and decreases the growth performance of carnivore species, including rainbow trout (*Oncorhynchus mykiss*). Whole insect meal (black soldier fly larvae, BSFL) and organic acid (sodium butyrate, SB) exhibited positive effects in terms of improving the growth performance and health of fish. This study aimed to investigate the effects of supplementation of BSFL meal and SB in SBM-based diets on growth performance, nutrient utilization, and distal intestinal histology of trout.

Nine experimental diets (42% crude protein and 20% lipid): fish meal-based diets (FM, control), SBM-based diets (30%SBM and 40%SBM), 30%SBM+5% BSFL, 40%SBM+5% BSFL, 30%SBM+0.2%SB, 40%SBM+0.2%SB, 30%SBM+5% BSFL+0.2%SB, and 40%SBM+5% BSFL+0.2%SB are being fed twice at satiation level for 16 weeks. Each diet was fed to five replicated 19 fish (~30 g) per tank. At the end of the feeding trial, the growth performance, feed utilization, and distal intestinal histology of the trout were measured.

The results showed that there is no significant difference in average weight (AVG) and weight gain (%) at the 5th, 8th, and 16 weeks among the dietary groups ($p > 0.05$). However, in the 12th week, the trout fed SBM40 registered a significantly lower value, while those fed SBM30+SB recorded a higher response ($p<0.05$). The feed intake, feed conversion ratio (FCR), protein efficiency (PER), and HSI value did not differ ($p > 0.05$) among the dietary groups. The histological results did show that the trout fed soy diets appeared to have a loss of vacuolization in the villi and increased lamina propria thickness within these folds (Fig 1).

Shortening and thickening of the folds were most apparent in the SBM40 + I5, the SBM40 + SB, and the SBM30 + I5 + SB, but the SBM30 + I5 appeared similar to the control diet. Based on the results obtained in the present study, it is suggested that 5% insect meal or 0.2% sodium butyrate may be used as a feed additive in high soybean meal diets for rainbow trout.

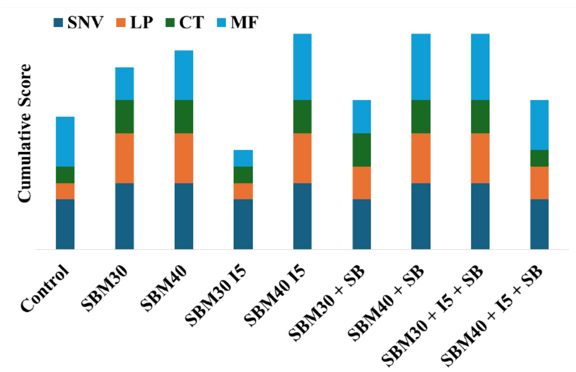

Figure 1: Cumulative histological scores of distal intestinal of rainbow trout fed experimental diets for 16 weeks. MF: mucosal folds; CT = connective tissue; LP = lamina propria infiltration; SNV = supranuclear vacuoles.

A COMPUTER-VISION-BASED BEHAVIOUR ANALYSIS OF LARGE NUMBERS OF ATLANTIC SALMON (*Salmo Salar*) HELD IN RECIRCULATING AQUACULTURE SYSTEM (RAS) TANKS

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Introduction: Advancements in algorithms and practical applications within aquaculture highlight its potential for the digitalization of the industry. However, accurately detecting and tracking fish in higher-density environments remains a challenge. This study proposes a pose estimation method for monitoring the behaviour of high numbers of fish stocked in RAS tanks.

Materials and Methods: RAS tanks (2m wide and 1m deep) holding ca. 1,000 Atlantic salmon (*Salmo Salar*) were continuously recorded using overwater dome cameras for 60 days. To analyse individual fish pose accurately, we employed the state-of-the-art pose model based on Yolo8 architecture, trained to detect fish key points. Using the coordinates of the obtained key points from a one-hour test video, we constructed a vector between the dorsal and snout keypoints for each fish, representing their swimming orientation. Our study investigated fish behaviour based on orientation. We observed rheotaxis (swimming against the current) and cohesive schooling. The cosine similarity of orientation vectors quantified how similarly neighboring fish were oriented. A threshold on the orientation score identified fish deviating from the school. Finally, orientation vectors and keypoints were visualized to analyse behaviour.

Results and Discussion: For the test video the model detected upto 226 out of 1000 fish. While a detection rate of 226 fish (22.6%) might appear low, it is important to consider that this successfully captured most visible fish at the surface, with high pose precision (96.7%). Visualizing different events in the video revealed a strong correlation between the number of detections and orientation scores. This suggests the model has potential to understand fish behavior related to feeding and disturbances.

Table 1. Model Evaluation

Precision %	Recall %	mAP(50-95)%
96.7	91.5	94.7

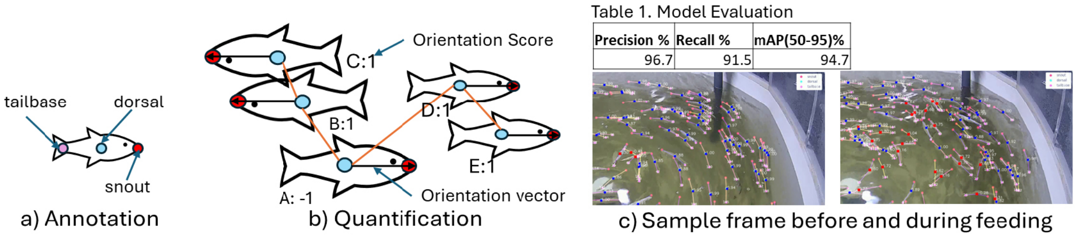

Figure 1. a) The employed annotation scheme b) The representation of orientation vector and orientation scores of different fish c) A comparison of orientation score when used with a threshold value of 0.8 before feeding and during feeding. The fish with red dot highlights the fish below orientation score.

THE IMPACT OF IRRIGATION AND INFRASTRUCTURE DEVELOPMENT ON RIVER ECOSYSTEM AND MIGRATORY FISH POPULATIONS

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River development for irrigation and water supply has a profound impact on global aquatic resources. The expansion of irrigation infrastructure is crucial to meet the increasing food demands of a growing global population, and it is expected to grow substantially in the next two decades. However, it is important to acknowledge that this infrastructure can have adverse effects on aquatic fauna, particularly fish. Fish are highly vulnerable to changes in flow patterns, obstructions in migration pathways, and limited access to vital habitats. Failure to consider and protect the migration needs of fish populations during the design and installation of infrastructure can result in significant environmental, social, and financial consequences. This issue is prevalent worldwide, as evidenced by numerous examples. Two key points to highlight are the shared challenges faced by many countries about fisheries impacted by river infrastructure development, and the potential for substantial environmental, social, and financial costs if the migration needs of fish populations are not taken into account and safeguarded during the design and installation of infrastructure. The expansion of irrigation infrastructure poses a threat to the diversity of freshwater fish on a global scale, as it creates barriers that impede their access to crucial nursery, feeding, and spawning habitats.

Fish passage involves movement in both directions, not just upstream migration. While there is typically a focus on helping fish move upstream past barriers, it is equally crucial to facilitate downstream movement. This is especially critical for diadromous species like eels, which must migrate downstream to reach their spawning grounds. It is essential to comprehend the impact of hydropower and irrigation systems on downstream migration to devise successful solutions.

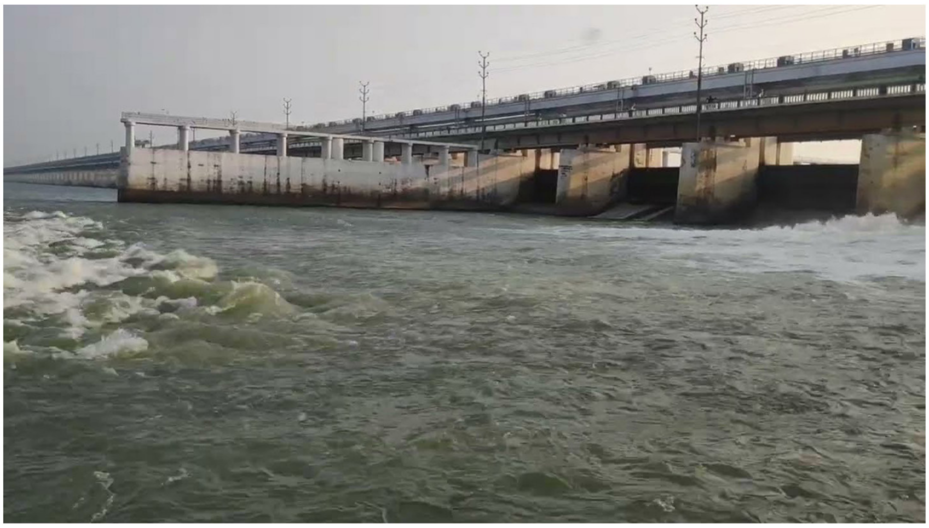

Fig: Barrage

REVIEW OF APPLICATIONS AND PROPERTIES OF THE NATURAL NUTRIENTS FROM MICROORGANISM *Paracoccus carotinifaciens* IN AQUACULTURE

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Main insights of industrial production of carotenoids using *Paracoccus carotinifaciens*

Paracoccus carotinifaciens is an aerobic Gram-negative bacterium that was isolated from soil early in the 1990s[1]. It mainly produces astaxanthin, which is well known as a strong antioxidant. We conducted classical mutation breeding without genetic engineering technology and optimized manufacturing conditions, and successfully started the industrial production of astaxanthin as non-GMO product in 2000s.[2] Dehydrated *P. carotinifaciens* is currently sold under the name Panaferd®AX as a natural pigment for fish and crustaceans.

P. carotinifaciens is cultured under optimized conditions and internally accumulates more than 10 kinds of carotenoids, such as adonirubin, canthaxanthin, and adonixanthin. Hence, *P. carotinifaciens* is suitable for producing desired several kinds of carotenoids.

Carotenoid applications in aquafeeds

According to a feeding test on rainbow trout (*Oncorhynchus mykiss*), Panaferd®AX brought higher CF (Color Fan) score than synthetic astaxanthin because other carotenoids such as adonirubin/adonixanthin besides astaxanthin were also accumulated in the muscle (Fig. 1).

We also explored that Panaferd®AX has additional effects on improving productivity, reducing stress, and increasing immunity of animals. First example, based on one experimental trial on red sea bream (*Pagrus major*) , Panaferd®AX improved the digestibility of SPC (Soy Protein Concentrate) better than only astaxanthin. The test implied that Panaferd®AX components other than astaxanthin were also effective to improve FCR (**Fig. 2**). As second example, Panaferd®AX benefits to whiteleg shrimp (*Litopenaeus vannamei*) were assessed and produced significant resistance to *V. parahaemolyticus*. Panaferd®AX is not only a valuable natural source for pigmentation, but also features properties as a multi-functional feed additive.

[1] A. Tsubokura, et. al., *Int. J. Syst. Bacteriol*. **1999**, 49, 277–282.
[2] M. Hayashi, et. al., *Carotenoids: Biosynthetic and Biofunctional Approaches. Advances in Experimental Medicine and Biology*, Springer, Singapore. 1261, 11–20.

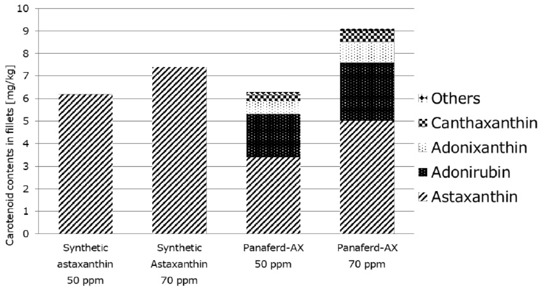

Fig. 1 Carotenoid contents in fillet

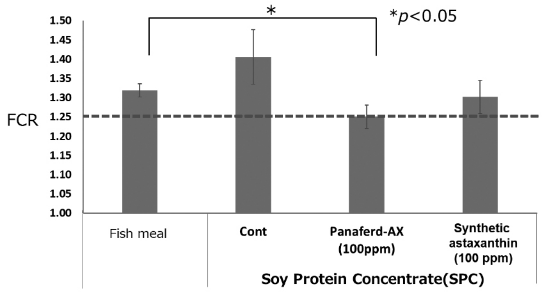

Fig. 2 FCR improvement for red sea bream

BEHAVIORAL RESPONSE OF NILE TILAPIA (*Oreochromis niloticus)* TO SIMULATED AVIAN PREDATION: EFFECTS OF HYPOXIA AND FEED AVAILABILITY

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Fish exhibit complex behaviors in response to hypoxia and predation risk. Most fishes actively swim to the water surface to gulp atmospheric oxygen during hypoxia and feeding episodes, this phenomenon increases their exposure to avian predators. While chemical cues have been studied for understanding antipredator behavior, the responses of Nile tilapia to predation risk in the wild and aquaculture have received little attention. In a recent study, the behavioral response of Nile tilapia to simulated avian predation, hypoxia, and feed availability was investigated. The presence of an avian predator significantly affected the fish's feeding behavior, with different responses observed based on the fish's size. Additionally, the distance of the avian predator from the water surface affected the fish's surface respiration behavior. These findings highlight the intricate interplay between hypoxia, predation risk, and fish behavior, suggesting the need for further research on the individual differences among fish groups and their impact on the predator-prey relationship ventilation frequency when the avian predator was simulated except for small-sized fish. Future studies can focus on how the individual differences among fish groups interact and affect the predator–prey relationship.

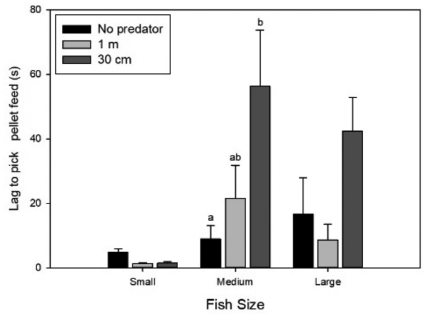

Figure 1: Lag to pick the first feed with avian predator simulated at different distances from the water surface.

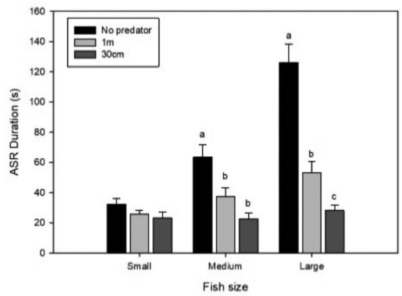

Figure 2: ASR duration of O. niloticus with avian predator simulated at different distances from the water surface.

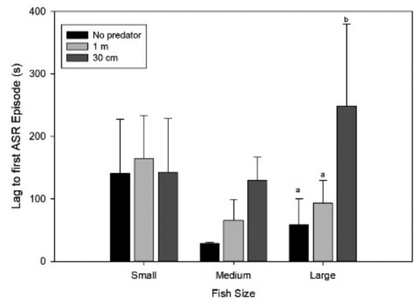

Figure 3: Lag to perform the first ASR with avian predator simulated at different distances from the water surface.

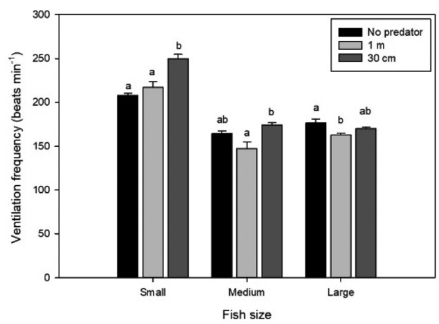

Figure 5: Gill ventilation frequencies (beats min-1) of O. niloticus with avian predator simulated at different distances from the water surface.

AQUACULTURE IN SALINE-ALKALINE LAND AND ECOLOGICAL IMPROVEMENT

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Soil salinization poses a threat to agricultural production, food security, provision of basic ecosystem services, and achievement of sustainable development goal (SDG). The United Nations Food and Agriculture Organization (UNFAO) published a global map of saline soil distribution on October 20, 2021. According to the map, it is estimated that there are over 833 million hectares of saline soil worldwide, which means that over 1.5 billion people worldwide are facing significant challenges in food production due to soil salinization. China has approximately 100 million hectares of saline-alkaline soil, which needs to be developed, utilized, and ecologically improved.

In order to scientifically utilize saline-alkaline soil resources and explore new methods for improving the ecological environment of saline-alkaline land, coupled with field engineering or salt washing engineering, is carried out ecological improvement model of reducing salinity and alkalinity with Pond-Rice Field and Pond- Lifting field aquaculture.

Ecological Improvement Model of Reducing Salinity and Alkalinity with Pond-Rice Field

It consists of pond engineering and rice field engineering, as shown in Figure 1. The pond is connected to the rice field through drainage channels, and aquaculture is carried out using the saline-alkaline water from the rice field. The aquaculture species include fish, shrimp, crab etc. Intermediate test was conducted in Tangshan, Shizuishan, and Dongtai in China. The ecological improvement effects are shown in Table 1.

Ecological Improvement Model of Reducing Salinity and Alkalinity with Pond-Rice Field

It consists of pond engineering and lifting field engineering, as shown in Figure 2. After digging soil to build ponds, lifting fields for land reclamation, and using altitudes differences, saline-alkaline water is transferred to ponds for aquaculture. The aquaculture species include fish, shrimp, etc., Intermediate test was conducted in Jingtai in China. The ecological improvement effects are shown in Table 2.

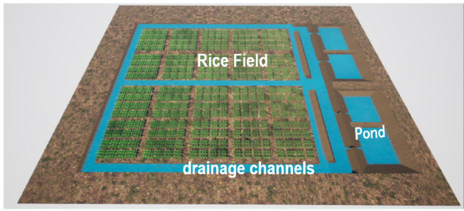

Figure 1 Sketch map of ecological improvement model of Reducing Salinity and Alkalinity with Pond-Rice Field

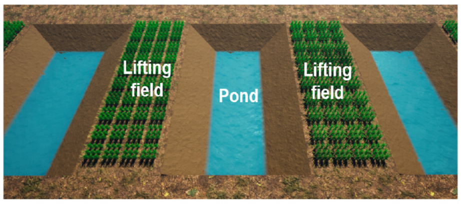

Figure 2 Sketch map of ecological improvement model of Reducing Salinity and Alkalinity with Pond-Lifting Field

Table 1 Ecological Improvement Effect of Pond-Rice Field Model in Saline-Alkaline Land of Tangshan

	Soil				Water		
	Before Salt washing	After Salt washing	Percent (%)		Before Aquaculture	After Aquaculture	Percent (%)
Salinity (g/kg)	8.54±0.10	8.34±0.09	↓2.34	Salinity (g/L)	9.30±0.01	8.65±0.16	↓6.99
C_A (cmol/kg)	3.66±4.43	1.49±0.88	↓59.29	C_A (mmol/L)	6.21±0.08	4.60±2.75	↓25.93
pH	0.78±0.65	0.56±0.21	↓28.21	pH	1.34±0.04	1.05	↓21.64

Table 2 Ecological Improvement Effect of Pond-Lifting Field Model in Saline-Alkaline Land of Jingtai

Soil	Before lifting field	After lifting field	Percent (%)	Water	Before aquaculture	After aquaculture	Percent (%)
pH	8.79±0.07	8.49±0.05	↓3.41	pH	8.17±0.89	8.04±0.11	↓1.59
Salinity (g/kg)	15.0±0.10	3.65±0.52	↓75.67	Salinity (g/L)	4.58±0.12	4.64±0.01	↑1.31
C_A (cmol/kg)	0.47±0.05	0.23±0.06	↓51.06	C_A (mmol/L)	2.17±0.38	1.74±0.18	↓19.82

DEVELOPING AN EFFECTIVE SHARK DETERRENT AGAINST SPINY DOGFISH (*Squalus acanthias*) FOR AQUACULTURE INSTALLATIONS

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Introduction
The fish farmers in western and southern Norway report that spiny dogfish often bite through the net and get in the fish cages. The holes in the cages can 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. Therefore, this is a financial, welfare and ecological challenge. To date there is no effective shark deterrent for fish farms. Hence, it is crucial to test and develop an effective method 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 electromagnetic (EM) pulse, 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 been tested to keep sharks away from bait and catch in line and net fishing. Their effectiveness varies depending on the species and geographical area; none of them provide a full deterrence. Here, by evaluating the candidate shark-deterrents by quantifying the behavioral and physiological responses, we find that both EM and skin extract could be used as effective shark deterrent. Further field trials in an affected production farms are necessary to evaluate the effectiveness of these methods from aquaculture installations.

Acknowledgement:
Funding: Norwegian Seafood Research Fund, Ref.- 901704

INCREASING FEMALE PARENTAGE CONTRIBUTION IN GREATER AMBERJACK *Seriola dumerili* THROUGH THE ADMINISTRATION OF GnRHa IMPLANTS

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The greater amberjack (*Seriola dumerili*) is a group spawner, and in the wild it forms large aggregates during the spawning season. In the present study, we acquired knowledge on the spontaneous spawning kinetic and parental contributions under aquaculture conditions, and after spawning induction using exogenous hormones.

Two breeding groups were formed in 75-m^3 tanks. The first group was allowed to spawn volitionally (Spontaneous; 5 males, M, 19.0 ± 0.5 kg mean (SD) body weight; 4 females, F, 29.7 ± 0.9 kg). The second group was treated with GnRHa implants (Induced; 6 M, 22.1 ± 1.6 kg; 4 F, 25.0 ± 2.0 kg). Spawning was monitored for 24 days and eggs were collected. Broodstock and egg genomic DNA were extracted for genotyping and parental assignment.

About 75% of the eggs obtained from the Spontaneous group were assigned to only one female (F14), while one female did not spawn (F21, **Fig. 1A**). On the contrary, all females from the Induced group spawned on multiple days (**Fig. 1B**). All males from both groups participated in the fertilization of the eggs (**Fig. 1C** and **D**). Considering the maximum number of crossings attainable from the Spontaneous and Induced groups during this study, the 13 and 20 families obtained, corresponded to 65 and 83% of the possible outcome, respectively.

The results of the current work demonstrated that GnRHa-implants were effective in increasing the number of females that spawn within a few days, making it possible to generate a greater number of families. This method may be a practical tool for increasing genetic contribution in breeding selection programs for this species.

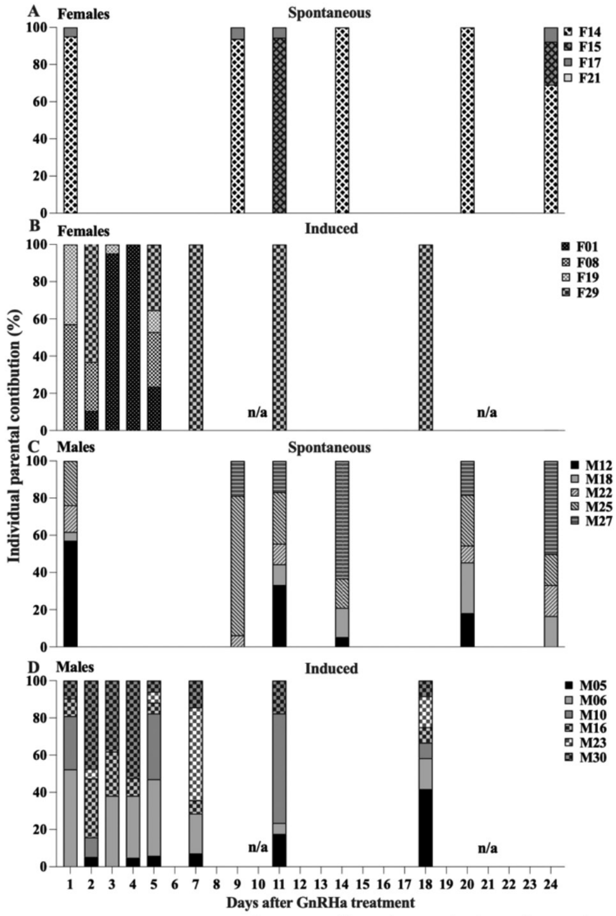

Figure 1. Parental contribution (%) of male and female greater amberjack from the Spontaneous and Induced group.

Financial support has been provided by the European Union's Horizon 2020 grant agreement No 862658, NEWTECHAQUA project.

THE CASE OF BLACK SOLDIER FLY AS A NOVEL INGREDIENT IN THE FEED AND FOOD INDUSTRY: HOW TO APPROACH NOVELTY

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The Black Soldier Fly (BSF) has emerged as a promising feed ingredient because of unique characteristics, including high protein content, rapid growth cycle, and ability to thrive on organic waste. This presentation looks into the opportunities and challenges associated with incorporating BSF-derived products into feed and food production systems, focusing on effective strategies for approaching novelty in this context.

Looking first at a comprehensive overview of the BSF's biology, emphasizing its efficient conversion of organic waste into valuable biomass. This aspect is particularly relevant in the context of sustainable food production and circular economy principles, where BSF larvae play a crucial role in nutrient recycling and bioconversion.

Next, looking at the nutritional profile of BSF-based products, highlighting their rich protein and lipid content, essential amino acids, and beneficial fatty acids. These attributes make BSF a compelling alternative to conventional protein sources such as soybean meal and fishmeal, especially amid concerns about resource scarcity and environmental impact associated with traditional feed ingredients.

Regulatory frameworks governing insect-based products vary globally, requiring clear guidelines and standards to ensure food safety and consumer confidence. Additionally, consumer perceptions and acceptance of insect-based foods and feeds represent a significant barrier that must be addressed through education, transparency, and product innovation.

Moreover, understanding consumer preferences, communication strategies, and product positioning are vital for market success. Leveraging sustainability narratives, emphasizing nutritional benefits, and engaging in dialogue with consumers can foster acceptance and market growth for BSF-derived products.

In conclusion, the integration of Black Soldier Fly as a novel ingredient in the feed and food industry offers promising avenues for sustainable innovation. By addressing technical, regulatory, and consumer-related challenges through collaborative efforts and strategic approaches, stakeholders can unlock the full potential of BSF-based products, contributing to a more resilient and environmentally responsible food system.

EVALUATING THE IMPROVEMENT OF AQUACULTURE WATER QUALITY AND FEED CONVERSION RATIO: A SYMBIOTIC RELATIONSHIP BETWEEN *Oreochromis aureus* & *Phycophyta*

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Red Tilapia (*Oreochromis spp*.) is the one of the most sorted after fish species in fisheries aquaculture because of its brilliant red colour, its ability to withstand high salinities and rapid growth rate. Void of the constant need to select cohorts to maintain the favorable colour, in St. Kitts & Nevis and the wider Caribbean it's the freshwater species of choice. Our fish imports into St. Kitts & Nevis are around 250 metric tons per annum, to serve a combined population of around 54,000 people.

One thousand (1000) Tilapia fingerlings were selected and divided into two groups: Group 1: full enclosure, biological and mechanical filtration, two (2) feeding per day. Group 2: 50% shaded plastic roof, mechanical filtration, one (1) feeding per day. Feed for both groups consisted of 38% protein. The source of water for both systems was rainwater that was harvested and stored in large quantities and the source of power was a 5k solar system. The systems were both recirculation systems and they were located on the same farm and ran for a period of 7 months.

TABLE1: FCR, water changes and average weight per cohort (7 months)

Cohort	**Group 1**	**Group 2**
Feed (lbs./kg)	600lbs/272.15kg	370lbs/167.82kg
Water changes	5	2
Fish weight	1.35lbs.0.61kg	2.13lbs/0.96kg
FCR	1.2	0.74

EXPERIMENTAL BIOSECURITY BREACH IN A BRACKISH WATER RECIRCULATING AQUACULTURE SYSTEM REARING ATLANTIC SALMON SMOLTS

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Adaption of land-based recirculating aquaculture system (RAS) has been pivotal in the significant development of Atlantic salmon production in the last decades. Since fish have little contact with the surrounding environment, the systems offer stringent biosecurity measures against diseases and fluctuating environmental conditions. Despite this advantage, RAS is not risk-free for disease outbreaks. There are still some potential transmission sources, including the intake of water and infected fish stocks, among others. High organic loads in these intensive systems likewise offer a favourable environment for opportunistic pathogens to thrive, establish and eventually infect the fish. Pathogen outbreaks in RAS have been reported, however, we have limited understanding of the extent of their risks since simulating these scenarios under controlled conditions is challenging.

A trial was conducted where a pathogen breach was simulated in brackish water recirculating aquaculture systems rearing Atlantic salmon smolts. Fish either received a traditional smolt feed or feed developed for RAS. *Yersinia ruckeri*, the causative agent of enteric red mouth disease (ERM) in salmonids was employed as the test pathogen. The pathogen was introduced into the system through the sump tank, simulating a pathogen breach via the intake water. The development of the disease was followed for 3 weeks. Thereafter, all remaining fish were taken out and the systems operated for another two weeks before the systems were disinfected through pH manipulation.

Preliminary results indicate that the pathogen did not manage to establish in the system as shown by its limited detection in the swab samples collected from the different locations, including, tank wall, drum filter, sump tank, biofilter wall and biomedia. Microbiota analysis provided further support for this. Key microbial phyla showed temporal variations during the trial, but the type of feed showed no clear effects. Key water qualities remained favourable and within the limits. Overall, the chemical composition of the sludge did not change, although, the phosphorus levels decreased during the disease development phase. There was no mortality following infection and fish did not show the classic pathologies for ERM. Despite the sporadic detection of *Y. ruckeri* in some fish samples, the load was relatively low. Infected fish mounted mucosal and systemic immune responses based on antibody and gene expression analyses, though the changes appeared not to be heavily affected by feed types. This was supported by minimal changes in the metabolomic and proteomic profiles of skin mucus. Biofilm carriers placed in the biofilters and sump tanks to monitor the establishment of pathogen and biofilm formation overtime, also confirmed the limited detection of pathogen, but showed stronger biofilm establishment in both biofilters and sump tanks for the traditional smolt feed RAS in comparison to the RAS feed ones. Infected biomedia were subjected to different disinfectants approved for use in Norway and those based on peracetic acid appeared to have the highest disinfection potential.

The work was supported by the Norwegian Seafood Research Fund (FHF 901826).

OMEGA-3 EPA AND DHA FROM ALGAL OIL IMPROVE PACIFIC WHITE SHRIMP ZOOTECHNICAL PERFORMANCE AND NUTRITIONAL QUALITY WITHOUT CHANGING SENSORY QUALITY

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Although the benefits of incorporating algal oil (AO) in plant-based aquafeeds have been established for most commercial fish species, knowledge on the effects of such incorporation in shrimp feeds is scarce. The present study assessed how AO inclusion at different levels in a plant-based shrimp feed could promote growth and FA deposition in the muscle. Pacific white shrimp, *Litopenaeus vannamei*, were fed a reference diet with marine animal ingredients (F0) or a plant-based diet with vegetable oil alone (P0), or in combination with 1% (P1) or 2% (P2) AO. After 51 days of culture, zootechnical and nutritional performance of shrimp fed with AO supplemented diets was significantly improved. The sensory attributes of shrimp fed P1 and P2 were slightly improved relative to P0 while the EPA and DHA content was three to four times higher, thereby increasing the nutritional value of shrimp. Therefore, plant-based feeds can sustain an efficient production of *L. vannamei* with controlled fatty acid profiles, provided they are supplemented with AO. In addition, the use of AO eliminates the reliance of shrimp feeds on the limited supplies of marine ingredients while maintaining the forage fish dependency ratio for fish oil of zero and allows for the use of more flexible formulae based on different meals of terrestrial origin.

In conclusion, the incorporation of algal oil in plant-based shrimp feeds offers a sustainable and nutritionally adequate alternative to traditional marine-based feeds. This approach supports the efficient production of *Litopenaeus vannamei*, enhances the nutritional value of the shrimp, and contributes to the overall sustainability of the aquaculture industry. As the demand for seafood continues to rise, innovations such as these will be essential in ensuring the long-term viability and environmental stewardship of global food production systems.

APPARENT DIGESTIBILITY OF TOASTED COWPEA SEED (*Vigna unguiculata*) IN WHITE-LEGGED SHRIMP (*Penaeus vannamei*)

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Introduction
The cost and availability of protein rich ingredients is a challenge for the development of the aquaculture feed sector, especially in developing areas. As a result, alternative protein sources are being used such as cowpea seeds, which are commonly found in sub-Saharan fish feed factories. This legume contains essential amino acids and it is quite rich in proteins (25.2±2.2, according to Feedipedia). However, cowpea is little studied and contains anti-nutritional factors (1). The purpose of this study is to determine the apparent digestibility of cowpea in shrimp.

Material & methods
168 shrimp (0.94±0.6g) were distributed in 12, 60L tanks with 14 shrimp per tank, i.e. a biomass of 13,16g per tank. Tanks were randomly divided into 2 groups, 1 control group (CTRL) and 1 test group (COW) (n=6 tanks/treatment).

A base diet for shrimp was formulated (37.3% proteins, 7.8% lipids). CTRL feed is composed of this base diet and 0.05% Yttrium Oxide (OY). The COW diet is composed of 30% roasted cowpea meal, 0.05% OY and the base diet.

After one-week of acclimatization with experimental diets, faeces were collected by siphoning twice a day at t+1h30 and t+2h30 *post prandium*. Faeces were then cleaned with distilled water and frozen at -20°C through the collection period. The collection continued until an estimated total dry matter of 8g of faeces per modality was obtained.

Perspectives
This trial started in March 2024 and will end in May 2024. This experience will help to fill the data gap while providing important information on the use of cowpea in shrimp diets. in sub-saharan Africa and contributing to local feed sustainability. The use of locally produced ingredients is a powerful way for improving the sustainability of aquaculture feed manufactured in sub-Saharan Africa.

(1) J.Viruska, *et al*. 2020. « Effect of Heat Processing on the Nutritional and Anti-Nutritional Factors of Cowpea (Vigna Unguiculata) ». The Annals of the University Dunarea de Jos of Galati. Fascicle VI - Food Technology 44 (1): 165-77. https://doi.org/10.35219/foodtechnology.2020.1.10.

Apparent digestibility of nutrient (ADC) in diet was calculated as followed:

$$ADC_{n,diet} = 1 - \frac{\text{marker}_{\text{diet}} \times N_{faeces}}{\text{marker}_{faeces} \times N_{diet}}$$

ADCn, diet=apparent digestibility of nutrient n in the food; marker= yttrium measured in food or faeces (%DM); n=nutrient in food or faeces (%DM)

Apparent digestibility of nutrient (ADC) in cowpea was calculated as followed:

$$ADC_{n,cowpea} = \frac{ADC_{n,COW} \times N_{COW} - ADC_{n,CTRL} \times 0{,}7 \times N_{CTRL}}{0{,}3 \times N_{cowpea}}$$

ADCn, cowpea=apparent digestibility of nutrient n in cowpea/COW diet/CTRL diet; n= nutrient (%DM) in cowpea/COW diet/CTRL diet

A VIEW OF EXTRUDED FISH FEED PRODUCTION UNITS IN SUB-SAHARAN AFRIAN THE EXAMPLE OF IVORY COAST

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Introduction

Ivory Coast is a major importer of fish, with a national consumption of 650,000T for a production of 110,000T in 2021, of which 4% comes from aquaculture (1). Ivorian aquaculture is characterized by extensive systems (2). In order to meet market demand, farmers are intensifying their system by using feeds. This study focuses on extruded fish feed factorises, which flourished across Ivorian territory since 2018. The objective is to characterize this new production.

Material & Methods

Six production units located in the southern half of the country between July and August 2023have been investigated. Producers were interviewed, the characteristics of the production tools were characterized and sampling of raw materials and feeds were carried out. Due to the lack of laboratories in the region, these samples were evaluated on various physical and visual criteria to obtain an estimation on their quality. When possible, fish farmers using feeds from these units were also interviewed.

Results and Discussion

Production units have various profiles: half of them were less than a year old at the date of the study, 3 were on-farm feeds production units, 2 of which produced exclusively for self-consumption. Declared production varies from 500 kg/day to around 1T/day. The facilities have various levels of equipment with installation costs (equipment + infrastructure) ranging from 9.103 USD to 273.100 USD (1 USD= 604,19 FCFA, 4th July 2023).

Raw materials (RM) used are mainly agricultural by-products (fig.1). Proteins are expensive and hardly available. It encourages feed producers to limit their inclusion in formulas or to use inexpensive, poor-qualitative ingredients.

RM are often obtained from artisanal processes. It is therefore impossible to get a standardized composition. It partly explains the unmastered nutritional quality of the final products.

Storage conditions are generally inappropriate with high moisture and temperature, which enables the development of mold. Warehouses were often colonized with insects. Such conditions lead to the loss of valuable nutrients.

Pellets physical characteristics were not homogeneous, in particular buoyancy (fig2), durability and length.

FIGURE 1: Frequency of use of different raw materials encountered in feed production units

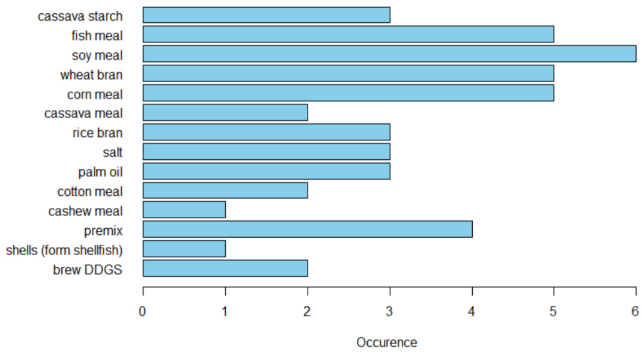
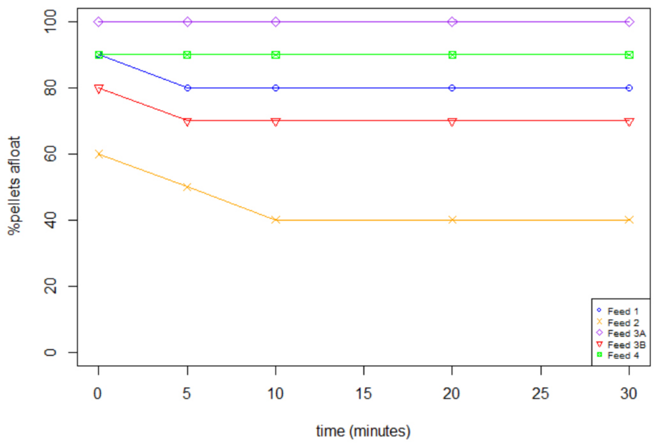


FIGURE 2: Comparative buoyancy of sampled extruded fish feeds



Interviewed fish famers clearly indicate their will to buy and support local feed producers, but they consider that local feed prices are high and zootechnical performances are unsatisfactory. These feedbacks matches the constatation made in factories.

(1) FAO- FISH4ACP, 2023
(2) Yao, et al. 2017

PRELIMINARY STUDY FOR THE DEVELOPMENT OF A PASSIVE SORTER FOR USE WITH JUVENILE WHITE SHRIMP (*Penaeus vannamei*)

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The farmed shrimp market has been growing for over 20 years, with 85% of volumes coming from aquaculture (1). This type of farming is now also expanding in Europe. One obstacle to rearing juvenile *Penaeus vannamei* shrimp is the farmer's ability to monitor the growth of his animals and thus optimise zootechnical performance (WG, FCR…). From time to time, hatcheries can deliver batches composed of non-homogeneous animals to fattening farms and research centres. In order to limit this variability for production or trials, the use of a passive sorter is a solution that limits handling and therefore maximizes animal welfare. To develop this type of sorter, it is necessary to better know the possible relationships between different parts of P vannamei physiognomy.

Materials and methods:

Biometrics were carried out on juvenile *P. vannamei* shrimp taken from different batches reared at an experimental station (Halieutica, France) soon after deliveries.
The following parameters were recorded:

- The length of the shrimp (from the tip of the rostrum to the tip of the tail).
- The width of the cephalothorax.
- The height of the animal at the reproductive organ level.
- Animal weight.

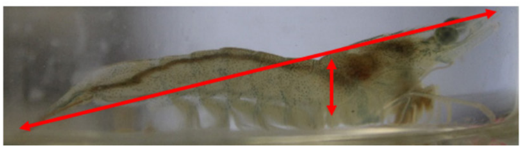
Results:

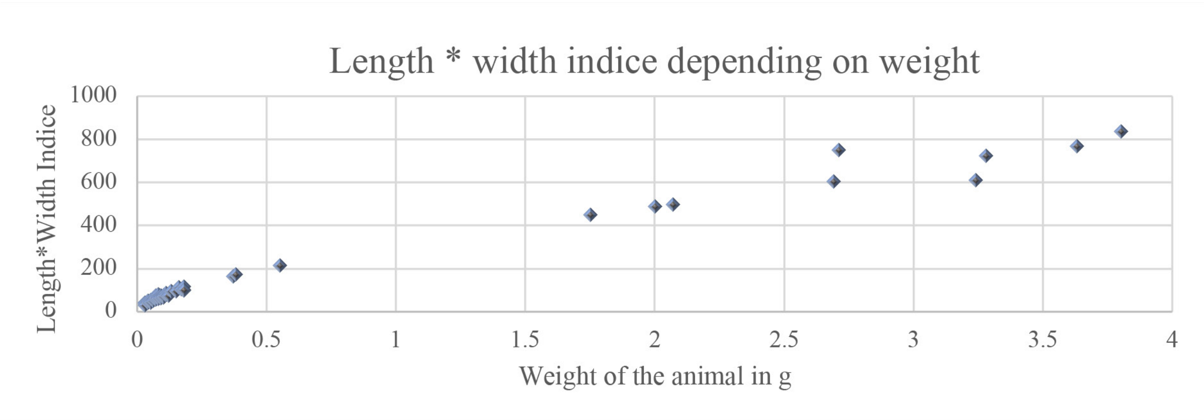
The measurements carried out to date enable us to identify some strong and logical trends. Length is strongly correlated with mass (corr=91%, R2=0.87). Width (the most important parameter for the sorter) is correlated with mass (corr=88%, R2=0.78). The length*width index is strongly correlated with mass. This correlation is more interesting than that between length and width and mass individually, as it is much stronger (corr=0.99, R2=0.98).

Discussion:

These measurements enable us to determine the characteristics of the passive sorter. First prototypes are currently tested. Different version of this new tool is under calibration for shrimp weighing less than 3 g.

(1) FAO, EUMOFA 2017/2020





COMBINATION MUST MAKE STRENGTH: A WORKFLOW TO DESIGN NEW FISH POLYCULTURES

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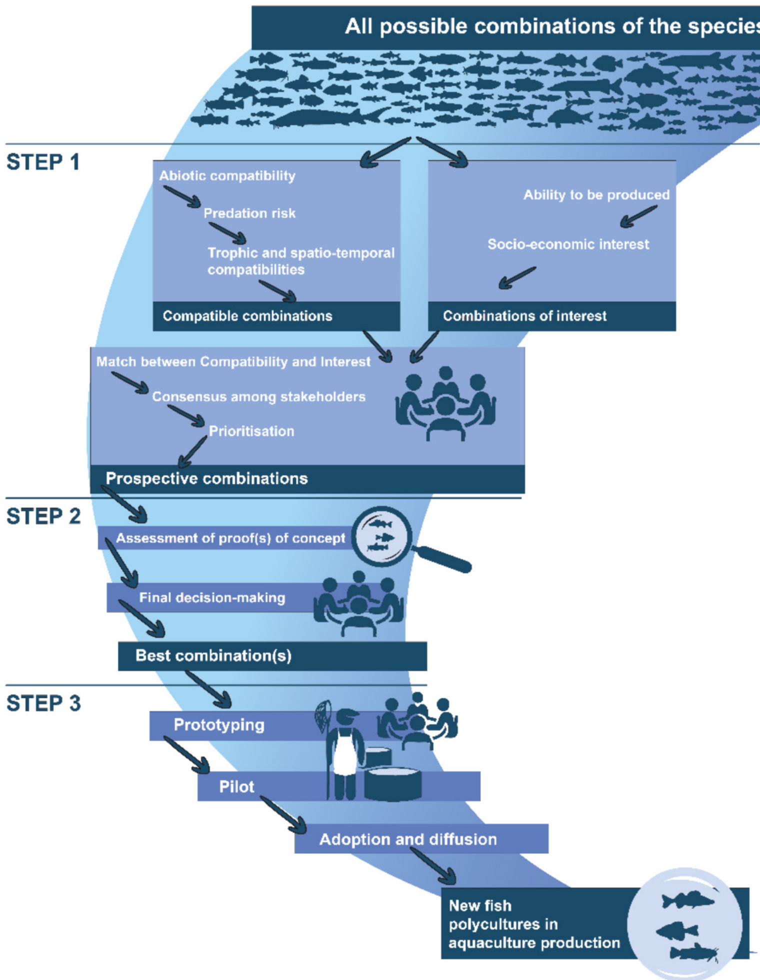
Polyculture is a relevant practice for improving the aquaculture sustainability, generating interest in its implementation in a variety of production systems. However, polyculture is a complex approach that can lead not only to complementarity between species, but also to interspecific competition and animal welfare issues. Potential benefits can be expected from polyculture provided there is compatibility and complementarity between the species combined. It is therefore essential to identify the best species combinations for a given aquaculture system.

In this context, we develop a conceptual integrative workflow for standardizing and planning the development of new fish polycultures. This workflow is designed to select all possible combinations of a set of species on the basis of three successive evaluation steps. Overall, these steps consider the compatibility and complementarity of co- farmed species, as well as stakeholder requirements, sustainability and fish welfare. Step 1 selects the most promising combinations of compatible species (i.e. "prospective combinations") based on the opinions and expectations of stakeholders, using databases and surveys. Step 2 validates the effectiveness of potential combinations on the basis of bioassays, considering species complementarity and animal welfare. Step 3 implements the best species combination(s) in aquaculture production, during which prototyping is used to study the sustainability of the resulting commercial production system. The resulting workflow aims to be a valuable tool for innovation in aquaculture by exploiting the opportunities and strengths of polyculture.



GATHERING TO SERVE: TOFF, A DATABASE ON FISH FUNCTIONAL TRAITS TO PROMOTE THE DEVELOPMENT OF FISH FARMING

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Functional traits are phenotypic characteristic of an organism that (in)directly influence its performance or fitness in its environment. These traits are typically related to how an organism interacts with its surrounding environment, acquires resources, and responds to ecological challenges. Functional traits include behavioral, morphological, phenological, and physiological traits (BMPP traits) that affect an organism's growth, reproduction, survival, or competitive ability.

Functional traits are valuable pieces of information for aquaculture research and management. They allow understanding or predicting the interactions and suitability of species in a farming environment, in order to improve the efficiency and sustainability of a production. For instance, estimating the feasibility and efficiency of combining new species using an approach based on functional traits is a useful tool for promoting polyculture practices that aim to farm species with complementary resource uses and minimal competition between resources. Similarly, the analysis of functional traits makes it possible to predict the consequences of modifications to the culture environment, such as those triggered by climate change, on species production. Overall, the functional trait approach can limit time-consuming and costly bioassays to evaluate alternative scenarios for aquaculture development. It is therefore essential to compile species trait datasets with broad taxonomic coverage in species groups important for aquaculture, such as fish, to foster applied developments through meta-analyses based on big data.

Although fish traits have been the focus of an abundant research, an overview of broad-scale insights is poorly available because useful datasets are scattered over several decades of literature in many languages. Datasets of fish functional trait are thus difficult to access and often unpractical to achieve meta-analyses without a time-consuming extensive review. Already available large-scale compilations include trait information for many fish species but not as detailed as required for aquaculture purpose.

In this context, we develop TOFF (i.e. Traits OF Fish), a database focused on functional fish traits that aims to bring together behavioral, morphological, phenological and physiological traits always coupled with environmental measures in a single, open-access repository. TOFF hosts data from published experimental and field studies. It already gathers data on 241 traits for 248 species extracted from 547 publications and present a collaborative platform. Further development of TOFF requires the foundation of a community of users and data providers. This community is open to all stakeholders in the aquaculture industry and research. Our ultimate goal is to provide a comprehensive and accessible data resource to facilitate progress in aquaculture development.

HOW DOES DOMESTICATION CHANGE THE BEHAVIOUR OF THE FIRST GENERATION OF FISH IN CAPTIVITY?

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Domestication establishes a new context of evolution in which species adapt to conditions chosen, design and controlled by humans. For fish, it is a continuous process divided into five levels from the first acclimatisation attempt to captivity (Level 1), to the completion of the life cycle in captivity (Level 4), and even to the implementation of selective breeding programs (Level 5). This leads to genetic and phenotypic changes over generations in domesticated species. Behavioural traits were among the first to change at the outset of domestication. However, in fish, potential changes in behavioural traits have been little studied at the very beginning of domestication. Therefore, the aim of this study is to investigate whether there are any behavioural changes in the first generation of domestication of a model species, the zebrafish (*Danio rerio*).

A wild population (F0) was acclimatised to captive condition and used to establish the first generation (F1) born in captivity. The conditions of captivity were constant and controlled among generations. A population (Lab), derived from different laboratory strains, was used as “domesticate reference”. The tests were of two types: a mirror-test to evaluate behavioural traits involved in activity, aggression, and stress and an open-field test to assess behavioural traits related to exploratory behaviour and stress. The mirror-test was performed at 120 days post-fertilisation (dpf) and the open-field at 127 dpf.

Principal component analysis (PCA) suggests a trend towards differentiation between the Lab population, the F0 and the F1. This difference is confirmed by the pairwise multi-response permutation procedure (MRPP) which showed a significant difference between Lab/F0 and Lab/F1 ($p < 0.05$). The contribution of the behavioural and activity-related traits to the PCA axes showed that F0 and F1 being more stressed than the population adapted to captivity conditions over several decades who appeared more aggressive. However, no significant difference was found in terms of activity (total distance covered by the individual).

It is essential to consider the expression of these behavioural traits in fish candidates for a new domestication programme and determine if their evolution during the domestication process leads to improved production and life cycle control of organisms. As previously discussed in the literature, fish domestication usually results in a decrease in stress response to aquaculture-related stress over generations, making it easier to maintain animal welfare and continue producing organisms. However, it is important to note that our study indicates that there were no substantial changes in the stress response of the first generation born in captivity compared to the wild population. This highlights the significance of minimising potential stress to fish populations at the beginning of the domestication process.

IN SILICO MODEL TO PREDICT COMPATIBILITY IN FISH POLYCULTURE

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Aquaculture is experiencing remarkable growth, particularly in South East Asia. In this region, the rearing of minimum two species at the same time (polyculture) in ponds is largely widespread. It improves the resource use, fish welfare and the system resilience. Nonetheless, this system is complex not least because wild fish population (associated biodiversity) can enter into the pond. Therefore, potential benefits can be expected provided the fish introduced by the farmers (planned biodiversity) and the associated biodiversity are compatible (i.e. able to live in the same system without detrimental interaction and with a minimized competition) or even complementary (i.e. able to use different portions of available resources or display commensal/mutualistic interactions). Therefore, it is important to identify the most promising combinations of species that may be introduced for a better productivity in this open system.

Compatibility is a prerequisite for any polyculture in an aquaculture system. However, its evaluation cannot be carried out by empirical testing alone for pragmatic reasons (e.g. considering 7 species, 120 possible combinations of 2, 3, 4, 5, 6 and 7 of these species would have to be tested) and ethical reasons (i.e., welfare concern by combining not compatible species). One solution lies in a preliminary *in silico* assessment of compatibility without bioassays, so as to focus experimental evaluations or field tests solely on the most promising combinations.

Informatic tool (i.e., AquaDesign) has been already developed to assess the abiotic compatibility (i.e., ability of living in the same abiotic environment) of given fish combinations. However, there is currently no tool available to evaluate the biotic compatibility between species (i.e. ability of living together system without detrimental interaction and with a minimized competition). We thus introduce an *in-silico* model for assessing biotic compatibility using fish functional traits. We apply it for the improvement of fish polyculture in pond systems in Cambodia.

The rearing system will consist of cages with Nile tilapia (*Oreochromis niloticus*) within a pond containing associated biodiversity that comes from flood during the rainy season and 2 or 3 species from the planned biodiversity. The implemented data in the model are functional traits from scientific literature mined in the database TOFF.

The outcome of the model is a compatibility index for each combination that incorporate the risk of predation and competition for trophic and spatio-temporal resources. In the context of Cambodia aquaculture, the next step will be to select combinations based on socio-economic expectations before performing field tests to validate the model's predictions against real-world outcomes.

CONTEMPLATING THE RESPONSE OF THE MARINE COMMUNITY TO THE POSSIBLE ADAPTION OF GLOBAL FISHERIES TO CLIMATE CHANGE: A STUDY FOCUSING GREY MULLET OF NORTH-WESTERN PACIFIC TOWARDS SUSTAINABILITY

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The possible impact of increasing global temperatures and altering oceanic conditions on the distribution of marine organisms raises significant concerns about the effects of climate change on marine ecosystems. The flathead gray mullet (*Mugil Cephalus L.*) is a cosmopolitan fish that lives in the warm and temperate zones over 42°N–42°S. It is a key fish species for industrial fishing off coastal Taiwan. Gray mullets enter the coastal waters of the southeastern Taiwan Strait (22°N–25°N) to spawn in winter and feed in the coastal and tidal waters of China (25°N–30°N). From 1986 to 2010, the annual catch of grey mullet decreased substantially and remained low. Although the Pacific Decadal Oscillation and El Niño–Southern Oscillation are recognized to affect gray mullet migration, the increase in sea surface temperature may be the main cause of the aforementioned decrease. We explored how weather changes affect fishing conditions and patterns at the gray mullet fishing grounds in Taiwan's coastal areas. Because of the decrease in gray mullet catches, the most common method for catching gray mullet in Taiwan's coastal areas between 1990 and 2010 was the use of drift or trawl nets instead of two-boat purse-seiner fleets. Since 2012, purse-seiner fleets have become the most common method for catching gray mullet. This trend indicates that the local fishing industry is adapting to changing environmental conditions. The grey mullet populations in the Taiwan Strait have experienced a complete collapse. Therefore, it is imperative for fisheries management organisations to adopt ecosystem-based management strategies in order to ensure the maintenance of biologically viable levels of species stock. By integrating current knowledge into managerial plans, decision-makers may make informed decisions that will also protect the other fisheries of the Taiwan Strait and the people who rely on them amid changing climatic conditions.

EFFECTS OF DIETARY CARNITINE SUPPLEMENTATION ON GROWTH, BODY COMPOSITION, AND LIPID METABOLISM IN GIANT GROUPER, *Epinephelus lanceolatus*, FED WITH HIGH PLANT PROTEIN DIET

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Replacement of fish meal with plant protein source in aquafeed may lead to carnitine deficiency which is a nutrient involved in lipid metabolism in fish. The study was to evaluate the effects of dietary carnitine supplementation on growth performance, body composition, and lipid metabolism in giant grouper, *Epinephelus lanceolatus*, fed with high plant protein diet. The diet containing 29% soy protein concentrate and 25.5% fish meal as main protein sources was used as control. Juvenile grouper (initial weight: 19.50±0.03 g) were fed with five experimental diets with 0.07 (control), 0.18, 0.83, 1.58, and 2.13 g/kg of carnitine in triplicate groups in a recirculation system for 8 weeks. Growth performance, body proximate composition, hepatic thiobarbituric acid-reactive substances, superoxide dismutase and catalase activities in grouper were not significantly ($p>0.05$) different among all dietary treatments. Muscle C16:0, total saturated fatty acid, plasma triglycerides, hepatic and muscle carnitine concentrations increased by the increment of dietary carnitine levels. However, while dietary carnitine levels increased, hepatic γ-butyrobetaine hydroxylase 1 (BBOX1), carnitine palmitoyltransferase-I (CPT-I) and fatty acid desaturase gene expression were significantly decreased. Results indicate that dietary carnitine supplementation can accelerate saturated fatty acids accumulation through the depression of gene expression for fatty acid desaturation and oxidation. Grouper can synthesize required carnitine by inducing BBOX1 gene expression when fish fed the high plant protein diet.

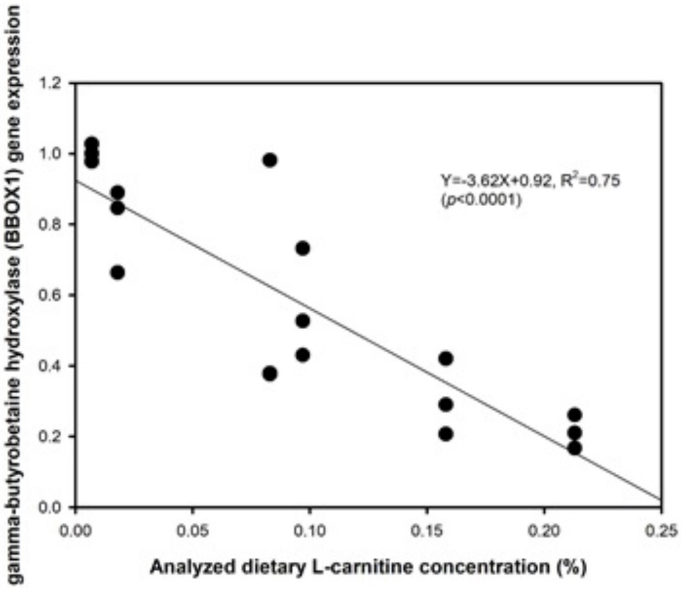

FIG. 1. Linear regression analysis of actual carnitine concentration (%) in diets and BBOX1 gene expression.

TARGETING OF TRIF SIGNALLING IN VIRAL AND BACTERIAL INFECTION: A STUDY ON TRIF$^{-/-}$ ZEBRAFISH

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TRIF serves as a crucial adaptor protein in TLR signaling pathways. TLR3 recruits TRIF directly to initial downstream signaling events leading to the expression of interferon-stimulated genes (ISGs). In aquaculture, it is essential to prevent and control, especially, infections as they can cause high mortality and economic losses in fish.

Gene editing using CRISPR/Cas 9 resulting from the 5 bp frameshift mutation in trif ORF renders it non-functional. The *trif* expression was observed from the two-cell stage, which may underscore its significance in early zebrafish developmental events. The detection of *trif* in neuromast signifies additional functions.

Disease symptoms and the mortality of both WT and *trif* knockout fish were evaluated following the challenge with VHSV and *E. piscicida*. The results revealed that *trif* KO had increased susceptibility with severe symptoms **(Figure A),** accompanied by alterations in downstream gene expression.

The caudal fin of the 5 dpf zebrafish larvae were amputated and immersed in the fluorescent-tagged VHSV (rVHSV). The *trif* KO had significantly higher infection due to the rapid penetration of VHSV through the caudal fin compared to the WT. The *trif*$^{-/-}$ *Tg (mpeg1:GFP;mpx;mcherry)* was developed for the simultaneous tracking of macrophages and neutrophils in real time **(Figure B).** The *trif* KO fish showed a reduced number of immune cells at the injury site when stimulated with poly I: C.

The findings proved that the intricate mechanisms by which Trif contributes to host defense against both viral and bacterial pathogens hold promising avenues for future research.

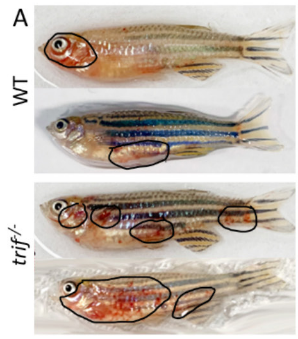

Figure A: Macroscopic appearance of 7 dpi WT and *trif*$^{-/-}$ KO fish infected with $5x10^6$ $TCID_{50}$/mL of VHSV. Hemorrhages are marked by circles

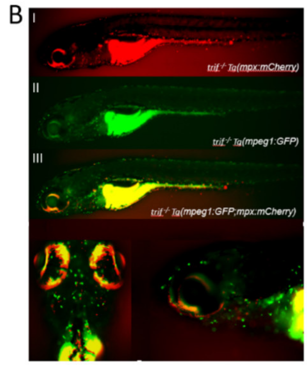

Figure B: Fluorescence imaging reveals signals from mCherry-labeled neutrophils (I) and GFP-labeled macrophages (II), along with merged views of both populations(III)

GENOMIC PREDICTION FOR GENETIC IMPROVEMENT OF LOW-FISHMEAL DIET ADAPTABILITY IN OLIVE FLOUNDER *Paralichthys olivaceus*

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Olive flounder (*Paralichthys olivaceus*) is a carnivorous marine flatfish one of the in-demand aquaculture species in East Asian countries. In most of the olive flounder farms in Korea, moisture pellets (MP) are used as feed. However, MP can easily deteriorate and catalyze microbial proliferation due to its high moisture content, which possesses negative impacts on the nutritional supply and augments the likelihood of disease transmission. Although numerous studies have been conducted on extruded pellets (EP) and fishmeal alternatives to reduce fishmeal content in EP, Korean farmers are hesitant to use EP due to lower growth rates compared to MP. Therefore, developing fast-growing olive flounder strains under low-fishmeal EP regimes is required. In this study, we conducted a genome-wide association study (GWAS) to elucidate the genetic architecture related to low-fishmeal diet adaptability and constructed a genomic prediction model to generate a fast-growing population under low-fishmeal EP regimes.

We used low-fishmeal EP with a 50% reduction in fishmeal content compared to commercial EP and fed from June to October 2023. We measured the growth phenotype every two months and collected caudal fin samples for genotyping using a 70K SNP chip designed by our laboratory. The phenotypic data used for analysis included final weight (WT), weight gain rate (WGR), and specific growth rate (SGR).

GWAS was performed using a linear mixed model with the gaston package in R. As a result, we identified 1, 3, and 1 significant SNPs for WT, WGR, and SGR, respectively, based on the Bonferroni cutoff (The result for WT is shown in Figure 1 as a representative example).

The genomic prediction models were built using 10 different algorithms, and the prediction ability was estimated through 3 repeats of 3-fold cross-validation. As a result, the prediction ability for WT was relatively high compared with WGR and SGR (Table 1). Especially, the Bayes B algorithm exhibited the highest value (Table 1). The findings of this study can be utilized in the development of new olive flounder strains with high low-fishmeal diet adaptability.

Figure 1. Manhattan plot for the final weight

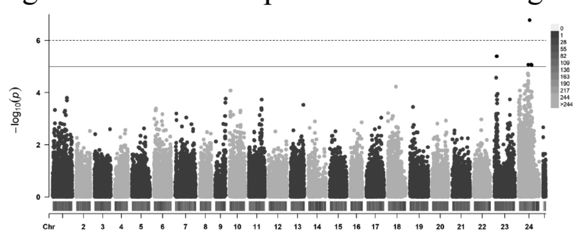

Table 1. Prediction ability estimation (mean)

Algorithms	WT	WGR	SGR
GBLUP	0.595	0.229	0.243
Bayes A	0.591	0.271	0.266
Bayes B	0.607	0.249	0.260
Bayes C	0.584	0.250	0.279
Bayes LASSO	0.592	0.225	0.275
Bayes RR	0.597	0.235	0.246
Elastic Net	0.556	0.238	0.240
Ridge regression	0.599	0.247	0.262
Random Forest	0.533	0.267	0.275
SVM	0.550	0.191	0.219

GENOME-WIDE ASSOCIATION STUDY (GWAS) ANALYSIS IDENTIFIES GENETIC FACTORS ASSOCIATED WITH ACUTE HIGH TEMPERATURE-INDUCED CORTISOL LEVELS

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The Republic of Korea leads the world in farm-raised olive flounder production, supplying nearly half the global output. However, the aquaculture industry in Korea faces significant challenges, including acute temperature elevation during transportation, the amount and form of dissolved oxygen, etc. These temperature-related stresses profoundly impact olive flounders' survival, growth, and immunity. To address these challenges, our study investigated the correlation between cortisol levels, indicating the high-temperature acute stress in olive flounders, using Genome-Wide Association Studies (GWAS) and genomic prediction strategies. By identifying genetic markers associated with stress resilience, we aim to develop acute high-temperature stress tolerance olive flounder traits and mitigate the adverse effects of temperature fluctuations in the Korea aquaculture industry. Therefore, in this study, 384 healthy fish were exposed to 29 °C, and blood was collected at 30 min and 1 h after the exposure for cortisol level measurement. Also, fin tissues were collected from all the individuals for the gDNA extraction. A high-quality 70K SNP chip was utilized to extract 57,638 high-quality SNPs from a cohort of 376 individuals. After the GWAS analysis, significant SNPs were identified based on the Bonferroni cutoff. Moreover, various genomic prediction methods were employed to determine the best model for analyzing the genomic estimated breeding values (gEBVs) within the study population. Our findings collectively indicate that genes significantly associated with high-temperature acute stress conditions and diverse prediction models hold promise for enhancing genetic diversity in flounder breeding within the aquaculture industry, paving the way for future genomic breeding programs.

SEA2SEE – TRACEABILITY FROM FARM TO PLATE IN MEAGRE (*Argyrosomus regius*) RAS AQUACULTURE

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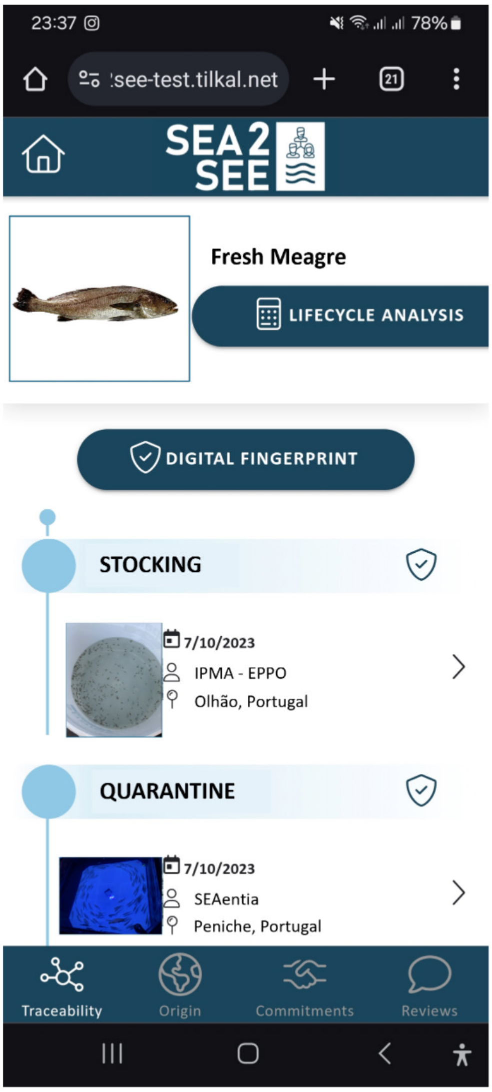
Current seafood traceability tools and services have the potential to take advantage of novel blockchain technologies to obtain a wide range of data and display sustainable seafood practices to consumers. The Sea2See project aims to fill in existing seafood traceability gaps through the development of an innovative end-to-end blockchain model and applications, to increase trust and social acceptance of sustainably fished and farmed seafood.

The project is focused on two main areas of intervention: 1- active commitment of stakeholders and consumer empowerment through the implementation of societal and sectoral strategies for co-creation, communication and awareness raising; 2- provide technological solutions to answer the need of a valuable data source collected throughout the whole seafood value chain and test these solutions in real-life environments.

One of the pilot case studies is SEAentia's RAS meagre farm in Peniche (Portugal), where sensor and production data are uploaded to a farm management software (Smartwater Cloud), and subsequently logged into a traceability platform through blockchain technology.

This network infrastructure allows any authenticated supply chain actors to input data and have complete auditability of the process from hatch until the fish reaches the consumer's plate, thus promoting trust and transparency throughout the whole value chain.

Figure 1 (to the right) - Sea2See traceability demo app.



Note: This demo for the aquaculture use case is still under development. We foresee to have the app ready for showcasing by the time of the AQUA 2024 congress.

Acknowledgements
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FISH FOR HEALTH – IMPROVED NUTRITIONAL QUALITY OF CULTURED FISH FOR HUMAN CONSUMPTION

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The project (5-yr) focusses on the promotion and increased use of fish and seafood products as a more healthy alternative to the consumption of processed red meat products and `fast-foods' in the fight against obesity, coronary heart disease, and associated ailments within the State of São Paulo, with obesity and associated ailments accounting for over 65% of total deaths in Brazil, and health-care costs dealing with overweight and obesity being over US$ 26 billion annually. The project aims to improve the health and well-being of people through the implementation of a multidisciplinary research approach, by 1) a socio-economic market survey concerning the main aquatic food products consumed within the State, 2) measuring the nutrient content and quality of the major aquatic food products consumed 3) monitoring the variability in the nutrient content and quality of major farmed and consumed aquatic food products and the nutrient composition and quality of the locally manufactured aquaculture feeds used to produce them, 4) enhancing the nutrient content and potential health value of farmed species through dietary fortification 5) development of sustainable products with a focus on reducing losses from fish industrialization, 6) improving the nutritional quality using genetic markers and genome wide association studies, and 7) increasing public awareness and understanding concerning the health benefits of fish through the dissemination and publication of project findings. After 20 months of project development results showed though a representative sample (questionnaire respondents) among the most abundant consumer classes (income based), São Paulo State citizens consumption mainly 1-4 times/month, mostly supplied by supermarkets (50%), equally fresh or frozen. The main reason for consumption of aquatic food is elevated nutritional quality and the price is considered high for the eight main species consumed in the state, sourced by fisheries or farming. A review of the locally published literature suggests a more consistent definition of nutrient content in the main aquatic food species consumed is needed and should also consider potential contaminants. In addition, growth of juvenile shrimp (*L. vannamei*) fed plant-based diets could be improved by supplementation of algal oil (EPA+DHA) with no effect upon sensory attributes in comparison to fish meal-based diets, while nutrient content in shrimp muscle responded to increasing dietary levels of algal oil. In genetics, the base population (BP) was established with tilapias from nine different origins covering a significant part of the national territory, and targeted matings were carried out among the BP animals, giving rise to the first selection generation (G_1). **Fish for Health** will keep generating results and new knowledge about the healthy consumption of fish, in addition to developing solutions to improve its nutritional quality, which can symbolize the food of the future, in the context of safety, nutrition and sustainability.

EFFECTS OF TRIBUTYRIN INCLUSION IN HIGH PLANT-BASED DIETS FED TO RAINBOW TROUT *Oncorhynchus mykiss* EVALUATED BY ^{1}H-NMR METABOLOMICS

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Short-chain fatty acids (SCFA) are described as promoters of good welfare state in animals. Butyrate is a SCFA that can be delivered to the intestinal tract through dietary tributyrin (TRB) supplementation. Through lipolysis in the intestine, TRB releases 3 butyrate fatty-acids (FA) and glycerol. TRB has been studied in fish, showing potential regarding its use as a supplement in high plant-based feeds, to ameliorate its adverse effects. The objective of our work focused on testing if the inclusion of TRB in high plant-based diets given to rainbow trout (*Oncorhynchus mykiss*) improved animal welfare and performance. Specifically, we used ^{1}H-NMR (Nuclear Magnetic Resonance) metabolomics to understand if different TRB inclusion percentages caused differences in the metabolite profile of plasma, intestine, liver, and muscle.

Juvenile rainbow trout were fed four experimental diets made up of only 10% fishmeal, 3% fish-oil, and 83% plant-based ingredients with increasing levels of supplementation of TRB, with concentrations of 0%, 0.1%, 0.2% and 0.4%. After 44 days, fish were euthanized at 6 h and 24 h after feeding and intestine, liver, and muscle samples were collected and stored. After separating the polar/nonpolar fractions, the aqueous fraction was analysed through ^{1}H-NMR metabolomics. Multivariate analysis was used to infer if there were differences in metabolite profile between diets.

At 6 h after feeding, we found differences in the metabolite profile of all tissues, regarding all diets. At 24 h after feeding, differences in metabolite profile between all diets were found only in the muscle. These results are in accordance with fish metabolism, that at 6 h post-feeding is at its absorption peak, and at 24 h is at a basal state. Although metabolite profiles presented differences between diets, the inclusion percentages of TRB tested, regarding this species particularly, did not reflect differences in performance parameters (growth performance and feed efficiency), as evaluated in a zootechnical trial (same experimental design). These results can be tied to factors like fish genetics (reared to be predisposed to high plant-based ingredients content), and general superior quality of the plant meals used. As a potential tool in fish nutrition studies, NMR metabolomics seems to complement other trials pinpointing underlying metabolic differences, otherwise unintelligible when focusing only on the classical zootechnical parameters (fish growth, survival rate, feed intake).

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COMMON CHICORY PRODUCTION AND NUTRIENT ACCUMULATION IN FLOATING RAFTS AND SUBSTRATE CULTIVATION METHODS IN AQUAPONICS

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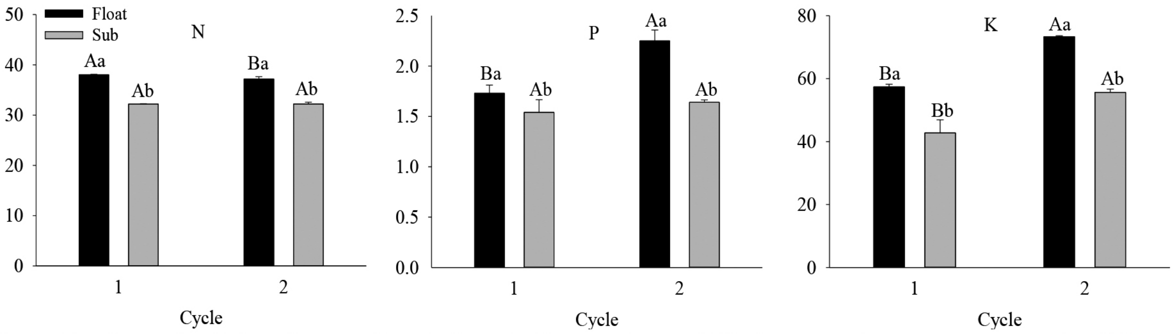
This study assessed the common chicory performance in aquaponics. The experimental setup consisted of an aquaponic system for farming tilapia (*Oreochromis niloticus*) and cultivating common chicory (*Cichorium intybus*), with two interdependent cultivation lines. The aquaponics component consisted of two treatments with six repetitions, comparing the two cultivation methods of (1) floating rafts and (2) substrate in two cycles. The results of the productive performance and the composition of the plant tissue were evaluated, comparing phyto-technical parameters (NL: number of leaves; LLL: longer leaf length; FM: fresh matter; and DM: Dry matter) of each plant between treatments and between cycles for each treatment. Higher yields (g plant^{-1}) were obtained in the aquaponics system with the floating method when compared to the substrate method in the first cycle. In the second cycle, there was a higher yield of vegetables cultivated with the substrate method perhaps due to the accumulation of organic matter in the substrate and mineralization of nutrients. The vegetables grown in the floating method accumulated higher concentrations of N, P and K in the leaf tissues in both cycles. Differences in the mineral composition of common chicory grown in aquaponics suggest that the floating system facilitates prompt absorption of these elements.

Table 1: Growth parameters of common chicory grown in aquaponics in the substrate and floating method in the 1st and 2nd cycle (n = 3).

Treatment	NL			LLL			FM			DM		
	1st cycle	2nd cycle	CV %	1st cycle	2nd cycle	CV %	1st cycle	2nd cycle	CV %	1st cycle	2nd cycle	CV %
Substrate	7.00 bB	9.00 aA	9.34	17.85 aA	17.71 aA	6.67	11.71 bB	14.28 aA	12.78	1.06 bA	1.00 aA	20.08
Floating	9.00 aA	8.00 bB	8.4	17.21 aA	12.42 bB	10.06	18.07 aA	7.07 bB	17.36	1.61 aA	0.93 bB	26.01
CV%	9.75	7.96		8.07	8.46		17.06	9.68		25.49	19.6	

NL: number of leaves; LLL: longer leaf length; FM: fresh matter; DM: Dry matter. Lower-case letters indicate difference between treatments for each cycle (lines) and upper case letters indicate differences between cycles for the same treatment (columns) (Tukey, p <0.05).

Figure 1: Concentration of N, P and K in the leaf tissue of the common chicory grown in aquaponics in the substrate and floating methods in the 1st and 2nd production cycle.



Float: floating rafts; Sub: substrate;Identical capital letters do not differ between the same treatment in different cycles and equal lower-case letters do not differ between treatments in the same cycle (Tukey, p <0.05).

COMMON CHICORY PRODUCTION IN SOIL FERTILIZED WITH AQUAPONIC SLUDGE: CULTIVAR PERFORMANCE AND NUTRIENT ACCUMULATION

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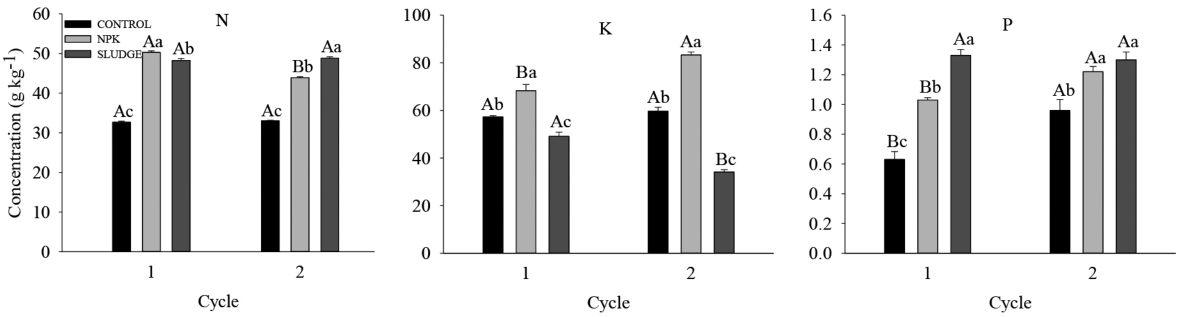
This study assed the common chicory (*Cichorium intybus*), performance in soil fertilized with aquaponics sludge. The experimental design consisted of three treatments with three repetitions: (1) fertilization with aquaponic sludge; (2) chemical fertilization (NPK); and (3) control (without fertilization). The sludge (80 L) was collected twice a week from the bottom of two clarifiers, homogenized, and later applied to the soil. The results of culture performance and the composition of the plant tissue were evaluated, comparing the phytotechnical parameters of each vegetable between treatments in the soil and a comparison between cycles for each treatment. The production of common chicory in the 1st cycle showed no differences between the Sludge and NPK treatments, and both were superior to the Control in all phytotechnical parameters. In the 2nd cycle, there were differences in the parameters of the length of the largest leaf, fresh mass and dry mass, with higher values registered in plants fertilized with sludge than in those treated with NPK or Control. This results suggests a cumulative effect of nutrients in the soil after successive sludge applications combined with the mineralization of nutrients, making them available in soil. Higher concentrations of P in the leaf tissue of the common chicory in the Sludge treatment suggests higher rates of absorption of this element. The aquaponic sludge can be used to fertilize vegetables in the soil and obtain yields equivalent to or even higher than those of mineral fertilization.

Table 1. Growth parameters of common chicory grown in the soil in the 1st and 2nd cycles:

Treatments	NL			LLL			FM			DM		
	1st cycle	2nd cycle	CV%	1st cycle	2nd cycle	CV%	1st cycle	2nd cycle	CV%	1st cycle	2st cycle	CV%
CONTROL	6.00 bB	8.00 aA	9.39	12.25 bA	12.87 cA	6.85	3.50 bB	5.12 cA	20.44	0.36 bB	0.68 bA	3.83
NPK	7.00 aB	8.00 aA	9	17.25 aA	15.62 bB	8.51	13.50 aA	10.25 bB	17.93	1.07 aA	1.00 bA	11.4
SLUDGE	7.00 aB	8.00 aA	8.57	18.25 aA	19.70 aA	9.64	15.12 aB	20.75 aA	18.15	1.18 aB	1.75 aA	24.95
CV%	10.1	8.03		9.74	7.94		25.36	14.93		19.74	27.83	

NL: number of leaves; LLL: longer leaf length; FM: fresh matter; DM: Dry matter. Capital letters indicate difference between the same treatment in different cycles (lines) and lower-case letters indicate differences between treatments in the same cycle (columns) (P <0.05).

Figure 1. Concentration of N, P and K in the leaf tissue of the common chicory grown in the soil in the NPK, Sludge and Control treatments in the 1st and 2nd production cycle.



Equal capital letters do not differ between the same treatment in different cycles and equal lower case letters do not differ between treatments in the same cycle (Tukey, p <0.05).

DEVELOPMENT AND APPLICATION OF HIGH-THROUGHPUT ENVIRONMENTAL NUCLEIC ACID (eNA)-BASED CHIP FOR PROFILING WATER QUALITY, OFF-FLAVOR AND PATHOGENIC AGENTS IN RECIRCULATED AQUACULTURE SYSTEMS (RAS)

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Debes Hammershaimb Christiansen, Louise von Gersdorff Jørgensen, Helene Larsen, Peter M. H. Heegaard

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Recirculated aquaculture systems (RAS) represent a promising approach to improving sustainable aquatic food production for human consumption through reuse of water resources and management of nutrient discharge. However, several challenges remain, including management of disease outbreaks, maintaining high water quality, and controlling microbial off-flavor compounds, calling for effective methods of early monitoring of microbial pathogen density and dynamics. The rapidly advancing field of environmental nucleic acid (eNA)-based monitoring offers opportunities for cost-effective and non-invasive tools for the identification and quantification of novel and known microorganisms. It is a promising method for the development of early monitoring and warning systems and for integrating high-throughput molecular technology in RAS facilities. eNA offers fresh perspectives on aquatic animal health condition monitoring and for advancement of aquacultural biosecurity.

This study focuses on a comprehensive, early eNA-based monitoring of pathogens and off-flavor producers, including method development and downstream application on RAS farms. Optimized procedures for eDNA collection and extraction methods were established to mitigate environmental inhibitors and to ensure adequate eNA yields. Subsequently, a novel eNA-based qPCR chip was developed by using a high-throughput Fluidigm platform. The chip is capable of rapid detection of multiple targets/indicators in parallel in large sample sets (e.g., 48 assay with 48 samples, generating 2304 datapoints), providing a powerful tool for comprehensive pathogen quantification and hence for monitoring health risks to improve management before uncontrollable disease breakouts and economic loss. Currently, a repertoire of 60 primer pairs has been devised to target an array of variables, including pathogenic (bacterial/viral/parasitic) agents, off-flavor producers, and associated factors (e.g., stress indicators). These assays are adaptable for customized combinations according to different host organisms and interests and can be seamlessly integrated into the chip platform for efficient implementation. Preliminary application has been carried out on RAS farms in Denmark, the Faroe Islands and Hungary, comprising rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and European catfish (*Silurus glanis*) aquaculture.

SYNERGIC EFFECTS OF DIETARY NUCLEOTIDES AND VACCINE TO BOOST HEALTH OF EUROPEAN SEABASS *Dicentrarcus labrax* JUVENILES

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Fish vaccines help enhance the immune response , thereby improving their disease resistance and overall health, reducing environmental pollution and disease transmission caused by high-density aquaculture. The use of vaccines may have some side effects such as adhesion and pigmentation. Nucleotides (NT), as a component of nucleic acids, are expected to serve as dietary supplements in aquaculture to enhance the immune and disease resistance of fish. This study aims to investigate the potential benefits of combining a diet enriched with nucleotides alongside vaccination. The effects of dietary nucleotides on mitigating side effects associated with vaccine injection are assessed through the evaluation of their combined impact on immune responses through the detection of specific immune markers.

45 fish juveniles (initial weight: 51.54 g ± 0.3g) were assigned into one of four treatments: (A) Diet with NT + Vaccinated fish (B) Diet with NT + Unvaccinated fish (C) Standard diet + Vaccinated fish (D) Standard diet + Unvaccinated fish. After feeding the control or nucleotide supplemented diet for 12 weeks, the fish in treatment A and C were injected intraperitoneally with the ICTHIOVAC VR/PD vaccine (0.2 ml/fish). The fish from treatment B and treatment D did not receive any vaccine, but were injected with PBS (0.2ml/fish). Fish were sampled 12 weeks after the beginning before vaccination (T1) and one month after vaccination (T2). To evaluate and monitor the development of potential side effect of the vaccine (pictures of the peritoneal cavity and blood analyses) between T1 and T2, 3 fish per tank were sampled weekly. While for initial (T0), before vaccination (T1) and final sampling (T2), growth performance indices, incidence of intra-abdominal adhesions and pigmentation (images), intestinal and skin histology, gene expression of skin and head kidneys, plasma, blood leukocytes and mucus were analysed.

Preliminary results showed that growth performance of the fish fed with nucleotides has no apparent difference after 12 weeks from the beginning of the trail (Fig. 1A). Vaccination caused adhesion and pigmentation, however we observed that, in the nucleotide diet treatments, the severity of those side affects was less than than in the control treatments. This may indicate that dietary nucleotides potentially reduce vaccine side effects. Similar occurance of side effects were observed in both treatments two weeks after vaccination.We expect that dietary nucleotides will mitigate stress reactions caused by vaccination, and further enhance the immune ability of the fish. Dietary nucleotides could play a positive role in promoting the recovery of European seabass from vaccine effects and further enhancing their immune capacity, exploring the role of nucleotides as a kind of aquatic feed additive.

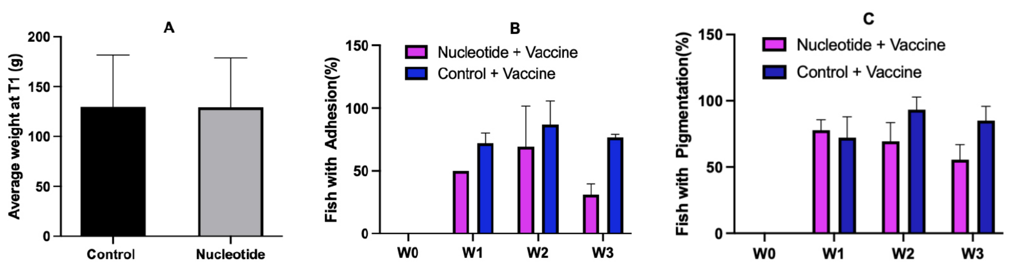

Fig. 1: Growth performance (A), Percentage of fish with adhesion (B), Percentage of fish with pigmentation (C).

GENERATION OF GOLDEN GOLDFISH *Carassius auratus* VIA TYROSINASE GENE TARGETING BY CRISPR/CAS9

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Goldfish (*Carassius auratus*), regarded as one of the world's earliest ornamental fish, has garnered significant attention from researchers due to its diverse range of color patterns and unique morphological variations. In our study, we have successfully developed a highly efficient and precise genome editing technology for *Carassius auratus tyr*osinase (*tyr*), resulting in the creation of a strikingly golden goldfish. The duplicated *tyr* genes (*tyrA* and *tyrB*) were first identified in *C. auratus*, and the CRISPR/Cas9 was used to disrupt both *tyr* genes. The edited albino mutants displayed a complete absence of melanocytes in both their eyes and body surface, whereas mosaic mutants exhibited varying degrees of melanin reduction. Notably, disrupting only *tyrA* or *tyrB* failed to yield a reduction in melanin content.

Six sgRNAs were designed to target the exons 1 and 3 of *tyr* gene using an online tool CRISPOR (Fig.1). The results of Sanger sequence revealed that *tyr*-sgRNA1 and *tyr*-sgRNA2 were effective in guiding Cas9-induced mutagenesis, showing evident multiple peaks near PAM sites of *tyr*A and *tyr*B (Fig.2). The color phenotypes of these edited fish were continuously monitored and documented at the time points of 10, 180, and 360 dpf (Fig.3). The observed phenotypes of the edited fish can be classified into two categories based on body and eye coloration: albino mutants and mosaic mutants. Complete albino mutants exhibited a complete absence of melanocytes both in their eyes and on their body surfaces. The mosaic mutants, also known as incomplete albino mutants, were characterized by varying degrees of decrease in melanin on the body surface and eye. Co-injection of the *tyr*-sgRNA1/*tyr*-sgRNA2/Cas9 mixture into embryos resulted in a phenotypic rate of 44.1% for albino mutants and 40.9% for mosaic mutants.

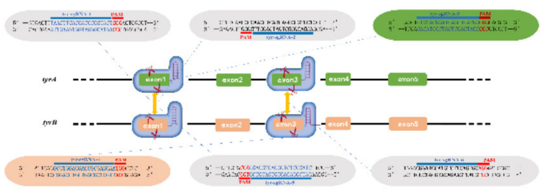

FIGURE1. The schematic diagram of *tyr*-sgRNAs.

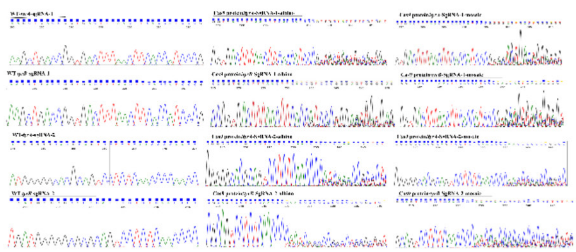

FIGURE2. The Sanger sequencing of PCR products in the injected embryos.

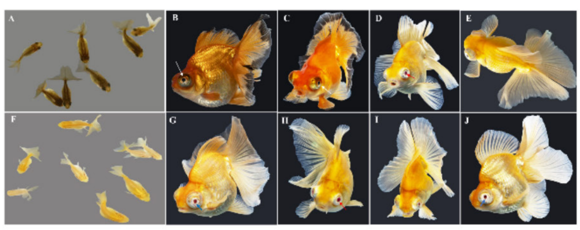

FIGURE3. The albino mutants injected with the mixture of *tyr*-sgRNA1/*tyr*-sgRNA2/Cas9 protein.

RECONSTRUCTION OF THE ORIGIN OF A NEO-Y SEX CHROMOSOME AND ITS EVOLUTION IN THE SPOTTED KNIFEJAW *Oplegnathus punctatus*

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Sex chromosomes are a distinct element of the genome, particularly due to the evolutionary forces that drive the fixation of the primary sex-determining gene, leading to genic degeneration and accumulation of junk DNA in the heterogametic sex. Among the most noteworthy occurrences in sex chromosome evolution is the emergence of neo-Y chromosomes, giving rise to X1X2Y sex-determining systems. These neo-sex chromosomes are critical in elucidating sex chromosome evolution, as they rejuvenate their genetic content.

The spotted knifejaw (*Oplegnathus punctatus*), a species with a cytogenetically identifiable neo-Y chromosome, has had its male and female genomes assembled at the chromosomal level. The comprehensive assembly and annotation of all three sex chromosomes have facilitated the reconstruction of their evolutionary trajectories. Unlike other neo-Y chromosomes, the fusion with X2 is remarkably ancient, dating back to approximately 48 million years ago (Mya). However, the genetic degradation of the neo-Y appears to be relatively moderate. Transcriptomic analyses reveal that the sex chromosomes harbor 87 genes, potentially serving crucial functions in the testis. The accumulation of such male-bias genes and the fusion with X2 appear to be the primary forces shaping the evolution of the neo-Y in the spotted knifejaw. Additionally, we have upgraded the genome assembly of the closely related species striped knifejaw (*Oplegnathus fasciatus*), achieving an almost complete T2T-level assembly. These provide an important foundation for further elucidating the origin and evolution of multiple sex chromosomes in fish.

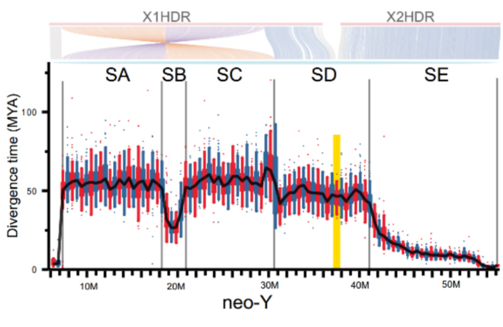

Fig. 1 Divergence times along the neo-Y in a sliding window of 100 kb.

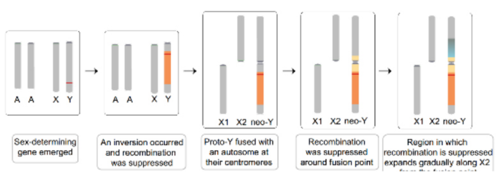

Fig. 4 Model for the evolution of the Y chromosome in spotted knifejaw.

ACHIEVEMENTS OF GENOMIC SELECTION FOR PARASITE RESISTANCE IN LARGE YELLOW CROAKER

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The large yellow croaker (*Larimichthys crocea*) is one of the most economically important marine aquaculture species in China, with an annual production exceeding 258,000 tons, the highest among all marine aquaculture species in China. However, the rapid development of the large yellow croaker industry has been hampered by outbreaks of various pathogenic organisms, leading to significant economic losses. The most severe disease affecting this species is white spot disease, caused by the ciliate parasite *Cryptocaryon irritans*. The prevalence of this disease is exacerbated by increasing temperatures.

C. irritans invades and colonizes the fish's skin, eyes, and gills, causing irritation, epithelial hyperplasia, respiratory difficulty, and skin lesions, which eventually lead to fish mortality. Due to the lack of cost-effective and labor-efficient measures for disease prevention and control, our research group aims to utilize genomic selection (GS) to develop new strains of large yellow croaker with enhanced *C. irritans* resistance.

To achieve this goal, we first conducted a genome-wide association study (GWAS) on resistance to *C. irritans*, finding that the trait is controlled by a few quantitative trait loci (QTL) with small effects and numerous loci with micro-effects. Using this information, we established and optimized a GS model to calculate genomic estimated breeding values (GEBV), enabling the selection of candidates with the highest likelihood of possessing parasite resistance. With the assistance of genomic selection, we were capable of generating two independent resistant strains within two years.

Over an eight-year period, we developed two resistant strains of large yellow croaker through three generations of genomic selection, which exhibited significantly improved resistance to *C. irritans* after challenge tests. One strain showed a survival rate of 43.6% at 96 hours post-challenge, compared to 6.3% in the control group. The parallel resistant strain displayed a survival rate of 89.9% at 96 hours post-challenge, while only 14.2% of the control group survived. These consistent results highlight the potential of GS applications in the genetic improvement and selective breeding of large yellow croaker for resistance to *C. irritans*. Our study also demonstrates the successful application of genomic selection for disease resistance, contributing to sustainable aquaculture and blue food production in the context of global climate change.

PREDICTING AQUACULTURE CONDITIONS AND QUALITIES OF PACIFIC WHITE SHRIMP USING DEEP LEARNING AND MULTISPECTRAL IMAGING

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The Pacific white shrimp (*Litopenaeus vannamei*) is a globally prominent aquaculture crustacean adaptable to diverse habitats. Identifying common features for predicting production methods, spoilage indicators, and freshness in shrimp is crucial for enhancing quality and consumer satisfaction. However, the influence of environmental factors during growth on aquatic product quality remains unclear.

This study aimed to develop a deep learning model for predicting aquaculture conditions directly from raw Pacific white shrimp. Raw shrimp samples and corresponding aquaculture data (type, salinity, pH, sampling month) were collected from seven farms across three Taiwanese counties. Compact deep learning models with 6 to 12 hidden layers and less than 350k parameters were built and trained using custom Python code based on TensorFlow-Keras libraries. ReLU activation function, Adam optimizer, and mean squared error were employed for training the regression models.

Multispectral imaging (MSS), a non-invasive technique, was used to capture reflection spectra (26 wavelengths between 369490 nm) from 970 raw shrimp samples. All four tissue regions (eye, head, trunk, tail) and their combinations exhibited similar Mean Absolute Percentage Error (MAPE) during model validation, ranging from 0.7% to 12.2%. Subsequently, the best models were tested on data from two new shrimp farms, achieving a MAPE of 21.8±11.5% (best) for the eye-trunk combination and 35.1±17.0% (worst) for the head-eye combination.

Our compact deep learning models effectively predicted aquaculture conditions with acceptable error using raw shrimp data. These findings suggest a potential link between aquaculture conditions and post-harvest product characteristics like shelf life and quality, which is valuable for both producers and consumers. The developed models hold promise for optimizing white shrimp aquaculture practices and promoting sustainable aquatic food production

EFFECTS OF POPULATION AND PHOTOPERIOD ON ACCUMULATION OF POTENTIAL TOXIC ELEMENTS IN SUGAR KELP

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Sugar Kelp (*Saccharina latissima*) dominates as a commercially produced seaweed species in Norway, and accumulation of potential toxic elements (PTE) is one of the obstacles for a wider use of the biomass. The aim of this study was to compare PTE accumulation from two geographically distant Sugar kelp populations in Bergen (west in Norway) and Tromsø (north in Norway) when grown in a standardized environment to reveal possible population effects on PTE accumulation.

From each population ~800 young sporophytes were produced and divided into 10 flow-through seawater tanks with overhead lighting, 5 tanks with 8 hour light per day and 5 with 12 hour light per day. After 7 weeks sporophytes from each population-tank combination were pooled and analysed for arsenic, cadmium, and iodine content.

While arsenic was unaffected by population and photoperiod, increased daylength tended to increase cadmium content while reduce iodine content (Figure 1). The Bergen population accumulated more cadmium and less iodine than the Tromsø population and was more sensitive to photoperiod. This implies that genotypes may vary in chemical composition when grown under the same conditions. Selecting the optimal genotype may reduce PTE content and increase the value of the sugar kelp biomass for food, feed, soil amendment or other purposes. The population differences may be local adaptation of the populations to different environments along the Norwegian coast.

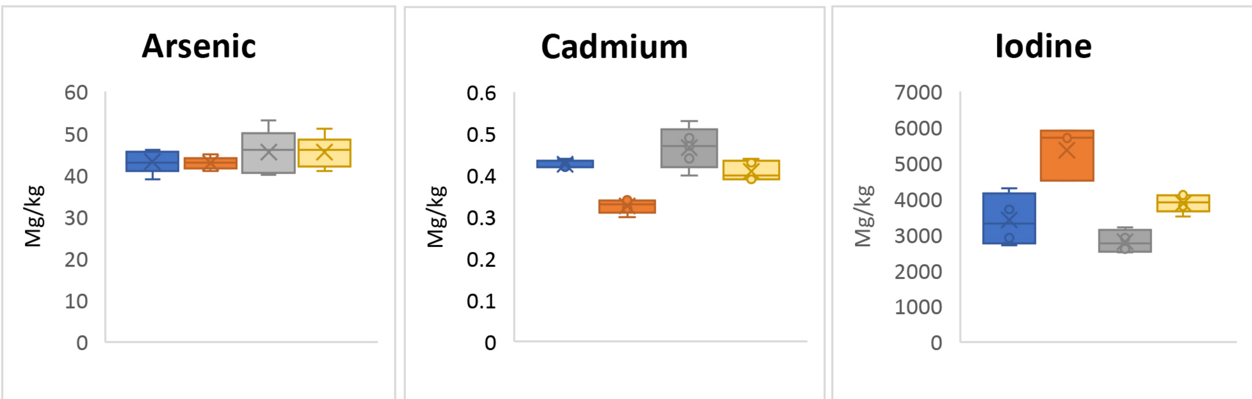

Figure 1. *Concentration of Arsenic, Cadmium and Iodine at the end of the experiment.* ***Blue box****: Bergen population on 8 hour light;* ***Orange box****: Tromsø population on 8 hour light;* ***Grey box****: Bergen population on 12 hour light;* ***Yellow box****: Tromsø population on 12 hour light.*

GENOME-WIDE ASSOCIATION STUDY OF VHS (VIRAL HEMORRHAGIC SEPTICEMIA) RESISTANCE TRAITS USING HIGH-DENSITY SNP ARRAY IN OLIVE FLOUNDER (*Paralichthys olivaceus*)

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Viral Hemorrhagic Septicemia (VHS) represents a significant threat to aquaculture, causing substantial losses in olive flounder (*Paralichthys olivaceus*), a species of paramount importance in marine aquaculture of South Korea. In this study, Genome-Wide Association Study (GWAS) for VHS resistance traits (survival: SUR, days post challenge: DPC) was conducted to uncover genetic parameter and direct or indirect connection between genotype and phenotype of the target trait.

This study aimed to assess performance of 4 different GWAS models (Simple Linear Regression: SLR, Linear Mixed Model: LMM, Logistic model: Logit, Bayesian model: Bayes) for detecting significant SNPs related VHS resistance traits in olive flounder.

A total of 2,398 olive flounders, including VHS-resistant and susceptible groups, were genotyped using a 120K SNP chip designed specifically for this species. In the case of SUR trait, 4 SNPs showed significance ($p < 1 \times 10^{-6}$) in the logistic regression model, while mixed logit model revealed only 1 SNP. SLR and LMM model detected 96, 80 significant SNPs associated with DPC trait respectively and the effects of SNPs using Bayes model were shown. As SLR and LMM were used for SUR trait, lambda was found to be 2.043 and 1.001, respectively. For DPC trait with the same models, lambda was confirmed as 1.207 and 0.983, respectively. Both SUR and DPC traits showed that the expected and observed p-values exhibit non-linear when using SLR, with lambda values deviating from 1 compared to results of LMM. This indicates that overestimation is relatively severe, suggesting it might not be suitable for exploring VHS resistant trait-associated variations.

The findings from this study not only enhance our understanding of the genetic background of disease resistance in olive flounder but also provide valuable markers for selective breeding programs aimed at improving VHS resistance in this species.

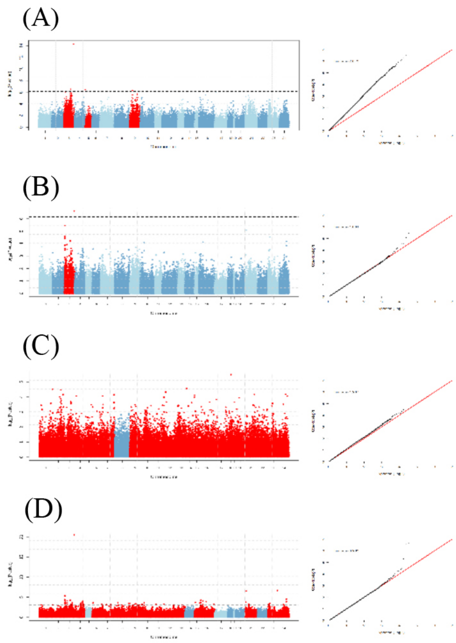

FIGURE 1. Manhattan plots and qq plots for VHSV resistance traits(SUR, DPC). (A) and (B) were shown result for SUR trait using logistic regression model and mixed logit model respectively. (C) and (D) were shown result for DPC using SLR and LMM.

ENHANCING FISH VACCINE EFFICACY THROUGH IMMUNE MOLECULAR AUGMENTATION: INSIGHTS FROM GENE EXPRESSION ANALYSIS AND CHALLENGE TRIALS

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In the aquaculture industry, disease control remains a significant concern, with vaccination being a pivotal strategy due to its cost-effectiveness and long-lasting protection. However, limited understanding of fish immunology has hindered the development of effective vaccines, often relying on trial-and-error methods. This study aimed to investigate immune-related gene expression during the vibrio vaccination incubation period, elucidating key immune pathways in orange spotted grouper (*Epinephelus coioides*). By comparing survival rates in challenge trials and identifying highly expressed genes associated with vaccine protection, we found distinct differences in the expression of immune-related genes, notably IL-6 and TNF-α.

Subsequent administration of recombinant IL-6 and TNF-α proteins revealed their ability to induce $CD4^+$ and $CD8^+$ lymphocyte proliferation via the Th2 and Th1 pathways, respectively. Further experiments involved adding these proteins to vibrio vaccine contain commercial adjuvant Seppic Montanide™ ISA 763A to enhance immune effects. Results showed that IL-6 addition surpassed commercial adjuvants alone, and surpassed TNF-α addition in specific antibody potency, while TNF-α addition outperformed IL-6 in terms of protection, and better than commercial adjuvants alone. These findings underscore the importance of modulating Th1 and Th2 lymphocyte proliferation for vaccine efficacy. Incorporating these immune molecules into vaccines significantly improved survival rates in pathogen challenge tests, offering insights into fish immune responses and suggesting a promising approach for enhancing vaccine efficacy.

NATURAL ANTI-PARASIDIC AND ANTI-MICROBIAL INGREDIENTS SOLUBILIZED BY A NATURAL SOLUBILIZER FOR CONVENTIONAL AND ORGANIC AQUACULTURE APPLICATIONS

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Many essential oils are known to possess anti-parasitic and anti-microbial properties including the monoterpene-dominated Tea Tree Essential Oil (TTEO) and phenol-abundant Clove Bud Essential Oil (CBEO). However, water-solubility limits uniform dispersion thus bio-accessibility of these compounds in the first place with microbial degradation that follows. Enhancing solubility and maintaining stability in aquacultural environments apparently hold the key to successful medications. Compared with the use of emulsifiers such as Tween 80, a natural solubilizer isolated from Stevia leaves was used to create water-soluble essential oils. TTEO and CBEO were each solubilized to a 5% water-soluble concentrate. The 5% concentrate of TTEO was diluted to 1, 0.5, 0.1, 0.05, and 0.01% without losing clarity (Figure 1). The 5% CBEO concentrate (Figure 2) behaved the same, completely dilutable. The wide range of clear dilutions from 50,000 to 100 ppm would allow accurate design of desired concentrations for achieving anti-parasitic and anti-microbial efficacy with minimal unwanted toxicity or side effect attributed from product ingredients. Even though TTEO is reportedly anti-microbial and can be used alone but CBTO was found to be at least 10-fold more potent. The two water-soluble concentrates are freely miscible, enabling any desired combinational concentrations composed of two classes of volatile compounds. The solubilizer is generally regarded as safe (GRAS) by the US FDA. Clean-label products can be built by using water-soluble TTEO and CBEO ingredients. Efficacious and safe concentrations can be delivered orally or topically. Immersion in TTEO at 40 ppm (0.004%), for example, was reported effective against the parasitic Dactylogyrus spp[1]. Both the concentrate and its dilutions were found stable physically (no separation), chemically (no degradation), physiologically (across pH from 1.8 to 8), and biologically (no microbial growth). Moreover, such formulations for oral or bath are organic solvents-free, chemical preservatives-free, and emulsifiers-free. In fact, it is 100% botanical. Obtaining samples for evaluation toward collaborative development is open.

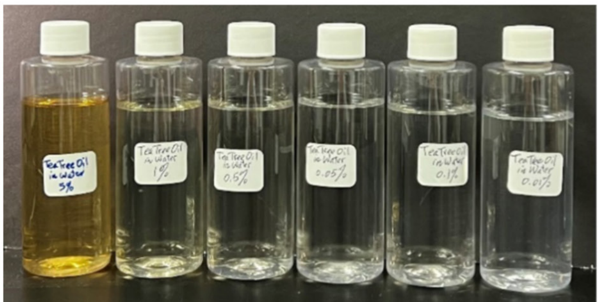

Figure 1. Water-soluble TTEO concentrate and its dilutions enabled by the use a GRAS solubilizer. They are stable without any use of organic solvents, emulsifiers, or chemical preservatives. TTEO is reportedly anti-parasitic.

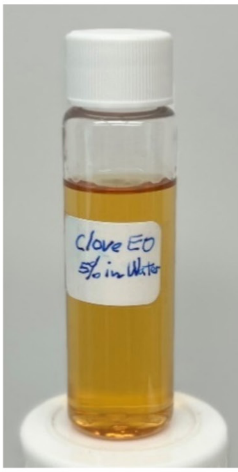

Figure 2. Water-soluble CBEO concentrate enabled by the use a GRAS solubilizer. It is freely dilutable without losing clarity. CBEO is one of the most potent anti-microbial essential oils.

PROGRESS IN TREATING AMOEBIC GILL DISEASE USING TRYPANOCIDAL DRUGS: SUCCESS IN VITRO AND IN VIVO

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Amoebic gill disease (AGD) is a devastating disease that causes multi-million-dollar losses annually in salmonid fish farming. The causative agent of AGD in Atlantic Salmon is *Neoparamoeba perurans* which belongs to the *Paramoebidae* family. An interesting feature of most of the *Paramoebidae* is the endosymbiotic relationship they have with the *Perkinsela-like organism* (PLO). The PLO is a kinetoplastid, distantly related to diseases causing parasites in humans such as *Leishmania* and *Trypanosoma* spp. and fish diseases such as *Ichthyobodo* spp. As there appears to be a high level of metabolic interdependence between host and the symbiont, eliminating the PLO, which resides adjacent to the nucleus of its host, will hypothetically eliminate *N. perurans*. In pursuit of this goal, we have opted to utilise frontline and experimental trypanocidal drugs targeting the PLO both *in vitro* and *in vivo*. As *N. perurans* cannot be cultivated axenically, conventional drug screening methods face hindrance due to bacterial activity within the amoebic culture. To assess *in vitro* efficacy, holographic microscopy was utilised to evaluate cell motility and viability under drug pressure. Drugs demonstrating promising *in vitro* efficacy (figure 1) were subsequently tested in two rounds of *in vivo* trials on the West Coast of Ireland which had exhibited encouraging signs of effectiveness (figure 2). It is anticipated that establishing a market for trypanocidal drugs in aquaculture could enhance fish welfare and potentially reduce production costs for the application of these drugs in a medical context within the tropics.

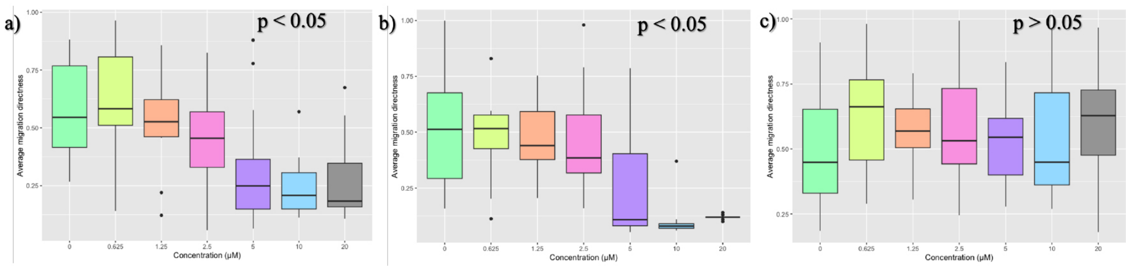

Figure 1: the average migration directness of *Neoparamoeba perurans* after 6 hrs of being treated with different trypanocidal drugs: a) Drug A, b)Drug B, and c) Drug C, at concentrations ranging from 20 μM to 0.625 μM..

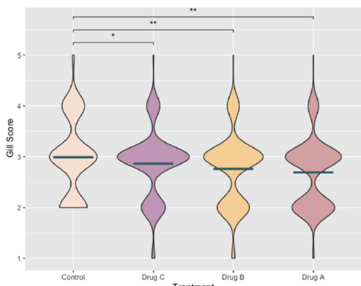

Figure 2: the gill scores obtained from an *in vivo* drug trial in which fishes (n= 1231) with AGD received two doses of either Drug A, Drug B, Drug C, or control over a two-week period. A statistical analysis was done to assess for significant differences in gill scores among fish receiving different treatments; * indicates $p < 0.05$, ** indicates $p < 0.01$.

RESEARCH ON THE DOUBLE CARBON GOAL AND REALIZATION PATHS OF FISHERIES IN CHINA

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Under the background of carbon neutralization, setting and implementing the dual carbon goal with the characteristics of the fishery industry has practical significance for promoting the green and high-quality development of China's fishery and assisting the national "dual carbon" strategy. Based on the existing carbon emission and carbon sink accounting methods, combined with the reality of the industry, this paper explores and clarifies the "double carbon" goal of China's fishery and the path to achieve it. The research found that: (1) From 2011 to 2020, due to the double influence of the expansion of fishery economy and the policy of "reducing ships and changing production", the carbon emissions of fisheries increased first and then decreased, but it remains to be seen whether the carbon emissions reached a peak; first carbon source. (2) Affected by the rapid development of aquaculture, the fluctuation of fishery carbon sinks has increased, and aquaculture has become the primary source of carbon sinks. (3) The fishery has dual attributes of carbon source and carbon sink, and it is more a carbon source, with over 30 million tons of carbon not being neutralized. (4) Based on the medium- and long-term demand pressure for aquatic products and the green and high-quality development of fishery, set industry-specific dual-carbon goals for fishery. Based on the research conclusions, this paper proposes the energy saving and consumption reduction of fishing boats, aquaculture power consumption, pollution control and carbon reduction, as well as the ecologicalization of aquaculture, optimization of structure, and increase of ocean pasture sinks, etc., in order to promote the practice of the industry and achieve the dual carbon goal.

ARCTIC CHARR PERSONALITY TRAITS OVER DEVELOPMENT: INFLUENCE OF SHELTER PRESENCE AND RELATIONSHIP WITH WELFARE AND PERFORMANCE IN AQUACUTURE

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Animal personality refers to behavioural differences that are consistent across time and contexts. Consistent traits and their link with farming performance have already been shown in several fish species. However, the underlying mechanisms of fish personality and its development over time remain unclear. Covering this gap would improve fish welfare and its link with farming performance.

Moreover, the consistency of fish behavioural traits is often examined over short periods of time, despite the goal of improving welfare throughout the entire development of the fish, from hatching to slaughter. Thus, examining personality traits over longer time periods would enhance current knowledge about fish welfare.

Juvenile Arctic charr (*Salvelinus alpinus)* from an Icelandic aquaculture strain were raised either in plain tanks or with the presence of a shelter. The fish were kept in a bi-parental design to account for family effects. Shortly after first feeding, eight fish per tank were VIE tagged. Then they were individually submitted to an open field test and an emergence test to assess boldness and exploration, respectively. Each fish underwent both tests three times to evaluate the repeatability of these traits. We then assessed the relationship between boldness, exploration, and individual growth. Additionally, we compared boldness and exploration of fish between the two treatments and among families. To assess long-term repeatability of those traits, the same fish will be tested for these personality traits again later this year. This oral presentation aims at providing the preliminary results obtained for boldness, exploration and growth in the early stages of development.

ON-FARM GENOMICS FOR SELECTION AND MANAGEMENT IN AQUACULTURE: TANK-SIDE GENOTYPING

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Aquaculture breeding programs are increasingly using genomic technologies, for applications such as parentage assignment, marker assisted selection and genomic selection. Currently, samples are shipped to genetic labs for genomic processing. The turnaround-time to receive genotyping data can be variable and lab work can be expensive. The major drawback of this process in the context of breeding programs is that large numbers of selected animals to be potentially selected as broodstock must be maintained during this time in dedicated facilities until their estimated breeding values (EBVs) can be determined, which can be costly with daily husbandry and hatchery maintenance.

A potential solution to improve the efficiency of the selection process aquaculture breeding programs that incorporate genomics, could be the use of portable next-generation-sequencing (NGS) technologies. These tools can rapidly and affordably derive genotypes for parental verification and calculate accurate EBVs for genomic selection. In addition to sequencing selection candidates tank-side, these tools can also be used for disease monitoring. The ability sequence, identify, and treat infected organisms immediately could save aquaculture enterprises from detrimental losses.

The first step for “tank-side genotyping” is rapid extraction of DNA from fin clips. We have tested a range of rapid extraction protocols, including kits available on the market and one rapid extraction method (Table 1). The method known as the “dipstick method” (Zou et al., 2018), was created to extract DNA with minimal lab equipment.

The dipstick method with an additional bead clean up shows promise that rapid DNA extractions are possible for tank-side genotyping. This could be the start of addressing current limitations in genomics for aquaculture.

Table 1. Results of DNA extractions from fin clips of the best preforming kits and methods.

Kit	Sample #	Protocol completion (mins)	DNA (ng)	260/280	260/230
PureGene kit	1	3,200	1,330	1.7	0.82
	2	3,200	2,350	1.89	1.04
	3	3,200	1,825	2.14	1.09
Dipstick method	4	250	1,160	1.77	0.91
	5	250	880	1.73	0.87
	6	250	800	1.9	1.05
Dipstick method + bead clean up	7	390	8,600	1.93	2.02
	8	390	15,600	1.83	1.78
	9	390	8,250	1.7	1.08

GONAD DEVELOPMENT, REPRODUCTIVE PHYSIOLOGY AND GONAD LIPID COMPOSITION DURING GAMETOGENESIS OF FLATHEAD GREY MULLET *Mugil cephalus* MOVED FROM FRESHWATER TO MARINE CONDITION

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In euryhaline teleosts, salinity may affect gonadal development and reproductive steroidogenesis. The interest on the euryhaline flathead grey mullet (*Mugil cephalus*) is growing due to its highly sustainable and profitable rearing. However, applicable protocols for commercially farming the animals are currently absent as mullet fails to complete gametogenesis in captivity. This study aims at i) verifying if the transfer of fish from fresh- to saltwater conditions triggers the gonad development; ii) describing the physiological cascade leading to sexual maturation; iii) assessing the lipidomic biochemical changes in gonads and fillets during maturation.

A sub-sample of 3 years old *M. cephalus* produced at IMC hatchery and farmed in freshwater ponds were transferred at the beginning of the reproductive season (July) into seawater in a lagoon enclosure. We monitored i) the gonad development ii) the physiological reproductive status (steroid hormones concentration and gonadotropins receptors gene expression), iii) the lipid composition of gonad and fillet. Equivalent samples were also collected from the population in freshwater and from wild animals during their seasonal reproductive migration.

After the transfer to saltwater, 14% of females developed vitellogenic oocytes. On the other hand, in freshwater only 4% of females had vitellogenic oocytes while 24% of wild females reached full maturity. In saltwater, the increase of plasmatic estradiol was significant at the beginning of vitellogenesis (1.14±0.52 ng/ml), causing a related growth of the oocyte diameters 3 weeks later (377±77 µm). Estradiol followed a similar trend in freshwater and wild females, although with no significant differences during maturation. *Fshr* expression in vitellogenic stages was down-regulated or not detected in seawater and freshwater farmed fish; *lhr* expression was up-regulated in maturing fish in saltwater and maturing wild females. Gonad total lipid content showed an increase in maturing stages. In saltwater females, there was an elevation of PUFAs during the immaturity phase, whereas SFAs and MUFAs remained stable in both gonadal and muscle tissues. During the late vitellogenesis stage, there was a significant shift in the gonadal fatty acid profile: an increase in mol% of total fatty acids for 18:0 and 18:3n-6, while 20:3n-6 and 18:1n-7 exhibited a decrease. In the muscle tissue of farmed females (both fresh- and seawater), there was a noted deficiency in EPA, ARA, and DHA when compared to their wild counterparts. Conversely, 18:2n-6 and 18:1n-9 were present in higher concentrations, mirroring the dietary inputs and suggesting that the dietary regime may not be optimally tailored to foster an ideal tissue fatty acid composition.

These results suggest that freshwater farmed mullet females can mature similarly to wild individuals in few weeks, if moved during the natural reproductive season to saltwater conditions as described. Our results also shed new light on the physiological and biochemical processes underpinning grey mullet gametogenesis, helping to define new protocols for induction of sexual maturation in captive conditions.

IMPACT OF VESSEL THRUSTER OPERATIONS ON ATLANTIC SALMON IN OPEN NET PENS

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Introduction

The expansion of aquaculture production into more exposed and harsher ocean environments increase the risk during vessels berthing and mooring operations alongside the cages. A possible solution to deal with this increased risk is to utilize Dynamic positioning (DP) systems, which allows vessels to maintain a specific position and orientation without having to moor the vessels alongside the cages. DP may lead to increased use of thrusters in vicinity of the cage systems, which may be negative for fish welfare according to existing knowledge on effects of anthropogenic noise on fish.

The knowledge about effects of anthropogenic sounds on farmed fishes is scarce. However, it is shown that high anthropogenic noise levels may alter behavioural response, increase stress and cause decreased growth rate for farmed fish. Atlantic salmon *(Salmo salar)* have been found to only detect low frequency sounds below 380 Hz, coinciding with the dominant frequencies from commercial ships at frequencies below 300 Hz. For other farmed fish species, it has been found that noise from vessels can lead to reduced foraging behaviour, whereas other studies has shown that farmed fish can adapt to anthropogenic sounds without any long-term effects on growth rate. The results from this project may be used for risk assessment of future vessel operations close to sea cages.

Method

Full-scale experiments are performed at a commercial open net pen sea farm for Atlantic salmon grow-out production to identify possible behavioural response during controlled vessel thruster activity towards a cage. Sensors and cameras are used to observe possible abnormal behaviour compared to reference measurements in undisturbed conditions. Expected measurable responses are groups of fish moving suddenly away from the noise or propulsion jet source and/or groups of fish suddenly unschooling. The physical properties of the thruster and propulsion jet, such as noise level, noise frequency and water speed will be measured at various positions within the cage. This provides useful information for evaluating the fish desire and ability to escape from the noise and propulsion jet within the cage.

Results and discussion

The results from the full-scale experiments are being processed. Preliminary results based on interviews with vessel captains and fish farmers to map current practice on use of vessel thrusters close to sea cages indicates that there have not been observed any occurrences of abnormal fish behaviour during or after vessel thruster operations. This may indicate that farmed salmon have the possibility to move adequately away from the disturbance sources and/or that the fish adapts to the noise levels present at existing sea farms. It should be noted that the results from these experiments could only be used to map the fish's immediate behavioural response and that any potential negative effects on fish welfare needs to be further investigated.

USE OF GENETIC SIMULATIONS AND BIOECONOMIC MODELLING TO INFORM AQUACULTURE BREEDING DECISIONS

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Scotland UKWith large number of animals per generation, and high genetic diversity, selective breeding programmes can deliver large returns on investment, even for modestly sized aquaculture producers. Genetic gain can be influenced by many factors including: candidate numbers; evaluation group sizes; selection intensity and trait weighting in the selection index. In addition, financial returns from the breeding programme will be influenced by the economic values of the traits being selected. Recent advances in genetic technology (particularly the cost of genotyping) have offered additional opportunities for investment in selective breeding with genomic selection becoming standard in many sectors within aquaculture.

With realised genetic gains taking up to 4 years to observe and only being available following a significant financial investment, accurate decision making tools are necessary for the design of an optimised breeding programme.

By combining high resolution genetic simulation software, with realistic aquaculture breeding programme constraints, we have been able to simulate multiple generations of selective breeding under realistic genetic parameters, allowing the effect of multiple selection strategies to be evaluated prior to committing time and resources.

By combing commercial economic data (e.g. production costs and market value) with realistic growth models, the outputs of genetic simulations can be expressed in financial units, allowing informed decisions aimed at maximising economic return.

EPIGENETIC STUDY OF FISH ERYTHROCYTES FOR THE SEARCH OF INNOVATIVE ANTIVIRAL SOLUTIONS IN AQUACULTURE

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Nucleated erythrocytes (RBCs), characteristic of fish, amphibians, reptiles and birds, are multifunctional cells, as in addition to participating in gas exchange and transport, they have also been described to respond to various pathogens, including viral infections.

On the other hand, recent studies in immunology have pointed to a process called trained immunity which is defined as the long-term functional reprogramming of cells of the innate immune system as well as other blood cells such as RBCs, induced by exogenous or endogenous challenges, leading to an enhanced response to a second challenge after a return to a non-activated state. Trained immunity is mediated by epigenetic reprogramming.

In the present work, rainbow trout, an important species in salmonid aquaculture, will be used as a model species that will be subjected to various stimuli such as: the viral hemorrhagic septicemia virus (VHSV), a DNA vaccine (pGVHSV), and an antiviral treatment based on green tea (*Camellia sinensis*). With this working model exposed to these stimuli, we will evaluate the role of epigenetic reprogramming of RBCs.

Our aim is to evaluate the epigenetic reprogramming of RBCs from fish exposed to different prophylactics and therapeutics by means of different epigenetic techniques: (i) study of epigenetic modifications by scATAC-seq; (ii) evaluation of the miRNA-ome; and (iii) analysis of the DNA-bound proteome by chromatin immunoprecipitation (ChIP) with mass spectrometry (MS).

The expected results will determine the differential gene expression of genes involved in the immune response in those RBCs exposed to the aforementioned stimuli. These results could lead to the development of new therapeutics and prophylactics in salmonid aquaculture.

PRELIMINARY RESULTS ON THE DEVELOPMENT OF AN RNA-SEQ-BASED BIOMARKER PANEL TO DIFFERENTIATE GROWTH RATES IN *Sparus aurata*

Morgana Angelo[1], Iris A.L. Silva[1], Marisa Barata[2], Florbela Soares[1,2], Bárbara Requeijo[1], Pedro Pousão-Ferreira[1,2], Cátia Lourenço-Marques[1,2,*]

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The development of a biomarker panel to differentiate fast- and slow-growing fish would be a significant advancement for aquaculture. The main objective of this work is to develop a tool, using RNA-seq technology, to predict growth rates accurately, aiding in genetic selection, reducing grading procedures, improving fish health and welfare, and lowering production costs.

Two trials using gilthead seabream (*Sparus aurata*) were conducted at the Aquaculture Research Station of the Portuguese Institute for the Ocean and Atmosphere (EPPO/IPMA). The trials began after initial weight sampling revealed growth differences within the batch (average weights of 74.22 ± 9.99 g for trial 1 and 14.46 ± 2.56 g for trial 2). After sampling, fish were sorted into slow- and fast-growing based on the average batch weight, with density maintained across all tanks. Liver and intestine samples from six fish per treatment in each trial were collected for differential gene expression (DGE) analysis. Total RNA was extracted, quantified and quality verified by gel electrophoresis before being sent for library construction and RNA sequencing (Novogene Co., Ltd).

Results show that fast-growing fish outperformed slow-growing ones, demonstrating better growth potential and physiological performance. Regarding RNA-seq results, the total gene count for each trial and tissue is shown in Table 1, along with the common DEGs between the tissues. Cluster analysis revealed that some groups of DGEs are tissue-specific (e.g. *ceacam5*, only expressed in the intestine), or size specific (e.g. *erbb3* only expressed in fast-growing fish – liver –, and *h4*, only expressed in slow-growing fish – intestine).

The Gene Ontology (GO) provides important information about the functional categorization of gene expression in the different tissues, highlighting key biological processes. The genes annotated to each significantly enriched GO term are presented in Table 2.

Biomarkers will be selected only if their expression is consistent in both trials and will be validated through qPCR. The preliminary results from the trials and RNA-seq analysis demonstrate the feasibility of this approach, showing clear distinctions in gene expression profiles between fast and slow-growing fish.

Acknowledgments: This study was funded by the project NanoPEIXE - Identification of biomarkers for the creation of a selection tool for giltheads with different growth profiles in aquaculture productions (ALG-01-0247-FEDER-070032) and INOVAQUA (MAR-021.1.3-FEAMPA-00004).

Table 1. Number of differentially expressed genes (DGEs) in each analyzed tissue for fast and slow-growing comparison in *Sparus aurata*.

DGES	Trial 1		Trial 2	
	Intestine	Liver	Intestine	Liver
Total	1997	1298	849	1682
Up-regulated	1006	687	392	864
Down-regulated	991	611	457	818
Shared	139		94	

Table 2. Key biological processes (BP), molecular functions (MF) and cellular components (CC) for fast- and slow-growing fish.

Tissue	Trial 1			Trial 2		
	Category	Description	Number of genes	Category	Description	Number of genes
Intestine	MF	oxidoreductase activity	72	MF	oxidoreductase activity	25
	BP	oxidation-reduction process	73	BP	proteolysis	24
	CC	intracellular organelle part	32	CC	extracellular region	15
Liver	MF	oxidoreductase activity	37	MF	oxidoreductase activity	57
	BP	transmembrane transport	33	BP	oxidation-reduction process	54
	CC	cytoplasm	31	CC	cytoplasm	42

EFFECTS OF DIETARY POTASSIUM DIFORMATE ON JUVENILE WHITE-LEG SHRIMP *L. vannamei* ON FEED EFFICIENCY AND SURVIVABILITY - A PERFORMANCE ANALYSIS

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Intensive production of the white leg shrimp, *Litopenaeus vannamei* (Boone 1931), in Central America and SE Asia is estimated to have reached 5.6 million t in 2023. The global shrimp market size in 2023 was valued at 73 billion USD and is suggested to reach 130 billion USD in 2032, thus having an estimated compound annual growth rate of 6.6%. In such intensive aquaculture production, bacterial diseases have been identified as a major cause of economic loss to producers. Feeding antibiotic-medicated feeds used to be a common practice to treat bacterial infections and the prophylactic use of antibiotics as growth promoters in aquaculture production has also been widely applied. However, growing awareness from both consumers and producers of all species grown in aquaculture has resulted in the current demand for responsible and sustainable aquaculture. Regulatory authorities in most exporting countries now focus on preventing misuse of antibiotic growth promoters in aquaculture, while public attention has shifted towards sustainable production methods. Dietary organic acids, and especially potassium diformate, which is the most widely tested organic acid salt in aquaculture, are among the various alternatives for environmentally friendly and nutritive-sustainable aquaculture approaches.

Dietary potassium diformate (KDF, traded as AQUAFORM, ADDCON) has been tested in shrimp aquaculture since 2006 and since then numerous publications, conference contributions and review-papers on the use of KDF in aquaculture have been published worldwide. This study analyzed the average impact of the additive, based on data collected from the published studies on its effects on performance parameters such as feed efficiency and survival rate, thus combining the most important parameters in shrimp production.

The final dataset contained the results of 9 published studies in which KDF was included, at dosages ranging from 0.1% to 0.8% and covered laboratory trials as well as usage under commercial conditions. Data were subjected to statistical analysis and a significance level of 0.05 was used in all tests. Results are expressed as percentage difference from the negatively controlled white-leg shrimp.

The average level of dietary potassium diformate from the dataset in all treated Vannamei shrimp was 0.33%. The performance of the white-leg shrimp, based on feed efficiency was significantly improved by 13.8% ($P<0.05$). Furthermore, the survival rate of the shrimp was highly significantly increased ($P<0.01$): this time the improvement was around 13.0%.

In general, results show significantly improved performance data in Vannamei shrimp fed with dietary potassium diformate. These findings support the use of KDF in shrimp feeding as a promising alternative in ecologically sustainable and resource-optimized shrimp production.

INTERACTIONS BETWEEN BIOPHYSICAL FACTORS, SCALE, PRODUCTION STRATEGIES AND ECONOMIC OUTCOMES IN OFFSHORE SALMON AQUACULTURE

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As onshore and inshore aquaculture sectors are experiencing growth bottlenecks associated with diseases and environmental emissions, the vast oceans available to offshore aquaculture seem to present opportunities for greater growth. Central drivers towards offshore oceans may be less conflicts with other users, greater distance to fish disease pressure from inshore, and opportunities to exploit economies of scale in an environment with no other aquaculture producers. This paper provides a comparative analysis of various offshore salmon aquaculture scenarios. We focus on scale and utilization of the fish farm and economic risks. Through comparative investment analysis of offshore projects our paper demonstrates the importance of upfront investment costs and capacity utilization for rates of return. We identify the following critical issues for development of offshore aquaculture – CAPEX of offshore fish farm concept, capacity utilization, risks and constraints in the offshore environment, different sizes of post-smolt and offshore fish farm capacities. Our analysis shows that the production strategies will have implications for post-smolt production strategies and investment decisions.

Our analysis shows that relevant offshore production strategies are highly dependent on the ocean environment. The production strategies and size of smolt or post-smolt have significant consequences for capacity utilization, cost productivity and financial returns.

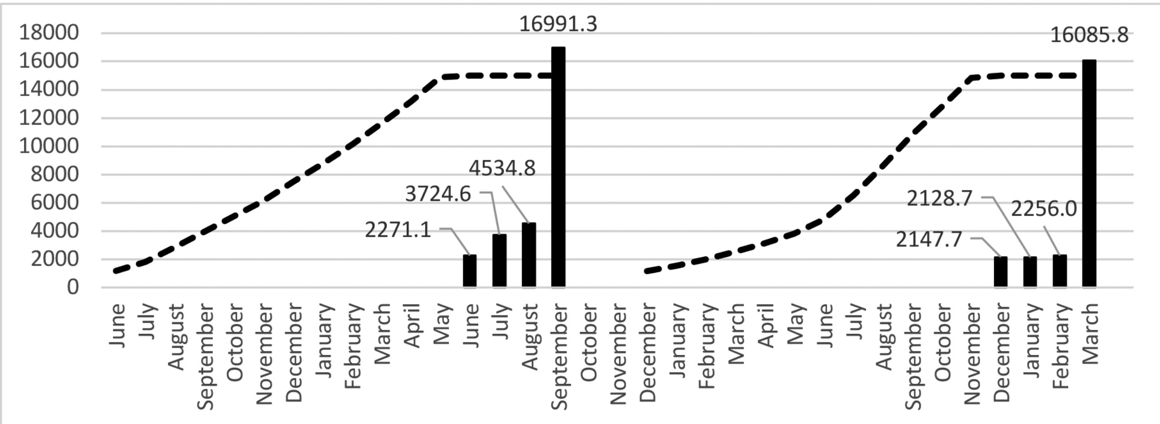

Figure 1 - Standing biomass (dotted line) and harvest for the production strategy 16 months of growth + 2 months of fallowing. Maximum Total Biomass of fish farm = 15,000 metric tons.

USING BLACK SOLDIER FLY LARVAE (*Hermetiaillucens*) TO CONVERT THE BIOFLOCS PRODUCED WITH SHRIMP SOLID WASTE

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Waste generated during aquaculture can be converted into bioflocs. Black soldier fly larvae (BSFL) are a valuable resource for the treatment of organic waste. This study evaluated the effect of feeding BSFL with bioflocs for the first time with the aim of providing a new approach for the resource utilization of aquaculture solid waste. Six treatment groups were established by feeding BSFL with fermented bioflocs (A1), bioflocs (B1), fermented wheat bran (A2), wheat bran (B2), fermented mixed diet (A3), and mixed diet (B3). The survival rates (SR) of the six treatment groups were not significantly different. The crude protein and lipid contents of the final BSFL were 50.9–63.94% and 3.96–7.73%, respectively, in the six treatment groups. The BSFL of B2 had the highest crude protein and lowest crude ash content while those of B1 had the lowest crude protein content and highest crude ash content. Oleic and linoleic acids were the predominant fatty acids in BSFL for all groups (more than 53.25%) and B3 BSFL contained three essential fatty acids (EFAs). By analyzing the effect of BSFL culture and biofloc reduction, we determined that bioflocs and wheat bran mixed at a ratio of 6:4 (dry matter) is ideal for cultivating BSFL. Overall we found that BSFL can directly treat bioflocs and the bioflocs intend provide ideal nutrients for BSFL, which implies a potential method for cleaner production in aquaculture.

The bioflocs were obtained from a commercial-scale *Litopenaeus vannamei* farming system (Chuzhou, China). *Hermetia illucens* eggs hatched with wheat bran (70% moisture) for 4–5 days. Newly hatched larvae were reared on wheat bran for 3 days before they were used in the experiments. For fermented wheat bran, dry wheat bran was dissolved in water to 30% dry matter. Six types of food substrate were designed for the experiments. Four materials (bioflocs, fermented bioflocs, wheat bran, and fermented wheat bran) described above as well as two mixed substrates: the combined substrate and fermented-combined food substrate. The combined substrate was created by mixing bioflocs and dry wheat bran at a dry matter ratio of 6:4. The fermented-combined substrate was created by mixing bioflocs and dry wheat bran at dry matter ratio of 6:4 before sterilization at 121°C for 15 min. The experiments were conducted in transparent plastic cans with perforated lids, controlled moisture at 70%, and at 28°C. In each can, 400 larvae (0.2–0.5 cm in size, 5 d old) were placed and the feeding larval density was 5.10 tails/cm^2. Feeding frequency was kept consistent for all groups. It began to harvest the BSFL when the first larval was found to pupate in all groups. The experiment lasted for 16 days.

The nutrition composition of final BSFL and initial BSFL (dry weights, %) (mean ± standard error; n = 3).

		Crude protein	Crude lipid	Crude Ash	Crude fiber	Total Phosphorus	Violate solid
Final BSFL	A1	46.67 ± 0.27^{a}	4.48 ± 0.42^{a}	31.75 ± 1.25^{a}	2.13 ± 0.38^{a}	1.20 ± 0.02^{a}	68.24 ± 1.25^{a}
	B1	43.66 ± 1.46^{a}	4.18 ± 0.04^{a}	32.12 ± 1.31^{a}	2.01 ± 0.57^{a}	0.98 ± 0.06^{a}	67.88 ± 1.31^{a}
	A2	51.55 ± 1.08^{b}	$4.48 \pm 0.\ 42^{a}$	10.25 ± 0.06^{b}	2.41 ± 1.02^{a}	0.97 ± 0.05^{a}	89.75 ± 0.06^{b}
	B2	52.97 ± 0.57^{b}	7.73 ± 0.55^{b}	9.67 ± 0.58^{b}	6.15 ± 1.14^{b}	0.79 ± 0.07^{b}	90.33 ± 0.58^{b}
	A3	44.51 ± 1.50^{a}	4.46 ± 0.27^{a}	27.64 ± 0.87^{c}	2.66 ± 1.02^{a}	1.07 ± 0.04^{a}	72.36 ± 0.87^{c}
	B3	45.59 ± 0.32^{a}	3.96 ± 0.26^{a}	26.46 ± 0.37^{c}	2.55 ± 0.39^{a}	1.10 ± 0.03^{a}	73.54 ± 0.37^{c}
Initial BSFL		45.10 ± 4.41^{a}	5.69 ± 0.26^{c}	11.60 ± 0.56^{b}	4.02 ± 0.30^{a}	1.04 ± 0.06^{a}	88.40 ± 0.56^{b}

Note: BSFL, black soldier fly larvae. The different alphabet represents significant differences. Group A1: BSFL was fed by fermented bioflocs, Group B1: BSFL was fed by bioflocs, Group A2: BSFL was fed by fermented wheat bran, Group B2: BSFL was fed by wheat bran, Group A3: BSFL was fed by fermented-combined substrate, Group B3: BSFL was fed by combined substrate.

Fatty acid composition of final BSFL and initial BSFL in six types of food substrate treatments (mean ± standard error; n = 3).

Fatty acid	Initial BSFL	A1	B1	A2	B2	A3	B3
C10:0	ND	ND	ND	0.18 ± 0.03^{a}	0.46 ± 0.05^{b}	ND	ND
C12:0	0.39 ± 0.02^{a}	1.63 ± 0.37^{b}	0.45 ± 0.12^{a}	11.15 ± 0.25^{c}	11.22 ± 1.28^{c}	1.69 ± 2.08^{b}	1.24 ± 0.62^{b}
C14:0	0.38 ± 0.04^{a}	0.66 ± 0.14^{b}	0.49 ± 0.016^{a}	2.43 ± 0.08^{c}	2.45 ± 0.25^{c}	1.43 ± 0.49^{c}	0.82 ± 0.28^{d}
C14:1(ω9)	ND	ND	ND	0.07 ± 0.011^{a}	0.14 ± 0.03^{b}	0.21 ± 0.03^{c}	ND
C15:0	0.18 ± 0.011^{a}	0.46 ± 0.052^{b}	0.76 ± 0.04^{c}	0.16 ± 0.013^{a}	0.21 ± 0.03^{a}	0.48 ± 0.03^{b}	0.82 ± 0.27^{d}
C16:0	15.13 ± 0.59^{a}	11.31 ± 0.13^{b}	11.06 ± 0.12^{b}	13.89 ± 0.25^{a}	14.97 ± 0.36^{a}	16.08 ± 1.46^{a}	17.71 ± 5.19^{a}
C16:1(ω9)	3.01 ± 0.13^{a}	9.50 ± 0.28^{b}	12.77 ± 0.83^{c}	2.61 ± 0.08^{d}	4.41 ± 0.15^{e}	6.15 ± 0.43^{b}	4.29 ± 1.25^{b}
C17:0	0.42 ± 0.002^{a}	0.93 ± 0.03^{b}	1.41 ± 0.079^{c}	0.27 ± 0.02^{d}	0.30 ± 0.02^{d}	0.69 ± 0.02^{e}	1.24 ± 0.34^{f}
C18:0	5.09 ± 0.11^{a}	6.47 ± 0.26^{b}	6.13 ± 0.18^{b}	4.25 ± 0.13^{c}	4.26 ± 0.13^{c}	6.49 ± 0.15^{b}	6.81 ± 1.90^{ab}
C18:1(t9)	0.36 ± 0.09^{a}	1.11 ± 0.32^{b}	1.19 ± 0.09^{b}	0.50 ± 0.21^{a}	1.69 ± 0.34^{b}	2.28 ± 0.27^{b}	6.36 ± 4.02^{c}
C18:1(ω9)	29.08 ± 0.11^{a}	36.76 ± 0.62^{b}	32.64 ± 0.46^{c}	21.48 ± 0.46^{d}	20.81 ± 0.36^{d}	26.35 ± 3.0^{a}	24.58 ± 6.13^{a}
C18:2 (t9 , 12)	ND	0.63 ± 0.95^{a}	0.02 ± 0.012^{b}	0.43 ± 0.19^{a}	0.11 ± 0.19^{b}	0.42 ± 0.50^{a}	ND
C18:2(ω9 , 12)	42.45 ± 0.95^{a}	20.65 ± 1.23^{b}	20.61 ± 0.81^{b}	40.09 ± 0.81^{a}	35.78 ± 0.46^{c}	31.29 ± 1.00^{d}	29.13 ± 4.52^{d}
C20:0	0.91 ± 0.04^{a}	1.33 ± 0.08^{b}	1.28 ± 0.10^{b}	0.32 ± 0.02^{c}	0.36 ± 0.04^{c}	0.77 ± 0.09^{a}	1.23 ± 0.47^{a}
C20:1(ω11)	0.13 ± 0.05^{a}	ND	ND	0.13 ± 0.01^{a}	0.15 ± 0.01^{a}	ND	ND
C18:3(ω9 , 12,15)	1.21 ± 0.04^{a}	0.63 ± 0.09^{b}	0.71 ± 0.03^{b}	1.44 ± 0.09^{c}	1.37 ± 0.03^{c}	0.97 ± 0.08^{d}	1.13 ± 0.21^{a}
C21:0	ND	ND	ND	0.13 ± 0.05^{a}	0.33 ± 0.04^{b}	0.18 ± 0.27^{a}	0.14 ± 0.17^{a}
C20:2(ω11,14)	ND	ND	ND	ND	0.20 ± 0.06^{a}	ND	ND
C22:0	0.71 ± 0.06^{a}	1.02 ± 0.10^{b}	0.96 ± 0.06^{b}	0.22 ± 0.01^{c}	0.22 ± 0.014^{c}	0.48 ± 0.07^{d}	0.65 ± 0.15^{d}
C22:1(ω13)	0.23 ± 0.04^{a}	0.83 ± 0.052^{b}	1.64 ± 0.28^{c}	0.17 ± 0.043^{a}	0.36 ± 0.039^{d}	0.40 ± 0.12^{d}	0.37 ± 0.21^{e}
C20:4(ω5,8,11,14)	ND	2.21 ± 0.033^{a}	2.69 ± 0.10^{b}	ND	ND	0.92 ± 0.12^{c}	1.03 ± 0.11^{d}
C24:0	0.15 ± 0.02^{a}	ND	ND	ND	ND	ND	ND
C20:5(ω5,8,11,14,17)(EPA)	ND	3.78 ± 0.11^{a}	5.01 ± 0.12^{b}	ND	ND	1.65 ± 0.26^{c}	1.83 ± 0.62^{d}

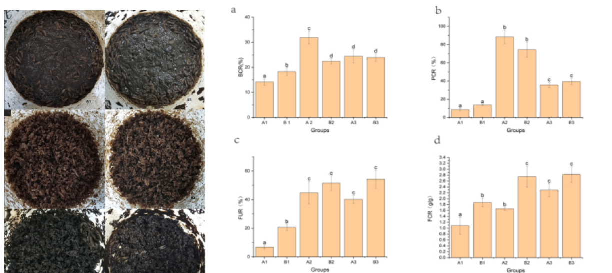

Fig. 3. Utilization efficiency of black soldier fly larvae (BSFL) on different type of food substrates treatments. The different alphabet represents significant differences between different food substrates treatments. The biomass conversion rate (BCR) of black soldier fly larvae (BSFL) in different food substrates treatments(a), Protein conversion rate (PCR) of black soldier fly larvae (BSFL) in different food substrates treatments(b), Feed utilization rate (FUR) of black soldier fly larvae (BSFL) in different food substrates treatments(c), Food conversion ratio (FCR) of black soldier fly larvae (BSFL) in different food substrates treatments(c).

ROLE OF RECIRCULATING AQUACULTURE SYSTEMS (RAS) IN THE GENERATION AND REMOVAL OF MICROPLASTICS: THE CASE FOR NORWEGIAN AQUACULTURE

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The proliferation of microplastics (MPs) in the environment is high on the global agenda. Identifying, quantifying, and applying mitigation and remediation measures to particular sources is receiving dedicated attention from researchers, industries and policy makers. RAS systems have been queried to present accumulation of contaminants, such as heavy metals, drug residues and metabolites. Since RAS heavily reply on plastic components, the release of plastics and associated additives should also be investigated. It is essential to inventory sources to determine the potential for RAS systems to up concentrate these pollutants. We hypothesised that RAS systems will have several potential sources of plastics and associated additives, such as water treatment (particle filtration), bioreactors, and other plastic installations. The efficiency of water treatment and filtration systems are likely to remove microplastics in the form of sludge. Thus, not compromising the production of commercial fisheries products. There may also be differences between the different bed types within a RAS which could influence the dynamics and sedimentation of microplastics. Therefore, we aimed to first determine the sources, and then quantify the MPs and additives. For this purpose, we used a robust methodological approach to fill the research gaps connected to MPs pollution in RAS and farmed salmon.

Plastic material was collected from three RAS and their infrastructure. All plastic sources were analysed to identify their polymer and additive contents and a reference library created. This was used for targeted analysis when investigating the presence of microplastics within RAS facilities, specifically looking at intake water, recirculating water, sludge, fish, and fish feed. Analysis of these samples showed low levels but identified several potential sources of microplastics, including plastic infrastructure and consumables. Potential sources originating from the RAS infrastructure were identified as the fix bed bioreactor (FBBR) and moving bed bioreactor (MBBR), and biomedia. Screening for additives identified that several plastic-related chemicals were ubiquitous in RAS recirculating waters, including selected antioxidants and plasticisers. The source and distribution of these chemicals was compound specific. Fewer chemicals were quantified in sludge and fish compared with water. But specific compounds including antioxidants and plasticisers were consistently found in fish.

Polymer	RAS#1	RAS#2	RAS#3
Polyester / polyethylene terephthalate (PES/PET)	No known sources identified from RAS. Not possible to identify source – also in inlet waters.	Low number of particles	Peak after water retention unit but environmental levels from inlet cannot be ruled out.
Polyvinyl chloride (PVC)	Low number of particles	Low number of particles	Only observed in inlet water
Polyamide (PA)	Slightly elevated numbers at after the MBBR/FBBR in both systems. PA feeding systems may contribute as source.	Elevated numbers after the MBBR. PA feeding systems may contribute as source.	Elevated after FBBR – unknown source in RAS
Polypropylene (PP)	Slightly elevated numbers at after the MBBR+FBBR in Påvekst. Biomedia may contribute as source.	PP was found throughout the samples, biomedia made of PP, not possible to distinguish source. Biomedia may contribute as source.	Low number of particles
Polyethylene (PE)* also used in PEX tubing	Low number of particles	PE appeared to peak after the freshwater inlet water (following the water treatment). Data linked to a single replicate.	Only observed in inlet water.

SHELLFISH WASTE AS A NEW ASSET FOR A SUSTAINABLE AQUACULTURE: THE MOLLUSCAN SHELL AND ITS ANTIBACTERIAL AGENTS

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With a production of around 250,000 tons per year, France is Europe's leading shellfish producer. Although conchyliculture is often described as a sustainable alternative to wild fisheries in the context of growing anthropic pressures, it generates a tremendous amount of shells, mostly considered as waste. While some efforts have been made to recycle it, this very abundant and cheap co-product has yet a low added-value: it is merely used for construction, soil amendment or as poultry feed calcium supplements. As the first shield against chemo-physical and biological threats, mollusc shells are formed through a highly controlled process involving the secretion - by the mantle - of macromolecules that regulate the deposition of the crystalline units and remain occluded in the mineral phase. The development of high-throughput techniques like transcriptomics and proteomics reveal the complexity of this calcifying matrix and further supports the hypothesis that the molluscan shell is not solely a physical barrier. Indeed, the presence of highly basic peptides suggests potential bactericidal properties. Antimicrobial peptides are some of the most effective innate immune system molecules in metazoans. Unlike traditional antibiotics, they do not induce any form of resistance: among the molecular processes often involved, destabilisation of bacterial membranes and their permeabilization constitute an unstoppable mechanism for bacteria. To date, bactericidal agents have only been identified in soft tissue in molluscs, but their putative presence in the shells may partly explain certain natural observations: although bivalve shells remain in aqueous environments conducive to microbial contamination, they are not very prone to the development of bacterial biofilms.

Here, we seek to demonstrate *in vitro* the antimicrobial capacity of calcifying shell matrices extracted from commonly consumed molluscs in Europe through isolation and characterization of these macromolecules. For this purpose, organic matrices were extracted from whole-shell powders and fractionated according to their solubility in dilute cold acetic acid, and, for the soluble fraction only, according to different ultrafiltration cutoffs. Common strains of marine pathogenic bacteria were selected (*Aliivibrio salmonicida*, *Vibrio tapetis, V. mytili, V. harveyi, V. aestuarianus*) and the effects of different shell extracts on bacterial growth were measured via disk diffusion assays. In parallel, all organic fractions were analysed by proteomics (LC-MS-MS). Positive results were obtained, showing bacterial growth inhibition of *A. salmonicida* and *V. harveyi* in the presence of insoluble matrices, in particular that of the oyster and of the cockle. Considering the role - sometime cooperative - of these strains in vibriosis outbreaks, and the rise of antibiotic resistance among *Vibrio* species, our results could lead to innovative and non-polluting applications in aquaculture, food safety, conservation science and health. This project intends to recycle co-products and make the most of them, therefore helping to establish a virtuous circular economy and a more sustainable aquaculture.

FOSTERING RESPONSIBLE ANTIBIOTIC USE AND SUSTAINABILITY IN AQUACULTURE: THE APPROACH OF THE AQUACULTURE STEWARDSHIP COUNCIL (ASC)

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The effects of, and enforcement measures needed to reduce antibiotic use in aquaculture remains limited in many regulatory frameworks and within the research community. Open aquaculture production systems are intimately interconnected to their surrounding environments which increases the risk of antimicrobial resistance and contamination. Semi-closed and closed production systems contribute to this issue as well as water, medicated feed, fertilizers, wastes and feed all can be sources of antibiotic resistant genes. As a result, it is pivotal for the aquaculture industry to know about the potential risks of antibiotic misuse and consider pre-emptive or alternative measures to antibiotic treatments. The Aquaculture Stewardship Council (ASC) acknowledges the critical role of antibiotics in fish farming for disease management while recognizing the concomitant risks including antibiotic resistance and environmental contamination among others.

Antimicrobial resistance is an issue for human, animal and environmental health. Understanding these interlinkages and building collaborations between different stakeholders to promote sustainable health outcomes is at the core of the One Health approach. Central to ASC's mission is the advocacy for responsible antibiotic use in aquaculture, underpinned by stringent standards and best practices. The ASC's standards incorporate antibiotic sampling protocols, ensuring regular monitoring of fish and environmental samples to assure compliance within the scheme. In this presentation, we explore the operations of the ASC regarding the collection of data on antibiotic use from farmers. Additionally, we will highlight our efforts to promote better animal welfare management to ultimately reduce the need for antibiotic treatments. Our vision is centred around the One Health approach where sustainability, human- and animal welfare, and environmental stewardship take precedence in aquaculture operations. ASC is committed to cultivating a global aquaculture sector that prioritizes responsible antibiotic use practices, minimizes environmental impact, and heightens the health and welfare standards of farmed fish. ASC underlines collaborative efforts with industry stakeholders, researchers, governments, and non-governmental organizations to forge a sustainable and resilient aquaculture sector, meeting the needs of present and future generations while safeguarding aquatic ecosystems and the communities reliant upon them.

WHOLE GENOME SEQUENCE REFINES GENOMIC ARCHITECTURE OF RESISTANCE TO *Flavobacterium columnare* IN RAINBOW TROUT

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Introduction

Columnaris disease (CD), caused by *Flavobacterium columnare* is an emerging disease affecting rainbow trout (*Oncorhynchus mykiss*) aquaculture worldwide (Declercq et al., 2013). Revealing the genomic basis underlying resistance to CD has important implication for selective breeding of trout and to better understand the genes involves in disease resistance. In a previous study a main QTL associated with resistance was detected on chromosome Omy3 (Fraslin et al., 2022) after a natural outbreak in commercial conditions with mortality phenotypes recorded on 3,054 individuals genotyped for about 28K SNPs. In the present study 45 parents out of 81 were sequenced to further refine the QTL location after alignment to the new reference genome USDA_OmykA_1.1 (Gao et al., 2021).

Materiel and methods

From the initial dataset composed of 3,054 offspring and 81 parents, 45 parents and 5 offspring were selected for whole genome resequencing (WGS) based on the number of offspring per family, survival rate and segregation of the resistance QTL detected in Fraslin et al., (2022). Alignment on the trout reference genome, USDA_OmykA_1.1, was performed with Bowtie2, SNP calling was performed using recommended pipelines with GATK and BCFTools, Genomic imputation from the 18,940 SNPs obtained by genotyping after alignment to the new genome assembly was performed using FImpute V3. The accuracy of imputation was analyzed using data from the 5 offspring that were sequenced. A genome wide associated study (GWAS) was performed using the imputed genotypes and a mixed linear model implemented in the GCTA software.

Results and discussion

About 24 millions SNPs were called by each software, only the common biallelic SNPs detected by both software were kept resulting in a total of 10,666,323 post quality controls (maf 10%, HWE $p > 0.001$, coverage 60% and depth between 8x and 30x). Imputation was performed correctly for the 5 individuals, with accuracy ranging from 78% for individuals with no parents in the sequenced dataset to 84% for individuals with both parents in the sequence dataset. The QTL on Omy3 was confirmed with the WGS based GWAS. Other QTLs on Omy5, 14 and 20 were detected. This study paves the way towards a better understanding of the genetic mechanisms underlying resistance to CD in rainbow trout. Further study is needed to identify positional candidate genes within the QTL and their implication in fish resistance.

Acknowledgment

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References

Declercq et al. (2013). Veterinary Research 44, 10.1186/1297-9716-44-27. Fraslin et al. (2022). Aquaculture 557, 738332. Gao et al. (2021). Genes Genomes Genetics 11, jkab052.

NITRIFICATION ALKALINITY EQUIVELANT – AN ALKALINITY BALANCE IN A CLOSED RAS

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pH and alkalinity management in recirculating aquaculture systems (RAS) is crucial for maintaining optimal conditions for nitrification and fish. The overall chemical equation for nitrification is likely the most well-known equation in RAS water management.

Equation 1: *Overall stoichiometry nitrification.*

$$NH_4^+ + 1.86O_2 + 1.98HCO_3^- \rightarrow 0.020C_5H_7NO_2 + 0.98NO_3^- + 1.88H_2CO_3 + 1.04H_2O$$

In theory, total nitrification consumes 1.98 mol of HCO_3^- for every mol of NH_4^+ oxidized, which equals 1.98 alkalinity equivalents used per mole of NH_4^+ (1.98 mole HCO_3^-/1 mole NH_4^+; equation 1). This 1.98 alkalinity equivalents consumed used for every mol of NH_4^+ oxidized will result in a pH drop unless the equivalents of a base (i.e. sodium carbonate) are provided. In practice however, only approximately half of the alkalinity equivalents are consumed per mol of NH_4^+ oxidized. It is suggested that fish excrete NH_3 rather than NH_4^+, removing one H^+ from the water to form NH_4^+ and consuming net only one alkalinity equivalent when oxidized.

Although nitrate, the ultimate product of nitrification, is not as harmful to fish as NH_3/NH_4^+, it still needs to be controlled in RAS since high levels might affect fish growth and health, which usually occurs through water exchange. Denitrification reactors can be used in aquaculture to remove nitrate, reducing the need to exchange water for nitrate control. Another benefit of using a denitrification reactor is the production of alkalinity.

Equation 2: *Overall stoichiometry denitrification.*

$$0.61C_{18}H_{19}O_9N + 4.89NO_3^- + 0.39NH_4^+ + 4.15H^+ \rightarrow C_5H_7NO_2 + 2.27N_2 + 5.98CO_2 + 5.15H_2O$$

Denitrification removes approximately 0.91 equivalents of acid (producing alkalinity) per mole of NO_3-N denitrified (4.15 mole H^+/4.54 mole NO^{3-}; equation 2). This implies that complete denitrification of produced NO_3-N can nearly entirely compensate for alkalinity loss if fish excrete NH_3, but quantitative data on this have never been published. An 8-week feeding experiment with tilapia in individual RAS (n=4) was performed, whereby the faecal waste produced was fed into an up flow sludge blanket reactor (no water exchange). An alkalinity balance was made using a nitrogen mass balance and alkalinity measurements. Because nitrate removal exceeded nitrate production during the experiment, more nitrate was added to the systems. Similar to the theory, higher nitrate removal relative to production increased alkalinity and pH while no base had to be added. To complete the balance, the alkalinity equivalent for nitrification was computed indirectly, using an alkalinity equivalent of 0.91 for denitrification, resulting in an average alkalinity consumption of 0.99. These results support the idea that fish excrete NH_3 and that the net alkalinity loss owing to nitrification is close to one.

IMPLEMENTATION OF A GIS COURSE FOR AQUACULTURE ANIMAL HEALTH

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INTRODUCTION:
A recent scoping review has revealed that while Geographic Information System (GIS) is utilized as a tool capable of supporting aquatic animal health activities, its application is not fully structured by operators involved in health surveillance and research activities in the aquatic domain. Therefore, it is considered crucial to establish a set of basic GIS-type knowledge to be used for projects of interest in the aquatic animal health domain.

In 2022, the "Aquae Strength" project, was launched to enhance aquaculture disease management in four beneficiary countries from the Far East, Middle East, and North Africa. One of the work packages included in the project aims at developing capacity building on the GIS body of knowledge to support aquatic animal health activities.

METHODOLOGY:
Two main activities have been identified: (i) the identification and development of the workforce issues useful for GIS application in the aquatic animal health domain, and (ii) the definition of training programs.

The identification of GIS workforce, which is not discussed in this communication, is based on expert elicitation sessions, development of feature concept documents, GIS project development framework, and development of best practices. Regarding the training programs, which are the topic of this communication, two types of courses have been defined: (i) in-person courses, and (ii) eLearning courses. Both approaches are focused on practical application and designed to overcome traditional obstacles to data availability and technological gaps, facilitating the development of GIS projects adapted to local needs. The course program focuses on the applications of GIS in various aquatic environments, including marine, transitional, and freshwater settings. It covers a wide range of topics starting from the fundamentals of GIS concepts and tools to practical data management and analysis that are relevant to fish farming, derived from laboratory outcomes, outbreak data, and fish farm registries. The course also discusses the use of GIS to support epidemiology activities, early disease detection, prevention of their spread, as well as the creation of thematic maps for decision-makers.

RESULTS:
The in-presence course was tested twice - first in November 2023 with a group of trainees from Cambodia and later in March 2024 with another group from Morocco and Tunisia. The participants had diverse professional competencies and backgrounds, working in the field of aquaculture animal health sector. Their feedback on the questionnaire administered at the end of the course was overwhelmingly positive.

CONCLUSION:
Enhancing diversity and building capacity in GIS within the aquatic animal health domain is the mission of the IZSVe-GIS aquatic group. Current GIS education fails to provide the necessary background to meet the needs of GIS technology users or the scientific community engaged in aquatic animal health. The program to implement a body of knowledge on GIS in aquatic animal health defined by IZSVe aims to fill this gap and promote a mature and efficient use of GIS technologies.

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BENEFITS OF CULTIVATING SEAWEED IN LAND-BASED PARTIALLY RECIRCULATED IMTA SYSTEMS

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Aquaculture plays a crucial role in food security and provision of income and livelihoods for communities. Following recognition as the most promising source of animal protein and essential nutrients worldwide, there has been significant investment and growth in this sector[1]. However, this increasing contribution to global aquatic food supply is hindered by sustainability challenges. Integrated multi-trophic aquaculture (IMTA) represents a sustainable production method combining the farming of multiple, complementary species from different trophic levels, and could reduce environmental impacts, diversify, and increase aquaculture production[2]. As part of the All-Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture (ASTRAL) project, we have investigated the benefits for the integrated cultivation of abalone (*Haliotis midae*) and sea urchin (*Tripneustes gratilla*) with the green seaweed *Ulva lacinulata* in land-based, partially recirculated aquaculture systems in South Africa.

Production of abalone or urchin in systems integrated with seaweed has shown numerous benefits. IMTA grown *Ulva* is an excellent source of minerals, vitamins, AA's, FA's, protein, complex polysaccharides, and other bioactive compounds. When used as a feed or a feed component, we have shown that *Ulva* enhances the chemosensory properties of formulated feeds, and significantly improves feed conversion ratio (FCR), feed consumption and digestible protein intake. Existing pigments in the seaweed (e.g., β-carotene) enhance the colour and marketable properties of urchin gonads. *Ulva* also has additional benefits in that it reduces nutrient release from farms into the sea (from 0.63-0.38t of N per urchin production cycle), reduces reliance on harvesting natural seaweeds and reduces dependence on the use of protein-rich formulated feeds (ca. 20% cost saving). The bioremediation ability of *Ulva* reduced energy consumption from 10.35-6.80 kWh per ton abalone biomass and 12.45-3.54 kWh per ton urchin biomass harvested when recirculation rates are increased from 0-50% and 0-90% for the abalone and urchin IMTAs, respectively. A perceived risk of growing *Ulva* in abalone effluent and utilizing it as a supplementary feed is biosecurity — which has hampered broader uptake of IMTA. However, our comprehensive microbiome studies have revealed high microbial diversity in all compartments of the IMTA, indicative of a healthy system, and *Ulva* exhibits a modulatory effect on the microbiome, reducing abundance of opportunistic pathogens (e.g., *Vibrio* spp.).

Collectively, our studies revealed several benefits associated with the cultivation of *Ulva* in IMTA systems and high potential for IMTA to support sustainable aquaculture and a circular bioeconomy.

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FAO (2020). The State of World Fisheries and Aquaculture 2020. FAO, Rome, 244 pp.
Chopin, T., 2015. Marine Aquaculture in Canada: Well-Established Monocultures of Finfish and Shellfish and an Emerging Integrated Multi-Trophic Aquaculture (IMTA) Approach Including Seaweeds, Other Invertebrates, and Microbial Communities. Fisheries 40, 28-31.

BEYOND THE CULTURED ORGANISM: SHIFTING THE PARADIGM FROM A FISH-CENTERED MICROBIOME MODULATION APPROACH

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Aquaculture is one of the fastest-growing sectors in the global food industry. Cultured aquatic organisms are already more than those captured by the fishing industry. Closed aquaculture systems, such as recirculatory aquaculture systems (RAS), are gaining traction as an interesting alternative to open-sea production. Yet, the intensification of the aquaculture industry, characterized by higher stocking densities, reduced water turnover, and high nutrient input, comes with several challenges. These include high pathogen prevalence, often leading to disease outbreaks, and high mortality rates, especially during larval and early development stages. Current solutions and research are hyperfocused on fish-centric approaches and often overlook the interconnections between microbial communities inhabiting different compartments of the production system, neglecting how these communities contribute to the overall health and stability of the entire system.

Here, we propose an innovative approach that conceptualizes the system as a holobiont, utilizing new technology to modulate the microbiome of biofilms (tank surfaces and biofilters), water, and cultured organisms in RAS at any animal developmental stage. This patent-pending technology involves the development of tailored porous mesh designed for the controlled release of biologically active beneficial substances. In this study, we present the results of our proof of concept for this innovative technology. Two validation trials with European seabass (*Dicentrarchus labrax*) in a lab-scale RAS and one trial in pilot RAS demonstrated that this mesh, loaded with humic substances, effectively manipulates the RAS microbiome (biofilms, bacterioplankton, and fish skin mucus), increasing the relative abundance of potentially beneficial bacteria, and suppressing putative pathogens, improved nitrite removal, exhibited immuno-modulatory effects and increased survival rates following a exposure to a *Tenacibaculum maritimum* infection.

The newly developed technology evaluated here offers a high degree of customization, with a diverse range of microbiome modulators tailored to specific needs. This innovation has the potential to shift the paradigm from a fish-centered microbiome modulation approach in aquaculture systems while requiring minimal changes to current system designs.

We acknowledge the support of BlueComposite (PdC nº 08.207, SAICT-45-2021-02), funded by Centro 2020 - Portugal2020, the European Regional Development Fund (ERDF), to CESAM (UIDP/50017/2020+UIDB/50017/2020+ LA/P/0094/2020) and CIIMAR (UIDB/04423/2020 and UIDP/04423/2020) funded by the Portuguese Foundation for Science and Technology (FCT/MCTES). Davide A. Machado e Silva was supported by a PhD grant (2020.05774.BD) funded by FTC/MCTES, and the European Social Fund POPH-QREN programme.

COMMERCIAL DEPURATION OF THE CARPET SHELL CLAM (*Ruditapes decussatus*) USING HUMIC SUBSTANCES AS A MICROBIOME MODULATOR

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Depuration is a vital process in providing safe seafood for human consumption. Coadjutants for depuration are sought out to improve depuration efficiency and enhance the shelf-life of seafood products. In this context, humic substances (HS) have potential to act as a cost-effective and environmentally friendly solution to enhance depuration and modulate shellfish microbiome. In this study, the potential use of HS as a depuration coadjutant is evaluated in respect to its ability to modulate the bacterial communities of the gastrointestinal tracts of clams after depuration.

Two depuration systems, each with three replicate tanks, were executed during twenty-six hours. No coadjutant was added in one system (i.e., Control: Cn), while in the other, a water-soluble HS commercial product (Hs; Humic Powder, FulviXcell) was added at final concentration of $2.5 mgL^{-1}$. After depuration, clams were stored in a climate chamber (5 ± 1 °C) for 6 days to simulate the commercial 'shelf-life' period. DNA was extracted from composite samples (n=4) of gastrointestinal tracts of clams sampled before depuration (St), after depuration (CnD and HsD) and at the end of the 'shelf-life' period (CnS and HsS). Bacterial composition was analyzed using high-throughput sequencing data of the hypervariable V3/V4 region of the 16S gene.

HS supplementation did not alter alpha diversity, but both factors: HS addition and 'shelf-life'; were found to have a significant effect on bacterial composition (Figure 1). Communities were mostly composed by sequences related to obligate intracellular symbionts, i.e, *Mycoplasma* sp. and *Ehrlichia* sp.. The family Metamycoplasmataceae emerged as a significant predictor of experimental variables according to the Boruta feature-selection analysis. We observed an increase in the dominance of this family in HS-depurated clams, but there was no difference observed in 'shelf-life' samples. Overall, our results indicate that HS has the potential to modulate the bacterial communities of the gastrointestinal tracts of clams, including intracellular host symbionts. Further studies should explore the potential of HS for development strategies to improve bivalve health in natural environments and aquaculture settings.

Acknowledgements to HUMICOIL (10.54499/2022.03081.PTDC) and CESAM (UIDP/50017/2020+UIDB/50017/2020+ LA/P/0094/2020), both funded by the Portuguese Foundation for Science and Technology (Ministry of Science and Higher Education - FCT/MCTES), through national funds.

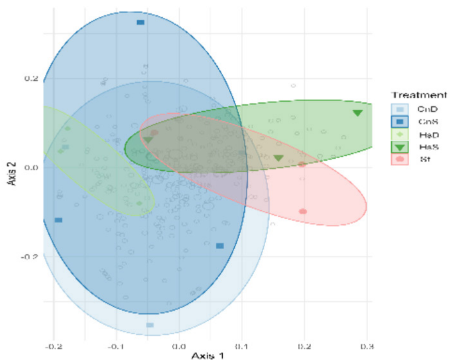

Figure 1 - First two axes of principal coordinates analysis (PCO) of ASV composition.

CONTROL MEASURES AGAINST INFECTIONS WITH *Flavobacterium psychrophilum* IN RAINBOW TROUT: SALTWATER OR RAISED WATER TEMPERATURE AS ALTERNATIVES TO ANTIBIOTICS

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High mortalities among rainbow trout (*Oncorhynchus mykiss*) fry are often seen as the result of infections due to *Flavobacterium psychrophilum*, and in Denmark this pathogen is one of the most common causes of disease in rainbow trout freshwater farms. The infection can be treated with antibiotics, but lower sensitivity against the infection is an increasing problem for some of the used drugs, wherefore alternative treatment methods are investigated. Studies showing how water temperature as well as salinity level in the water affect the outcome of an infection with *F. psychrophilum* will be presented, so infections with the bacterium can be controlled and high mortalities among the affected fish avoided.

The effect of salt water (1%) and warm temperature (18±1°C) in the control of *F. psychrophilum* in rainbow trout fry were investigated. Fish experimental trials were set up. In the first part of the experiment, fish (0.7 g) were at first infected by bath challenge and then exposed to either salt water (1 dpi – 12°C) or to increased water temperature (1 dpi or at onset of mortalities). Water parameters were changed gradually over 24 hours. Negative infection controls were included (freshwater and salt water at 12°C; warm freshwater at 18°C) and fish survival was followed over time. In the second part of the experiment, a cohabitation challenge was established and the effect of salt water (1%) on disease transmission evaluated (1.5-2 g/fish). Fish survival was followed over time.

Following bath challenge, the salt treatment delayed the appearance of clinical disease, while in the warm temperature groups fish survival decreased more rapidly than the positive control. During the co-habitation challenge, IP injected fish reached 0% survival within two weeks in both groups. Cohabitant fish swimming in salt water had a significant increase in survival (42.6%) compared to the positive controls (17.9%) kept in freshwater. Infected dead and moribund fish were confirmed positive for *F. psychrophilum*.

It was shown that increasing water salinity delayed and partly prevented RTFS. This delay can give time to achieve AMR test results and initiate treatment before reaching high mortalities in a fish batch. Further studies should evaluate the robustness of the preventive effect of this approach, its effect on the microbial communities (fish and farm environment), and whether it could be combined with other measures like e.g. phage therapy. The effect of the warm water was surprising, as *F. psychrophilum* favours colder temperatures.

DANISH CONSUMER PREFERENCES AND WILLINGNESS-TO-PAY FOR SEAFOOD TREATED WITH BACTERIOPHAGES

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Fish disease is a major constraint in sustainable growth for global aquaculture. Infectious bacteria causing significant economic losses are only treated by antibiotics. Excessive antibiotics use negatively influences the surrounding environment and enables bacterium to be resistant to antibiotics. Therefore, bacteriophages, natural enemies to specific bacteria, have been paid attention to aquaculture as an alternative to antibiotics. This paper will encourage a reduction in antibiotic use in fish farming by using the natural occurring bacteriophages to combat bacteria. However, marketability of bacteriophages is unknown since seafood products treated with bacteriophages are not commercially available yet. Though there are studies about consumer preferences for seafood attributes in some European countries, their main focus is not how fish diseases are treated, especially by bacteriophages. Thus, this study investigates the marketability of seafood farmed with bacteriophages by applying a discrete choice experiment (DCE) and Contingent Valuation (CV) to estimate the willingness-to-pay for rainbow trout fillet treated with bacteriophages in Denmark. This research contributes to revealing extra amounts of money consumers are willing to pay for avoiding antibiotics and making bacteriophages commercially available in the aquaculture industry. We believe this study promotes a more sustainable future for aquaculture by reducing the use of antibiotics.

TABLE 1. Attributes and Levels

Attribute	Description	Level
Health control	Indicates how trout is treated with (to combat diseases)	Antibiotics, Vaccination, Bacteriophages
Stocking density	Indicates minimum rearing space for the fish within the trout farming	Low: 25kg/m^3 Medium: 50kg/m^3 High: 80kg/m^3
CO^2 footprint	Indicates total amount of greenhouse gases that trout farming adds to the atmosphere (in kg of emissions)	Low: 2000-3000 CO^2-eq Middle: 3000-6000 CO^2-eq High: 6000-13000 CO^2-eq
Country of origin	Indicates country where trout is farmed	Denmark, Germany, Turkey
Technology of production	Indicates how/in which environment trout is farmed	Extensive, Semi-intensive, Intensive
Price (DKK)	Price for trout fillet (250gram)	16, 42, 67, 92

FROM CAGE TO TABLE: ASSESSING A NOVEL NUTRITIONAL AND FEEDING STRATEGY DURING PRE-HARVEST ON EUROPEAN SEABASS *Dicentrarchus labrax* STRESS INDICATORS AND MEAT QUALITY

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Introduction

Pre-harvest stress in aquaculture refers to the physiological and behavioural changes that occur in fish due to various stressors before they are harvested for human consumption. These stressors typically include handling, crowding, and air exposure and can affect the meat quality and the visual appearance of the fish, ultimately lowering its commercial value and acceptance by the end consumer. Consequences include bleeding, melanosis, texture loss and reduced fillet consistency. Nowadays, more welfare-friendly harvesting methods are being explored (e.g. stunning), but few fish farms have already adopted new equipment and procedures. To address this topic with a solution of comprehensive use in any fish farm, the present work aims at evaluating a novel nutritional approach consisting of a natural anxiolytic to be fed under a short-term protocol before harvesting. The main objectives are to assess the optimal dosage and feeding protocol in European seabass and to measure the impact on stress indicators and postmortem meat quality.

Material and methods

A commercial formulation with no anxiolytic additive was used as a control diet (CTRL). Three additional experimental diets were formulated to include 0.5, 1.0 and 2.5 g/kg of the anxiolytic additive (D0.5, D1.0 and D2.5 diets, respectively). The experiment was held in a marine RAS composed of 15 tanks of 400L capacity. Twenty European seabass of ca. 30 g initial body weight were stocked per tank. The trial had 3 periods: acclimation, phase 1 and phase 2. During acclimation (two weeks) all the fish were fed the CTRL diet. Fish were fed manually to apparent satiation twice per day. In phase 1, CTRL diet was tested in triplicate tanks, whereas the experimental diets were tested in four replicate tanks, and in two replicate tanks in phase 2. During phase 1, fish were fed for 10 days, and after a 48h-starvation, three fish per tank were sampled (sampling 1). During phase 2, the four replicate tanks of the experimental diets were divided in two feeding strategies (approaches A and B), fed for another 10 days, and after a 48h-starvation, six fish per tank were sampled (sampling 2). In the approach A, the two replicate tanks kept being fed with the respective experimental diet, whereas in the approach B the other two replicate tanks switched to the CTRL diet. Before each sampling, a pre-harvest stress event was simulated, consisting of crowding for 15 min, and air exposure for 30 seconds. In each sampling moment, fish were sampled to measure the cortisol level in the plasma, rigor mortis, QIM evaluation at 24, 72 and 168h postmortem, and overall fish quality through, sensory evaluation, texturometer analysis, water activity and total volatile nitrogen.

Results and discussion

Fish stocking occurred with no mortality incidents. The trial is ongoing with the first sampling completed and the samples currently undergoing analysis. The trial is expected to be concluded by mid-April. Upon thorough analysis, all results will be presented at AQUA 2024 as scheduled.

ASSESSING SENSORY EVALUATION AND CONSUMER PERCEPTION OF FISH FEED INSECT MEAL-BASED MEAL

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In order to overcome the problem of sustainability in aquaculture, new protein sources are being sought to replace classical protein sources in the feeding of farmed fish.

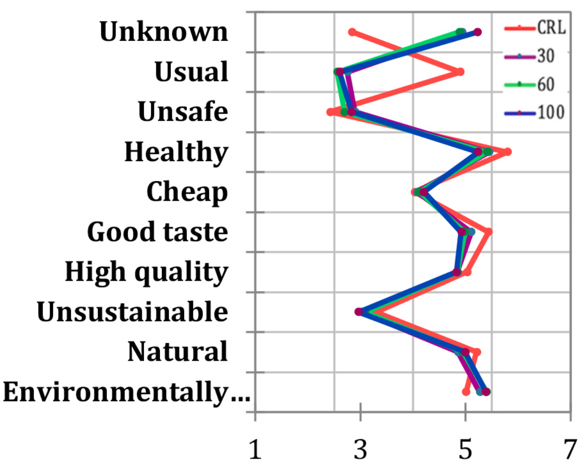
The aim of this study was to investigate the replacement of fishmeal with insect meal in fish feed by assessing the sensory profile of fillets and consumer perception of the use of this new protein source.

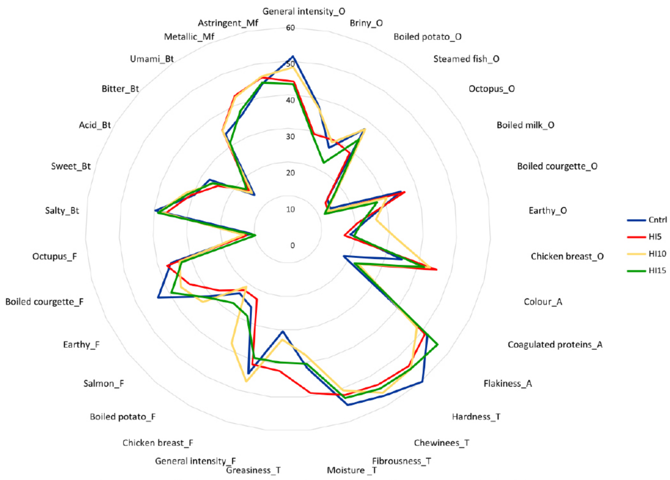
The method used to compare the products under investigation was quantitative descriptive analysis (QDA). For this purpose, a test was carried out with different levels of inclusion of larvae meal of both *Hermetia illucens* in sea bream, (CTRL, 5%, 10% and 15%) and *Tenebrio monitor* (CRL, 30%, 60%, 100%) in sea bass, instead of fish meal.

The fillets were gutted and cooked according to a standardised procedure. They were then examined by panelists, who identified discriminating sensory descriptors. Each sensory descriptor was linked to two references describing minimum and maximum intensity in order to optimize the repeatability and reproducibility of the sensory responses. In the sea bass trial we also set out to investigate the perceptions that this insect proteins might arouse in the consumer. For this trial we had the product tasted blind and immediately afterwards the same product accompanied by information, asking to score liking and perception according to a set of terms defined in the literature.

The results showed that the inclusion of up to 15% *Hermetia Illucens* in place of fishmeal found no significant difference between the sensory profiles of the four products under investigation. This result was corroborated by the second study on *Tenebrio molitor*, where no significant difference was found between the sensory profiles but showed that Western consumers still have a strong neophobia towards this protein.

It's evident that substantial effort will be required to refine the information presented to consumers to encourage acceptance of this protein. It's worth noting that the protein boasts an excellent nutritional profile.





ASSESSING SENSORY EVALUATION AND CONSUMER PERCEPTION OF FISH FED INSECT MEAL-BASED FEEDS

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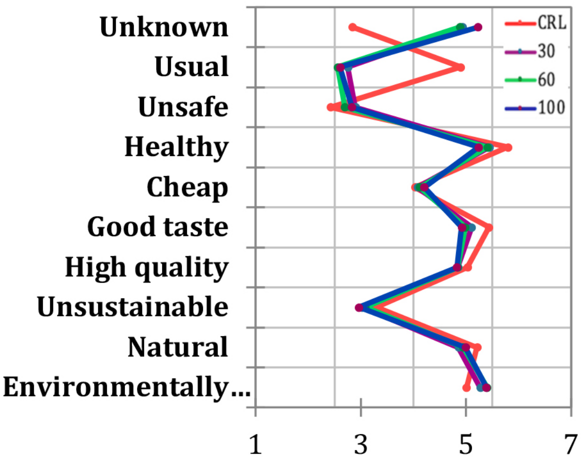

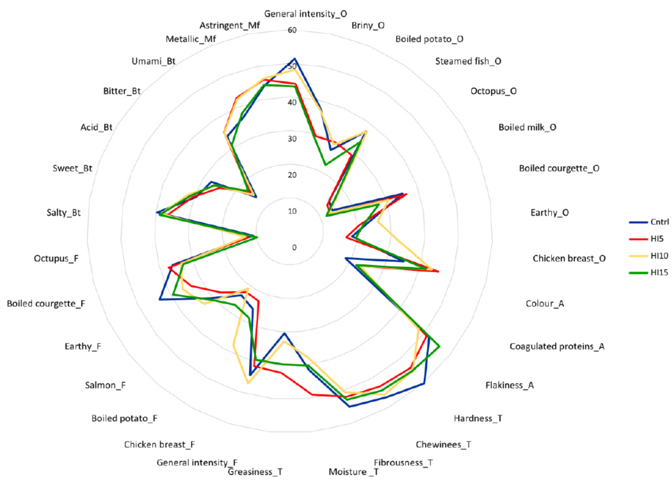

ENHANCING RESILIENCE OF THE EUROPEAN SEAFOOD MARKET THROUGH A SUSTAINABLE GROWTH FRAMEWORK FOR AQUACULTURE

Wesley Malcorps*, Richard Newton, Piotr Eljasik, Remigiusz Panicz, John Bostock, Stephanie Horn and Dave Little

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Global trade is central in supplying the European demand for seafood. Apart from the Norwegian (European Economic Area (EEA) member) Atlantic salmon sector, aquaculture in the European Union (EU) is lacking scale, and growth capacity is undermined by cheaper (imported) alternatives sourced from capture fisheries and aquaculture. Urbanisation and rising income levels are driving global demand for high-value carnivorous species already farmed in Europe, but there is a high dependency on imported feed ingredients, which are responsible for most costs and environmental impacts of fed aquaculture production. We combined a (scientific) literature and market observatory review with a secondary data analysis of the EEA seafood industry and markets to develop a comprehensive sustainable growth framework for aquaculture. We identified four main strategies to enhance the resilience of the European seafood market. Firstly, seafood from fisheries and aquaculture are being produced as a continuum, rather than a dichotomy. These systems are, and will remain, interlinked, in terms of production sites and nutrient flows. Therefore, it is crucial to optimise nutrient availability from both systems by encouraging consumption of the edible portion of a variety of wild fish but ensuring their processing wastes and fish species without a 'food' market are used in aquafeeds. Secondly, low input aquaculture species, such as common carp, seaweed and bivalve aquaculture might better fit within a landscape food production system, but demand needs to be stimulated through post-harvest processing that increases both economic performance and resource efficiency. Thirdly, the responsible sourcing of aquafeed ingredients within Europe, that does not compete directly with human food supply but is well integrated within a circular economy model, could play an important role. Fourthly, such a circular model could also be applied downstream in the value chain, which could benefit from the strategic processing and utilisation of aquaculture processing by-products into food, feed and industrial applications. Consequently, aquaculture volume and value could be increased without the need for additional resources, enhancing the economic and environmental performance. To unlock this potential, exchange of knowledge, technology and infrastructure across species and production systems in the EEA is crucial.

THE EFFECT OF PIKEPERCH (*Sander lucioperca*) BROODSTOCK ORIGIN ON THEIR ABILITY TO EXPRESS NATURAL REPRODUCTIVE BEHAVIOUR.

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Introduction

Despite the significant dependence on the expression of natural behaviour in pikeperch aquaculture production, the knowledge of its reproduction biology remains limited (Lappalainen, Dorner, and Wysujack 2003; Malinovskyi et al. 2023; Olin et al. 2018). Recent reports show that the reproductive performance of pikeperch males during natural spawning season could vary significantly, and as a result affecting females spawning decisions and the overall efficacy of reproduction, suggesting species sensitivity to shifts in reproduction behaviour (Malinovskyi et al. 2023; Teletchea et al. 2009)”ISBN”:”0044-8486”,”ISSN”:”00448486”,”abstract”:”A multiplicity of factors could modulate the quality of gametogenesis in fish. In the present study, we used a fractional factorial design (24-1, resolution IV. The potential impact of rearing history on spawning behaviour can have significant implications for the natural reproductive capacity of broodstock, raising concerns about the suitability of intensively-cultured fish for restocking and replenishment programs. Likewise, there are yet commercial farms using the hormone-free nest spawning technique that are intrigued by the potential of reproductive behaviour modulation.

Several studies found modified reproductive biology of intensively- vs extensively reared breeders (Khendek et al. 2018; Ljubobratovi□ et al. 2017, 2023), which might likewise imply modification of its reproductive capability in terms of practicing natural spawning. Thus, the study aims to investigate the potential influence of broodstock rearing history (pond- vs. intensively-reared) on their ability to express natural reproductive traits. The study seeks to understand whether it affects spawning decisions, reproductive effectiveness, and overall reproductive capacity in pikeperch broodstock during natural spawning on the nest.

Material and methods

To accomplish this, the study will use four sets of pikeperch broodstock for natural spawning on the nest, comprising fish with different rearing histories: (1) both sexes pond-cultured; (2) pond-cultured males and intensively-cultured females; (3) intensively-cultured males and pond-cultured females; (4) both sexes are intensively-cultured. The fish in each set were provided with artificial spawning nests and no hormonal induction of spawning. This approach allowed assessment of the capability of pikeperch broodstock to exhibit natural spawning behaviour, providing valuable insights into the potential influence of the rearing history on their reproductive performance. Each nest was photographed, and the image was analysed with the open-source image processor ImageJ. The analysis included the determination of the cleaned area and area of egg distribution relative to the total nest area.

Results and discussion

Results: Preliminary findings indicate a significant difference in the spawning success rate between pond-cultured and intensively cultured pikeperch broodstock. Pond-cultured fish demonstrated significantly better nest-cleaning efforts and more even distribution of eggs on the nest. Additionally, there was a considerable difference in the biochemical profile of blood plasma, particularly cortisol and glucose levels, which suggests a stress response in intensively cultured fish.

Discussion: The results suggest that the rearing history of pikeperch broodstock significantly affects their natural reproductive behaviour and spawning success. Pond-cultured fish not only performed better in terms of nest preparation and egg distribution but also exhibited lower stress levels, as indicated by their biochemical profiles. These findings highlight the importance of rearing conditions in aquaculture practices and their potential impact on the reproductive performance of broodstock. The observed stress response in intensively cultured fish raises concerns about their suitability for natural spawning and restocking programs. Further research is needed to explore the mechanisms underlying these differences and to develop strategies to improve the reproductive performance of intensively cultured broodstock.

(Continued on next page)

Acknowledgements

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References

Khendek, A., A. Chakraborty, J. Roche, Y. Ledoré, A. Personne, T. Policar, D. Żarski, R. Mandiki, P. Kestemont, S. Milla, and P. Fontaine. 2018. "Rearing Conditions and Life History Influence the Progress of Gametogenesis and Reproduction Performances in Pikeperch Males and Females." *Animal* 12(11):2335–46. doi: 10.1017/S1751731118000010.

Lappalainen, Jyrki, H. Dorner, and Klaus Wysujack. 2003. "Reproduction Biology of Pikeperch (Sander Lucioperca (L.)) – a Review." *Ecology of Freshwater Fish* 12:95–106.

Ljubobratović, Uroš, Nevena Kitanović, Sylvain Milla, Zoran Marinović, Georgina Fazekas, Jelena Stanivuk, Zoltán Nagy, and Ákos Horváth. 2023. "Predicting Population's Oocyte Maturation Competence and Evaluating Individual's Latency Time Using in Vitro Oocyte Maturation in Pikeperch (*Sander Lucioperca*)." *Aquaculture* 562:738851. doi: https://doi.org/10.1016/j.aquaculture.2022.738851.

Ljubobratović, Uroš, Géza Péter, Zoltán Horváth, Daniel Żarski, Tijana Ristović, Vanda Percze, Zsuzsana Sándor, Svetlana Lengyel, and András Rónyai. 2017. "Reproductive Performance of Indoor-Reared Pikeperch (*Sander Lucioperca*) Females after Wintering in Outdoor Earthen Ponds." *Aquaculture Research* 48(9):4851–63. doi: 10.1111/are.13305.

Malinovskyi, Oleksandr, Hadiseh Dadras, Tomáš Pěnka, and Tomáš Policar. 2023. "The Efficacy of Male's Nest Preparation and Its Effect on Female's Partner Selection during Semi-Controlled Reproduction in Pikeperch (*Sander Lucioperca*)." *Aquaculture* 565:739100. doi: https://doi.org/10.1016/j.aquaculture.2022.739100.

Olin, M., A. Vainikka, T. Roikonen, J. Ruuhijärvi, H. Huuskonen, M. Kotakorpi, S. Vesala, P. Ala-Opas, J. Tiainen, L. Nurminen, and H. Lehtonen. 2018. "Trait-Related Variation in the Reproductive Characteristics of Female Pikeperch (*Sander Lucioperca*)." *Fisheries Management and Ecology* 25(3):220–32. doi: 10.1111/fme.12280.

Teletchea, Fabrice, Jean Noël Gardeur, Martin Psenicka, Vojtech Kaspar, Yannick Le Doré, Otomar Linhart, and Pascal Fontaine. 2009. "Effects of Four Factors on the Quality of Male Reproductive Cycle in Pikeperch *Sander Lucioperca*." *Aquaculture* 291(3–4):217–23. doi: 10.1016/j.aquaculture.2009.03.029.

VALUE CHAIN AND EFFICIENCY ANALYSIS OF PANGAS AND TILAPIA MARKETING IN SELECTED AREAS OF BANGLADESH

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This article presents a framework for Pangas and Tilapia supply and value addition in Bangladesh based on different market prices. In this paper, we extend the analysis by assessing the different marketing costs of intermediaries and arathdar commissions. Using stratified random sampling techniques and structured questionnaires with face-to-face interviews, 950 samples were surveyed; these samples were collected from Dhaka, including Gazipur, Mymensingh, Cumilla, Bagura, and Khulna, as well as from Jossore, Chattagram, and Barishal, including Vhola and Dinajpur districts. Few actors are more powerful in relation to the supply chain in Pangas and Tilapia farming, and they earn more profit. The added value of Pangas *arathder* is Tk. 11017, Tk. 9506 and Tk. 8954 per ton according to the size of the fish, which were above 2 kg, 1-2 kg and less than 1 kg. However, the value added by the Pangas wholesaler was Tk 6613. 6859 and Tk. 6292 per ton, which is lower than that of *arathdar*. The value added by the retailer of Pangas is Tk. 13602, 12067 and 11504 per ton according to the size of the fish, which were greater than 2 kg, 1-2 kg and less than 1 kg, respectively. However, in Tilapia farming, the value added is greater than that of Pangas *arathder*, which is Tk. 15685, Tk. 10669 and Tk. 8041 per ton according to size of fish. The study also revealed that the value added by Tilapia wholesalers and retailers was greater than that added by Pangas wholesalers and retailers, for which Tk was 10496 and Tk, respectively. 9492 and Tk. 7916 per ton. Because the market price of tilapia is higher than that of Pangas. In the Pangas and Tilapia Value Chain, Farmer > Arathdar > Retailer > Hotel was the most efficient channel, and the least efficient channel was Farmer > Local Wholesaler > Retailer > Final Consumer.

Therefore, the government should monitor feed quality and improved the governance of pangas and tilapia value chain. Additionally, to explore the export potential of these fishes, DoF, BFRI and NGOs should fulfill their roles in training chain actors and providing extension services.

Table 1: Value added to Pangas marketing by different intermediaries

Markets	Particulars of Marketing	Tk/Ton		
		A Grade: >2 kg	B Grade: 1-2 kg	C Grade: <1 kg
Aratdar	Purchase Price	102381	91164	76628
	Marketing Costs	3444	3444	3444
	Arathdar Commission	2291	2041	1745
	Sales Price	114551	102073	87281
	Gross Margin	14461	12950	12398
	Value addition	11017	9506	8954
Wholesaler	Purchase Price	101060	90789	73636
	Marketing Costs	7527	7527	7527
	Sales Price	115200	105175	87455
	Gross Margin	14140	14386	13819
	Value addition	6613	6859	6292
Retailer	Purchase Price	104672	93131	76680
	Marketing Costs	2196	2196	2196
	Sales Price	120470	107394	90380
	Gross Margin	15798	14263	13700
	Value addition	13602	12067	11504

Source: Survey

Table 2: Value added to tilapia marketing by different intermediaries

Markets	Particulars of Marketing	Tk/Ton		
		A Grade: >1 kg	B Grade: ½-1 kg	C Grade: <1/2 kg
Aratdar	Purchase Price	135815	117535	98018
	Marketing Costs	3598	3598	3598
	Arathdar Commission	3041	2584	2150
	Sales Price	152057	129218	107507
	Gross Margin	19243	14267	11639
	Value addition	15685	10669	8041
Wholesaler	Purchase Price	131933	111603	92846
	Marketing Costs	8238	8238	8238
	Sales Price	150667	129333	109000
	Gross Margin	18734	17730	16154
	Value addition	10496	9492	7916
Retailer	Purchase Price	137793	132381	97982
	Marketing Costs	2246	2246	2246
	Sales Price	155579	150850	113227
	Gross Margin	17786	18469	15245
	Value addition	15540	16223	12999

Source: Survey

STRESSES AND RECOVERY PROCESS OF BLOOD COCKLE *Tegillarca granosa* IN HYPOSALINE AND TURBID WATER

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Blood cockle *Tegillarca granosa* is a popular shellfish fishery resource and cultured in the Asia region, but not in Japan, even though found in the bay of Ariake, Japan. This species inhabits shallow mudflats and is thought to have a high tolerance level against different types of stressors. However, with on-going changes to coastal marine environmental factors due to anthropogenic changes, climate change, etc., this particular species tolerance level to prolonged low salinity and high turbidity levels which occur during the rainy season is unknown. Therefore, this study focused on exposing individuals to low salinity and high turbidity conditions for a certain period and then examining if they recover after a recovery period.

A 2 x 2 experimental design with six replicate tanks were set up at water temperature at 20 ± 1°C. During the exposure period of 2-weeks, salinity (10, 30 psu) and turbidity levels (0, 900 mg L^{-1}) were prepared and stocked with 2 individuals $tank^{-1}$. After the exposure period, exposed individuals were then introduced to a 1-week recovery period where salinity and turbidity levels were restored to normal conditions of 30 psu and 0 mg L^{-1}, respectively. As physiological health indicators, gaping behaviour, clearance rate (CR), survival rate and glycogen content measurements were recorded.

Gaping behaviour results showed that 10 psu individuals, irrespective of turbidity, started opening their valves around Day 4. During the exposure period, salinity and turbidity independent effect on CR were detected only on Day 7 (P < 0.05). There were no significant differences in glycogen content of foot during the exposure and recovery period except for turbidity independent effect on the recovery individual. *T. granosa* was considered to have a high tolerance level against low salinity and high turbidity.

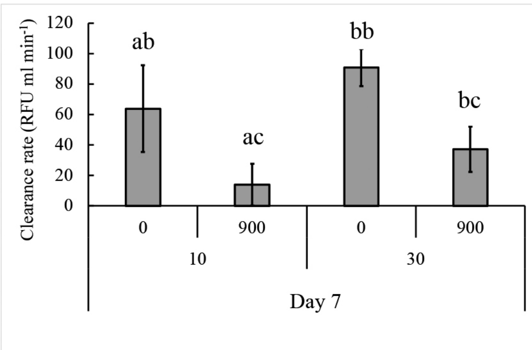

Fig. 1 Clearance rate of *T. granosa* on Day 7 exposed to salinity (10, 30 psu) and turbidity (0, 900 mg L^{-1}) levels. Different letters indicate significant differences (P < 0.05).

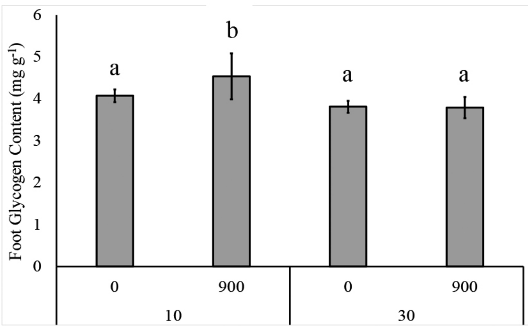

Fig. 2 Glycogen content of *T. granosa* foot taken at the end of the recovery period (21 days).

NOVEL INSIGHTS INTO PISCIDIN 1 OF GILTHEAD SEABREAM (*Sparus aurata*), A HOST DEFENCE PEPTIDE

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Host defense peptides (HDPs, also known as antimicrobial peptides) are crucial components of the immune system. To date, the piscidin family has been described exclusively in teleosts, and they are recognized for forming helical structures (Asensio-Calavia et al., 2023). In this study, our objective was to characterize the *in vitro* activity of two synthetic piscidins (piscidin 1 and piscidin 2) originally identified in the genome of gilthead seabream (*Sparus aurata*) (Serna-Duque et al., 2022). We studied their antimicrobial activity by determining the minimum inhibitory concentration (MIC) against three pathogens (*Vibrio harveyi, Vibrio anguillarum, Vibrio alginolyticus*) and one probiotic (*Shewanella putrefaciens,* SpPdp11), their hemolytic activity against gilthead seabream erythrocytes and cytotoxic activity against a fish cell line (SAF-1). Finally, we set out to explore their immunomodulatory properties that are less well known in HDPs, specifically, to study whether piscidins modulate the release of extracellular traps (ETs) from seabream head kidney leucocytes. Piscidin 1 has strong antimicrobial activity (MIC: < 50µM) against pathogenic bacteria and low activity against Pdp11. Piscidin 1 has a remarkable hemolytic activity (<40%) and modulates immune responses by promoting the release of ETs from seabream head kidney leucocytes. In contrast, piscidin 2 showed lesser effects than piscidin 1 in all activities tested. Therefore, the results suggest the different involvement of the two piscidins in immunity. Piscidin 1 could be a potential therapeutic candidate to address some of the current health challenges present in aquaculture production.

Bibliography

Asensio-Calavia, P., González-Acosta, S., Otazo-Pérez, A., López, M. R., Morales-delaNuez, A., & Pérez de la Lastra, J. M. (2023). Teleost Piscidins-In Silico Perspective of Natural Peptide Antibiotics from Marine Sources. *Antibiotics (Basel)*, *12*(5). https://doi.org/10.3390/antibiotics12050855

Serna-Duque, J. A., Cuesta, A., Sánchez-Ferrer, Á., & Esteban, M. Á. (2022). Two duplicated piscidin genes from gilthead seabream (Sparus aurata) with different roles in vitro and in vivo. *Fish & Shellfish Immunology*, *127*, 730-739. https://doi.org/https://doi.org/10.1016/j.fsi.2022.07.013

Acknowledgments

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IMPROVED SURVIVAL TO ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IN WHITE LEG SHRIMP, *Litopenaeus vannamei* DUE TO GENOMIC SELECTION

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Acute Hepatopancreatic Necrosis Disease (AHPND), caused by *Vibrio parahaemolyticus,* poses a significant threat to the global shrimp industry, primarily affecting white leg shrimp, *L. vannamei*. Despite stringent biosecurity measures and water quality management, outbreaks remain common. Well-structured selective breeding programs have the capability to select for improved survival to diseases provided that the trait is heritable and shows significant genetic variation. In this study we investigated the efficacy of genomic selection in improving survival under AHPND challenges.

Test groups of approximately 1000 juvenile shrimp from multiple families across several generations in the Kona Bay breeding program underwent AHPND challenges. Each challenge was preceded by LD50 trials to determine the optimal pathogen concentration, aiming for around 50% mortality. Time to death (expressed in hours) was recorded for each juvenile in the main challenge. Both challenged individuals and their siblings, selection candidates in the breeding program, were genotyped using a custom SNP chip. Genomic data were analysed using Genome Studio and SNP and Variation Suite. Selection candidates for the next generation were selected using GBLUP multi-trait genomic selection. Each subsequent generation was subjected to a new disease challenge, replicating the conditions of previous challenges, varying only the concentration of the pathogen.

The average heritability of time to death was 0.089(0.013). Despite increasing concentrations of *V. parahaemolyticus* over generations, time to death improved (Fig. 1), indicating improved survival against the specific pathogen. To demonstrate the effectiveness of genomic selection we also performed cross validation where 50% of the challenge data was used to predict the time to survival in the other 50% (masked) data. This was done in five random iterations to obtain an accurate predictive power of the data. Correlations between predicted genomic and estimated pedigree breeding values (corrected phenotypes) were on average 0.86 (sd = 0.023) which demonstrates that genomic selection can be used effectively to select for improved survival.

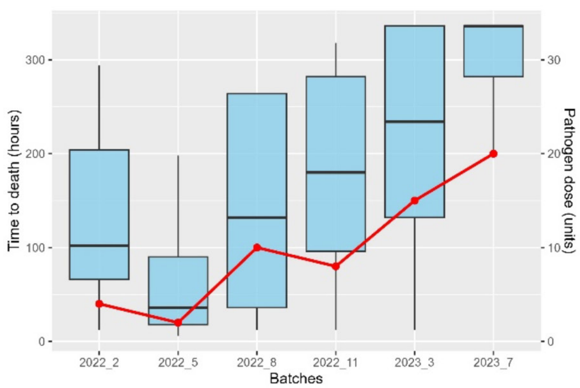

Fig 1. AHPND time to death in 6 batches (blue boxplots) and dose applied (red line).

PRECISION MICROPLASTIC SENSOR: AI AND OPTICS FOR ENVIRONMENTAL MONITORING

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Microplastic pollution poses a significant threat to aquatic ecosystems and human health worldwide. Originating from various sources, including breakdown of larger plastic debris, microbeads, and synthetic textiles, these minute particles, often less than 5 millimeters in size, can absorb toxic chemicals and enter the food chain, endangering wildlife, and human populations. Addressing this complex issue necessitates advanced detection and monitoring techniques.

The sensor offers precise identification of microplastics while distinguishing them from natural constituents. Operating as a compact, standalone device, it utilizes optical components and advanced software for systematic examination of water samples in continuous flow. Equipped with a peristaltic pump, the sensor propels water through different operational stages, including optical particle detection and imaging. UV and visible light enhance detection capabilities by facilitating differentiation of microplastics from organic materials through fluorescence signatures. The imaging component features a commercial microscope objective and high-speed digital camera, ensuring sharp image acquisition across the microplastic size spectrum. Moreover, the sensor's electronic unit orchestrates critical functionalities, ensuring smooth operation and real-time responsiveness. At its core lies an artificial intelligence (AI) processing system employing convolutional neural networks for image segmentation and multi-class classification, continuously learning, and adapting to emerging challenges to enhance accuracy over time.

Validation tests demonstrate the sensor's effectiveness in controlled environments, confirming its ability to accurately detect microplastics and differentiate them from other particles. Overall, the developed sensor offers a robust and efficient solution for environmental monitoring, contributing to efforts aimed at combating microplastic pollution in water bodies worldwide.

The microplastic sensor presented in this study represents a significant advancement in the field of environmental monitoring. By combining advanced optics, AI algorithms, and precision engineering, the sensor offers a reliable and efficient tool for accurately identifying and quantifying microplastics pollution in water environments. This technology holds promise for enhancing our understanding of microplastics distribution and its impact on ecosystems, facilitating targeted mitigation strategies, and ultimately contributing to the preservation of aquatic biodiversity.

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UNDER THE ASTRAL EU PROJECT: MICROBIOME STUDY OF SPECIES IN INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM AT DIFFERENT RECIRCULATION RATES IN SOUTH AFRICA

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Introduction
South African open water Integrated Multi Trophic Aquaculture (IMTA) system is one of the first commercial abalone farms in South Africa, consistently recirculating 50% of seawater, making use of Ulva´s bioremediation capacity. The system can go through increasing recirculation levels through time, from 50%, 75% to 100%. The resulting system is potentially more biologically efficient, environmentally sustainable, and more economically competitive. However, research needs to be developed to optimise these systems and understand their microbiome role and dynamics, as well as understanding the potential presence pathogenic bacterial outbreaks.

Material and methods
The experimental set-up consists of seven IMTA platforms, each composed of four clusters with one Ulva paddle-raceway and several abalone tanks per cluster. Ulva, abalone and seawater were collected from 3 independent platforms at 3 recirculation rates (50, 75 and 100%) and characterised for microbial populations. Microbial diversity was assessed through 16S rRNA and shotgun sequencing.

Results
Water recirculation rates significantly impacted the microbial biodiversity within the system. Increased recirculation resulted in decreased abundance of *Vibrio* in abalone microbiome, and decreased *Chloroflexi* in Ulva samples. *Vibrio* was consistently in low abundance in Ulva samples, controlling it through the system. Microbiome evolution was observed through the different time points of recirculation. No biofouling was observed.

Conclusions
Our study of the viability of running the system at 100% recirculation for several days show no risk of pathogenic dysbiosis. Furthermore, we provide deeper insights into the microbiome dynamics, contributing to the use of techniques to control disease risk for abalone farming. Microbial functionality in IMTA is being further assessed through whole genome sequencing.

MECHANISMS OF FOOD DETECTION IN RAINBOW TROUT: EXPLORING THE ROLE OF OMEGA-3 FATTY ACIDS AND THEIR SENSING RECEPTORS ON FEEDING BEHAVIOR REGULATION

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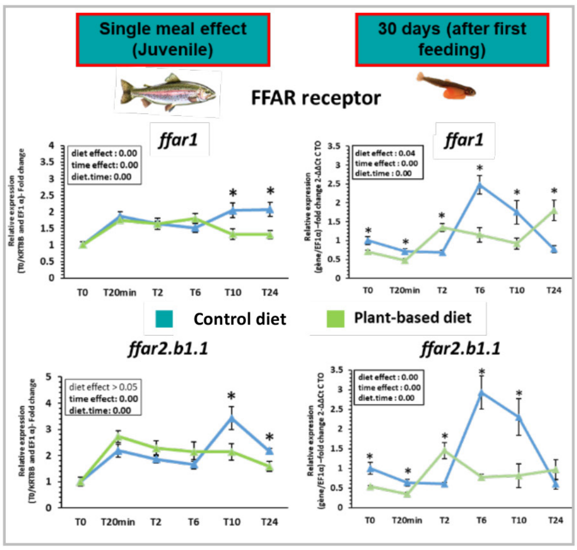
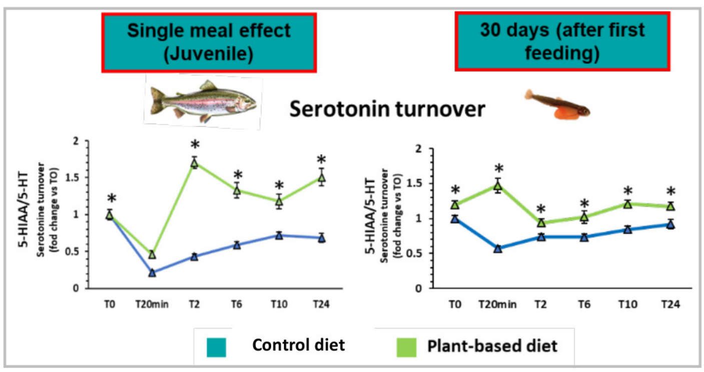
In the context of sustainable aquaculture development, it is crucial to reduce the use of traditional ingredients such as fish meal and fish oil. However, their total replacement with plant-based ingredients leads to a significant alteration in the survival and growth performance of rainbow trout, starting from the first meal and throughout their life cycle. Increasingly, studies are identifying altered feeding behavior as a hypothesis, although few mechanistic studies have confirmed this so far. While the removal of fish meal and fish oil results in the removal of omega-3 intake (DHA/EPA), few data exist regarding the impact of these nutrients on the feeding behavior of farmed fish. Yet, nutrient detection plays a crucial role in food selection, assessment, and ingestion in animals.

Therefore, understanding the mechanisms that regulate the feeding behavior of rainbow trout, such as the involvement of omega-3 fatty acids, is a prerequisite for acquiring crucial knowledge to develop new nutritional strategies aimed at improving the regulation of food intake throughout the rainbow trout's life cycle.



We investigated the effect of a plant-based diet on the mechanisms of food detection in rainbow trout. For this purpose, two postprandial kinetic studies (fasted, 20 minutes, 2 hours, 6 hours, 10 hours, and 24 hours postprandial) following a single meal (in juvenile trout) or after 30 days of feeding (following the first meal) were conducted.



Our results demonstrate that the nature of the diet (diet with fish meal and fish oil vs. plant-based) influences the expression of nutrient receptor genes, such as free fatty acid receptors, at the gustatory system level in trout, both following a single meal with a plant-based feed or after 30 days of feeding following the first meal. An alteration in serotonin regulation (a key neurotransmitter in taste information transmission and animal behavior) induced by plant-based feeding, particularly in areas associated with decision-making and behavior control, is also observed. These results not only show a deregulation of the integrated feeding response by plant-based feeding but also highlight the remarkable sensitivity of nutrient detection mechanisms in rainbow trout to new food sources.

Finally, additional *in vitro* data on the affinity of fatty acids on sensory receptors demonstrate that gustatory receptors could play a crucial role in the fine distinction of foods with different levels of omega-3 in rainbow trout, revealing increased sensitivity to certain fatty acids.

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SEX DETERMINATION IN FISH: NEW INSIGHTS FROM THE EUROPEAN HAKE *Merluccius merluccius* CHROMOSOME-LEVEL GENOME ASSEMBLY

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Sex determination (SD) mechanisms are exceptionally diverse and show high evolutionary rate in fish. At least 21 different SD genes have been consistently identified in more than 100 fish species to date. Exploring the intraspecific variation on the genes underlying SD in fish genomes as well as the environmental factors influencing sex could aid to understand this huge dynamism. Furthermore, sexual conflict regions that have been claimed as one of the initial triggers on new SD mechanisms should be inspected more extensively in fish genomes to identify potential candidate regions facilitating the transition to a new SD system. The European hake (*Merluccius merluccius*) is a species of great commercial value distributed throughout European coasts, which displays a significant sexual growth dimorphism and that has also been identified as a potential target for aquaculture diversification. Here, we present the first chromosome-level genome of the European hake assembled through long- and short-read sequencing. The genome was initially assembled in 162 scaffolds (N50: 32 Mb) that were anchored using Hi-C into 21 chromosomes spanning 715 Mb. Repetitive elements represented 43% of the genome. We performed RNA-Seq on muscle, liver, brain, spleen and gonad from pools of individuals along with proteomes from closely related species and gene predictors for improving annotation (26,625 protein-coding genes and 41,543 isoforms; 11,083 sncRNAs and 5,683 lncRNAs). Five males and five females were re-sequenced using 30X coverage 150 bp PE Illumina sequencing for sex-association genome screening to identify the SD region. Around 12 million SNPs were consistently called and, after filtering by MAF > 0.3, read depth (10 > x > 300) and no missing data in the 10 individuals, a total of 1,552,561 SNPs were retained for genome screening (1 SNP / 5.6 kb). We estimated genetic differentiation (F_{ST}) and intrapopulation fixation index (F_{IS}) using male and female populations across windows of 20 SNPs to identify candidate SD regions (for a typical XY/ZW SD system: $F_{ST} = 0.33$ and $F_{IS} = -1.0$). Chromosome 9 showed a significant differentiation between males and females between 10 and 20 Mb, which included several genes related to gonadal and sex differentiation (*ar*, *pgr*, *fox4*, *foxN3*, *tex11*, *sox3*, *mospd2* and *nr3c1*). After zooming, we identified a candidate region spanning 7 kb very close to *sox3* (20 kb distance), where most SNPs were heterozygous in males and homozygous in females compatible with a XX/XY system. Sex association of these markers was validated in a large sample of 45 males and 45 females using a MassARRAY genotyping platform. Further, several regions under putative sexual conflict were identified in the hake genome, and one of them in chromosome 5, including clusters of immune- and reproduction-related genes, was validated in the larger sample. This information supports a candidate gene for SD in European hake and provide new insights on fish SD through inspection of sexual conflict regions. Finally, a SD molecular tool could be derived from our data useful for fisheries management of European hake in a context of climatic change.

GENETIC EDITING FOR VIRAL RESISTANCE IN AQUACULTURE FISH

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Viral infectious diseases pose a significant threat to global aquaculture. Current vaccines and treatments are often ineffective and costly. Traditional genetic improvement methods, such as selective breeding, have been used to enhance disease resistance in fish, but they require sustained effort and only provide gradual improvements. Genomic editing emerges as an ideal complement to genomic selection, allowing for significant advancements in a shorter timeframe. Viral nervous necrosis (VNN) is a major viral disease affecting numerous fish species, including seabass and gilthead seabream. It is a neurological disease that severely impacts European seabass, accounting for 15% of infectious disease-related deaths in farms. The virus affects the brain, spinal cord, and retina, with mortality rates reaching up to 100% in juveniles and larvae. Biosecurity measures and vaccination are implemented to combat it, though they are not entirely effective. There is significant genetic variation in VNN resistance in European seabass, with heritability estimates ranging from 0.10 to 0.43. A QTL previously identified on LG12 explains over 33% of this variation and is associated with the ifi27l2a gene linked to resistance. Resistant populations show survival rates of 66-78%, compared to 40-45% in susceptible populations. The project aims to study the genetic variability of *ifi27l2a* and utilize genomic editing in seabass and gilthead seabream cell lines to confer VNN resistance. To apply genomic editing using the CRISPR-Cas9 system, optimization is crucial. Our preliminary research has focused on optimizing conditions for CRISPR-Cas9 electroporation and transfection in the DLB-1 cell line and generating genetic variants of the *ifi27l2a* gene. Despite identifying optimal conditions for transfecting sgRNA complexes, we have encountered low genetic editing efficiencies, analyzed through heteroduplex variant detection, ICE, and TIDE. However, at least three sgRNAs designed for this editing showed good performance, indicating their potential utility beyond our study in broader biotechnological applications. DLB-1 cells show high DNA sequence homology with those found in online databases, but we encountered differences in the amplification of one *ifi27l2a* gene fragment, an aspect still under investigation. Using CRISPR-Cas9, we aimed to create resistant mutants, having successfully designed and tested CRISPR-Cas9 tools, achieving a 38% editing rate in DLB-1 cell line. This is a promising step, but *in vitro* and *in vivo* challenges are still needed to validate the genetic function of *ifi27l2a*. Initially, we will conduct in vitro functional analyses through infection challenges to assess survival rates and perform molecular tests. Subsequently, we will transfer these results to *in vivo* models to validate resistance under real-world conditions. In conclusion, genetic editing is a powerful tool to address viral diseases in aquaculture, offering the potential to create resistant fish that can significantly reduce economic losses and improve industry sustainability. This research represents a significant advance towards healthier and more efficient aquaculture practices.

MARKETING AND SUPPLY NETWORK OF INPUTS AND EQUIPMENT IN SMALL-SCALE TILAPIA FARMERS IN GUERRERO AND OAXACA, MEXICO

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Small-scale aquaculture farmers face challenges related to low productivity, scarce economic resources, geographic isolation, limited bargaining power and little access to conventional marketing channels. These difficulties imply challenges for the commercialization of tilapia (CT) and access to inputs and equipment (I&E), necessary for their production process.

The objective of this paper is to show how the CT network and access to I & E of small-scale rural tilapia farmers (SSRTF) in Guerrero (Gro.) and Oaxaca (Oax), Mexico, is formed. The different TC modalities and the main localities of the network that participate in it are identified. Thirty-nine producers were interviewed, of which 30 are in Oaxaca and nine in Guerrero. The network was constructed taking as a reference the localities where these small-scale fish farmers carry out their activities: a total of to 83 localities in the states of Guerrero and Oaxaca as well as in neighboring states to which the network extends. For the network analysis, the degree, betweenness, and articulation points were used as central elements to situate the most important localities in the network.

The degree represents the number of exchanges made by the SSRTF in a locality, whether with consumers, intermediaries or suppliers. In the case of betweenness, it reveals a locality that functions as a link with one or more other localities that participate in the network, for the purchase and sale of tilapia and inputs and equipment, as well as the exchange of information with intermediaries or suppliers. Finally, the points of articulation represent the core localities that allow the network to be formed.

The results indicate that the TC operates with short market circuits and intermediaries and in some cases has a national market scope. In the supply of I&E network there is a broad presence of companies such as veterinaries, specialized distributors or intermediaries at the local and national levels. The network analysis made it possible to distinguish the main localities of the network (Table 1), which are all points of articulation; they are localities of relevance both for the social actors located in them and for the number of connections generated with social actors in other localities.

Table 1. Most important localities in the network according to their degree and betweenness

Degree	Value	Betweenness	Value
San Pedro de Ixcatlán (Oax.)	88	Oaxaca de Juárez (Oax.)	1465.76
Nuevo Pescadito de Abajo 2 (Oax.)	74	San Pedro de Ixcatlán (Oax.)	1397.18
Acapulco (Gro.)	56	San Juan de Mazatlán (Oax.)	1215.54
Buena Vista de la Salud (Gro.)	44	Nuevo Pescadito de Abajo 2 (Oax.)	845.78
San Juan Bautista de Tuxtepec (Oax.)	38	San Juan Bautista de Tuxtepec (Oax.)	604.39

Source: Own elaboration

USING PROTON-TRANSFER-REACTION TIME-OF-FLIGHT MASS SPECTROMETRY (PTR-ToF-MS) TO QUANTIFY MICROBIAL OFF-FLAVORS GEOSMIN AND 2-METHYLISOBORNEOL IN WATER. METHOD OPTIMIZATION, PERFORMANCE ASSESSMENT AND COMPARISON WITH ESTABLISHED GC-MS METHODS

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The microbial metabolites geosmin and 2-methylisoborneol (2-MIB) are of significant concern for two key industries worldwide: aquaculture, currently the fastest-growing food production sector, and the drinking water industry [1,4]. These compounds elicit well-known off-flavors that pose a major hurdle for the growth of aquaculture, particularly in recirculated aquaculture systems (RAS), as they greatly hamper fish marketability and thus the economic sustainability of the fish farms [1,2,3]. Their identification and quantification in water has hitherto only been reported using chromatographic methods [5,6,7]. With odor thresholds as low as 5-10 ng/L in water and 0.25 µg/kg in fish [3], robust and highly sensitive methods are necessary for a reliable quantification. Although chromatography offers comprehensive analysis, it is oftentimes slow and tedious. Therefore, the development of more rapid analytical methods to monitor off-flavor trace levels in both drinking and aquaculture water can be of great benefit for early outbreak detection and product quality monitoring. This study proposes a novel method for quantification of these terpenoids in water based on proton-transfer-reaction mass spectrometry (PTR-MS). First, the study investigated the fragmentation pattern of both compounds under standard proton-transfer-reaction conditions to determine specific ions suitable for quantification. Second, a static headspace (SH) method was developed after an optimization design that investigated the effect of three selected variables: temperature (°C), time (min) and NaCl content (g/L). Finally, optimal and suboptimal PTR-MS-based methods were evaluated from an analytical standpoint (repeatability, linearity, LOD and LOQ) and their performances were compared with an established GC-MS-based method [8]. Results, on the one hand, show the typical behaviour of these two microbial metabolites in H_3O^+-mediated reactions, dehydration, reported for the first time in PTR-MS studies. On the other hand, a sensitive (with LOD of 5 ng/L and 10 ng/L for 2-MIB and geosmin respectively) and fast method (10-15 min) for the quantification of these two metabolites in water samples was successfully developed. This opens the door for further developments in the quick monitoring of a wider range of contaminants and/or undesired metabolites in aquaculture and drinking water through PTR-MS.

References

1. Azaria, S.; van Rijn, J. *Aquacultural Engineering* **2018**, *83*, 57-64.
2. Abd El-Hack, M.E.; El-Saadony, M.T.; Elbestawy, A.R.; Ellakany, H.F.; Abaza, S.S.; Geneedy, A.M.; Salem, H.M.; Taha, A.E.; Swelum, A.A.; Omer, F.A.; et al. *Mar Pollut Bull* **2022**, *178*, 113579.
3. Jones, B.; Rocker, M.M.; Keast, R.S.J.; Callahan, D.L.; Redmond, H.J.; Smullen, R.P.; Francis, D.S. *Reviews in Aquaculture* **2022**.
4. Mustapha, S.; Tijani, J.O.; Ndamitso, M.M.; Abdulkareem, A.S.; Shuaib, D.T.; Mohammed, A.K. *Environ Monit Assess* **2021**, *193*.
5. Podduturi, R.; Petersen, M.A.; Vestergaard, M.; Jorgensen, N.O.G. *Aquaculture* **2020**, *514*.
6. Lindholm-Lehto, P.C. *Environ Sci Pollut Res Int* **2022**.
7. Bristow, R.L.; Haworth-Duff, A.; Young, I.S.; Myers, P.; Hampson, M.R.; Williams, J.; Maher, S. *Sci Rep* **2023**.
8. Petersen, M. A., Alam, M. A., Rahman, M. M., Ali, M. L., Mahmud, S., Schlüter, L., & Jørgensen, N. O. Aquaculture Environment Interactions **2014**, 5(2), 107-116.

CONTEXT OF INFORMATION TECHNOLOGY USE IN THE RURAL AQUACULTURE CHAIN OF THE SIERRA GUERRERENSE IN MEXICO

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Guerrero state is in the southeastern part of Mexico, it is renowned for its primary production sectors such as aquaculture and fisheries, particularly tilapia and shrimp. According to the 2019 Statistical Yearbook of the state, Guerrero contributed about 0.74% to the national production, positioning itself in fifteenth place (National Commission of Aquaculture and Fisheries, 2019). However, despite the potential benefits of information and communication technologies (ICT) to enhance efficiency and sustainability in supply chains, the aquaculture and fisheries sector in Guerrero exhibits limited permeability in rural areas, complicating the development of new commercial communication channels. To better understand the adoption of ICT in this sector, a survey is being conducted among aquaculture producers in the Costa Grande region in September 2022.

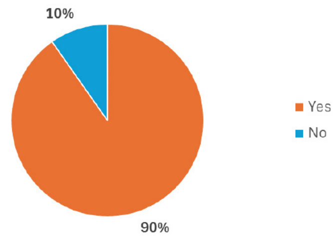

Figure 1 Percentage on mobile phone use.

According to a survey of 123 participants, 90% have their own mobile phone. This indicates that the majority of producers are already using mobile phones in their activities, including commercial ones.

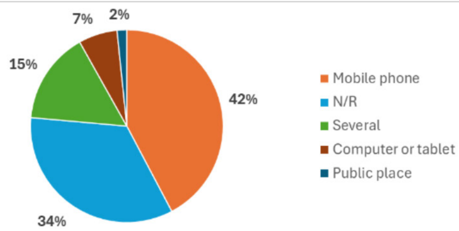

Figure 2 Percentage on means of internet access.

On the other hand, 42% access to internet by mobile phones, 7% from computers or tablets, 2% in public places, 15% use two or more of these methods, and 34% did not provide comments. This suggests that smartphones are the primary access to the internet for many producers.

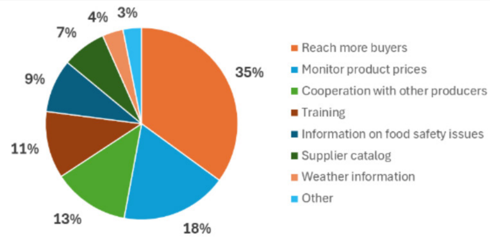

Figure 3 Needs to be addressed.

Finally, to understand the adoption of tools that could enhance their communication channels and benefit the marketing of their products in the Guerrero aquaculture sector, 35% would use an app to increase their reach to buyers, 18% to monitor market prices, and 13% to contact other producers and form alliances. Other uses include training (11%), information on safety and good practices (9%), access to a supplier catalog (7%), weather information (4%), and other purposes (3%).

This indicates that there is a broad need for the application of ICT to be addressed to enhance short rural aquaculture chains, with a greater interest in boosting commercial activity.

MICROSITE *TILAPIA* FOR THE PLANNING OF THE SUSTAINABLE DEVELOPMENT OF RURAL, SMALL-SCALE TILAPIA AQUACULTURE IN THE STATES OF GUERRERO AND OAXACA, MEXICO

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Planning for the sustainable development of rural aquaculture is a big challenge, considering all factors interacting in these territories, and external variables from environment, social and even political source. Hence, tools like microsites are very useful for the analysis and geographical representation of results. We present the microsite *Tilapia*[1], designed for our project and hosted in the *Southern Pacific Geoweb Platform*, with the idea of creating a site for information dissemination, run and operated by all the project´s technical responsible of the Project, with full access to information and knowledge generated in our project, from a geospatial and territorial perspective (Figure 1). This microsite also allows integration of diverse information formats and videos, which tell about processes established within each territory (*geospatial storytelling*).

Analyzing the territories, the adaptation capacity was inserted as a variable with two main objectives: to give a wider vision of vulnerability but also to recognize that factors influencing adaptation are more dynamic and of higher intensity, than the processes of Climate Change themselves[2]. A wider vision of vulnerability was adopted, not limited to the Climate Change agenda (mitigation/adaptation), but one that also includes the risks derived from extreme climate events, tensions on natural resources that generate degradation of water in quality and quantity, deforestation that affect microclimate and increases the risk of landslides, as well as soil loss that eventually impacts on the diversification of economic activities, reducing water infiltration and increasing the risks of landslides and floodings.

http://plataformapacificosur.mx/cms/tilapia
http://plataformapacificosur.mx/cms/multimedia/tilapia/menuSect-101-1952

DIETARY PROFILES IN RURAL HOUSEHOLDS WITH SMALL-SCALE TILAPIA PROJECTS: PRELIMINAR RESULTS FROM A CASE STUDY IN GUERRERO AND OAXACA, MEXICO

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In Mexico, tilapia is the main freshwater fish species for rural aquaculture projects. A benefit of many small-scale projects in rural territories in the country, is the supply of tilapia for consumption at local level, where usually fish was not available. Fish consumption is amply recommended for its nutritional value. In general, tilapia gives the consumer between 15 -17 grams of protein for each 100 grams of body weight, and other nutrients.

Latest world data from FAO (SOFIA, 2024) confirm the relevance of the aquatic animal in human consumption: aquaculture has already overpassed fisheries in total volume produced (51% to 49% respectively); inland aquaculture species provide 62.6% of total aquaculture, and the per capita consumption of fish already reached 20.7 kg., in a continuous growth for many years.

Fish is very important for Food and Nutrition Security. As part of the Blue Transformation seeked globably by FAO in the interaction of food-health and environment, achieving healthy diets is crucial and fish is a key element. The Blue Transformation is usually conceived as increased aquatic production, but other similar benefits are employment, economic growth, social development and environmental recovery. At country, region and territorial levels, there is a wide gradient of scenarios and realities that require detailed analysis in order to identify Government Public Policy.

We present preliminary results and methodology of a case study developed as part of our CONAHCYT 317100 research Project in the southern Pacific States of Oaxaca and Guerrero in Mexico, to reinforce nutritional education at household level where tilapia projects are in place. This is the preliminary step to assess feasibility of healthy diets in rural territories, focused on the role of the tilapia supplied by small-scale projects.

TOWARDS SUSTAINABLE SPANISH FOOD SUPPLY CHAINS: THE ROLE OF THE "SMART-FOODPRINT" PROYECT

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In the contemporary food industry, the importance of traceability and food supply chain management is widely recognized for their critical roles in safeguarding food safety, ensuring quality standards, and enhancing transparency throughout the production and distribution processes. However, environmental problems have been tackled in isolation, considering the production on the one hand, and the consumption of products on the other. Therefore, the most advanced management models are changing towards integrated approaches that allow for establishing relationships between production and consumption, as well as environmental and quality aspects. Furthermore, in response to the lack of transparency in the food supply chain, there is a need for the establishment of a science-based food traceability system, utilizing improved methods for authenticity testing.

In this context, SMART-FOODPRINT project aims to develop a user-friendly and high-quality traceability system to enhance transparency within the food industry. SMART-FOODPRINT comprises seven work packages (WP). The first two WPs are designed to ensure the feasibility and success of the project, as well as to maximize the impact of the project. Regarding the technical work, WP3 addresses analytical techniques and procedures for evaluating food traceability. Subsequently, WP4 focuses on environmental assessment, WP5 is oriented towards the creation of the ECO-SMART-FOODPRINT application, while WP6 aims to integrate an ecological labeling certification system. Finally, WP7 represents the case studies in which the SMART-FOODPRINT application will be tested. These WP are based on 5 key pillars (Figure 1) that will allow us to reach a successful industrial demonstrator: (i) development of testing methods; (ii) ICT infrastructure; (iii) creation of user-friendly interface; (iv) industrial demonstrator; and (v) management.

The expected results for the first period of the project include the design of a reliable analytical infrastructure and the formulation of the corresponding life cycle model. Regarding the first objective, we will start with the compilation of a DNA barcode library for fish authentication and the development of a NIR Standard Operating Procedure to determine information of seafood product. Subsequently, to verify the environmental aspects, the Life Cycle Assessment (LCA) methodology will be applied to calculate the impact of food fraud along this chain.

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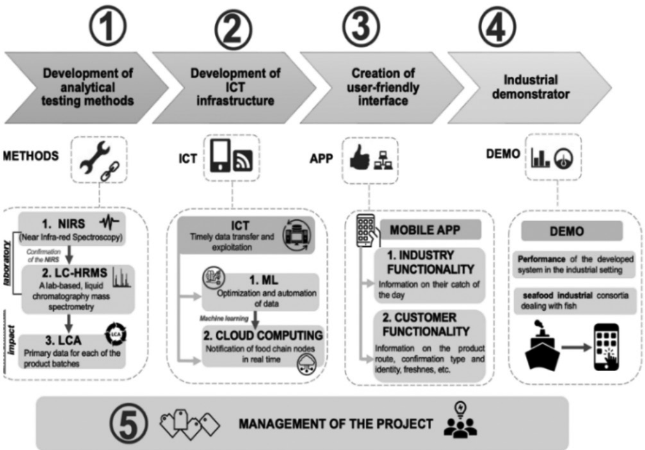

Figure 1. Project flow diagram.

INORGANIC PHOSPHATE USE IN DIETS FOR WHITE LEG SHRIMP *Litopenaeus vanamei* Boone, 1931 IN STANDARD DIETS

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From an economic and environmental perspective, P constitutes a significant proportion of the expenditure associated with mineral supplementation in diet formulations. The identification of P sources and their bioavailability in *Litopenaeus vannamei* diets assumes critical importance due to consequences of excessive phosphorus loads. The fishmeal replacement by alternative sources in shrimp diets has led to heightened interest in supplementing diets with inorganic phosphates, particularly in cases where high levels of vegetable meal are incorporated at elevated dietary levels. Thus, recent studies have assessed the availability of various inorganic P sources in shrimp feeds, investigating digestibility and growth performance with dicalcium (DCP), monocalcium (MCP), monoammonium (MAP), and monopotassium phosphates (MKP). Nevertheless, these studies predominantly utilized semi-purified diets (Milián-Sorribes et al., 2021) or suboptimal P levels in the feed (Lemos et al., 2021). Consequently, the objective of the current experiment was to assess to evaluate these aspects with standard diet formulations, considering both nutritional and environmental perspectives.

With this aim four trials were carried out: First and second trials were developed to establish the best inert marker (IM) for digestibility evaluation using four inert markers in a standard diet (Cr, Y, La and Ce) analyzing the diet digestibility and the inert marker retention in whole body, cephalothorax, and muscles (with and without intestine). The third and fourth trial were carried out to assess the P use of three P sources: monoammonium phosphate (MAP, $NH_4H_2PO_4$), monosodium/monocalcium phosphate (SCP-2%, $NaH_2PO_4/Ca(H_2PO_4)_2{\bullet}H_2O$ in proportion 12/88) and monosodium phosphate (MSP, NaH_2PO_4) using a Control diet without P supplement.
Regarding the IM results, Y resulted the best option to establish the digestibility due to the high solubility after acid digestion and the lowest Y retention in the shrimp whole body when was compared with the other markers.

Table 1. Apparent digestibility of diets (%) and inorganic phosphates used. Values are the mean (n=4) ± standard error (SE). Different superscripts in the same row indicate significant statistical differences with p<0.05. Newman-Keuls test.

ADC (%)	Control	*SE*	MAP	*SE*	SCP-2%	*SE*	MSP	*SE*
Dry matter	75	3	77.2	1.4	74.7	1.4	75	2
Protein	67.2	1.0	67	2	66.6	1.1	66.3	1.9
P	75.4[a]	1.2	84.3[b]	0.3	84.3[b]	0.4	86[b]	0.4
Ca	34[a]	3	40[ab]	0.9	55[c]	0.6	42[b]	4
P source digestibility								
P			96.1[a]	0.6	96.1[a]	1.0	100.0[b]	1.0
Ca					93.1	0.8		

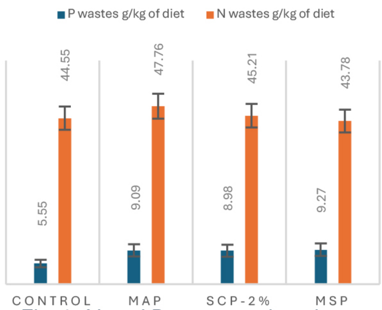

Fig 1. N and P waste estimation expressed in g N or P/kg of intake.

In the digestibility trial (*Table 1*) Control diet obtained the lowest P digestibility, because raw materials had lower P digestibility than the P from inorganic phosphates. Likewise, diet SCP-2% presented the best Ca digestibility values. The excretion of N or P did not present significant relevant differences, but when P and N excretion and retention was evaluated jointly with P and N digestibility (*Fig. 1*) the SCP-2% diet generated the lowest N waste in relation to the N intake. The MAP diet presented the highest amount of N residues due to N excretion, that was greater than in the rest of the diets, followed by the control diet.

Lemos et al. https://doi.org/10.1007/s10499-021-00651-3
Milián-Sorribes et al.10.3390/ani11061700.

ASSESSMENT OF INTESTINAL HEALTH THROUGH OMICS-BASED ANALYSIS OF WITH HIGH FISHMEAL SUBSTITUTION IN *Liza aurata*

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Introduction: The current challenge in aquaculture revolves around the quest for new, economically, and environmentally sustainable species. Species occupying lower trophic levels, such as *Liza aurata*, may represent promising candidates for aquaculture. Nevertheless, when formulating diets with high or total fishmeal substitution, it can potentially influence their growth and health status (Estruch et al. 2015). Thus, in this study the growth and health status through the transcriptome of posterior intestinal section was assessed in *Liza aurata* fed with two experimental diets containing 15% and 0% of fishmeal.

Material and Methods: Sixty *Liza aurata* (9.14 ± 1.52 g) 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. Monthly sampling was carried out to analyse fish growth and survival and specific 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 transcriptome of posterior intestinal section (FISABIO, Spain). We focused on transcripts with a padj value of less than 0.05. Enrichment analysis of GO and KEGG terms was conducted using the Fisher Exact Test.

Table 1. Growth performance and nutritional parameters of *Liza aurata*. Values represent mean ± standard error.

Diet	**Weight Gain (%)**
HP15	172.59 ± 33.55^{a}
HP0	98.16 ± 4.76^{b}

Values are the mean ± SEM (n=3). Different letters in the same column indicate significant differences at $p<0.05$. Newman-Keuls test.

(Continued on next page)

Results and discussion: Fish weight Gain, SGR and FCR showed significant differences (Table 1), indicating improved growth in the diet with higher fishmeal content. Despite being an omnivorous species, juvenile stages exhibited more carnivorous feeding habits.

Confirming these results, the intestinal transcriptome provided a total of 321 differential expressed transcripts (DETs). From the comparison HP0 vs HP15, 207 and 114 were were down and up-regulated, respectively. After GO enrichment analysis, results showed that 4 biological processes (BP) were significantly affected by the rearing conditions, mainly related to cell proliferation. Besides, KEGG analyses revealed 7 disturbed pathways, related to cellular proliferation and metabolism, particularly, protein digestion and absorption (ko04974). Therefore, a differential intestine expression was reported based on rearing conditions. In conclusion, relative high fishmeal requirements seem to be necessary in *Liza aurata* at juvenile state.

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EXTRACT *S. platensis* ADDITIVE USE IN PRACTICAL DIETS FOR *L. vannamei* Boone, 1931

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In aquaculture, feed additives have proved to be a useful tool as immune stimulants to improve intestinal health, stress, and disease resistance among other effects. Furthermore, *S. platensis*, it has been shown to reinforce the immune system of shrimp and improve nutrient bioavailability. Microalgae, in particular *Spirulina platensis*, possess high protein content and an ideal amino acid profile, comparable to the amino acid profile of marine protein sources origin such as fishmeal (Li *et al*., 2022). Thus, present study aims to investigate the use of extract spirulina additive inclusion of standard commercial shrimp diets and the effect on their shrimp growth.

Considering the goal of the experiment, a 35-day growth trial was carried out using a *Spirulina platensis* extract as an additive in 4 different diets (SP05, SP1, SP2 AND SP4) with 4 spirulina dietary inclusion (0.5, 1, 2 and 4 % of spirulina extract, respectively) and with a control (CON) diet without additive. All diets were assayed in triplicated tanks (15 animals per tank) in an open system with clear water. Sampling was carried out every 15 days and the increase in shrimp growth and survival of each treatment were monitored. Also, water quality parameters were measured and stable in all experimental tanks.

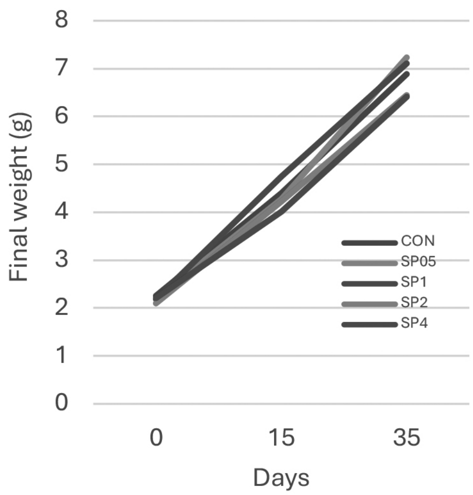

Figure 1.Evolution over time of shrimp weight fed with 5 experimental diets (CON, SP05, SP1, SP2 and SP4) for 35 days.

Table 1. Growth parameters (Final weight, survival, SGR, FCR and PER) of the 35-day experimental trial. Values are the mean (n=3) ± standard error (SE). Different letters in the same row indicate significant statistical differences (p<0.05). Newman-Keuls test.

Diets	Final weight (g)			Survival (%)			SGR (%/day)			FCR			PER		
CON	6.89	±	0.50	88.89	±	3.85	3.31	±	0.03	1.77	±	0.18	1.72	±	0.18
SP05	7.23	±	0.70	93.33	±	6.67	3.30	±	0.24	1.54	±	0.06	1.97	±	0.08
SP1	7.11	±	0.69	84.44	±	15.40	3.38	±	0.14	1.64	±	0.14	1.85	±	0.15
SP2	6.44	±	0.96	88.89	±	3.85	3.18	±	0.44	1.96	±	0.18	1.56	±	0.15
SP4	6.41	±	0.81	93.33	±	0.00	3.00	±	0.38	1.97	±	0.40	1.58	±	0.29

significant differences were found in any of the growth parameters (Table 1 and Figure 1). Overall, the dietary inclusion of spirulina did not have significant effect on survival data.

Li, L., Liu, H., & Zhang, P. (2022). Effect of spirulina meal supplementation on growth performance and feed utilization in fish and shrimp: a meta-analysis. Aquaculture Nutrition.

(Continued on next page)

At the end of growth trial, statistical analyses were done for SGR, FCR and PER however no significant differences were found in any of the growth parameters (Table 1 and Figure 1). Overall, the dietary inclusion of spirulina did not have significant effect on survival data.

Li, L., Liu, H., & Zhang, P. (2022). Effect of spirulina meal supplementation on growth performance and feed utilization in fish and shrimp: a meta-analysis. Aquaculture Nutrition.

ASSESSING THE IMPACT OF ATMOSPHERIC HEATWAVES ON INTERTIDAL CLAMS

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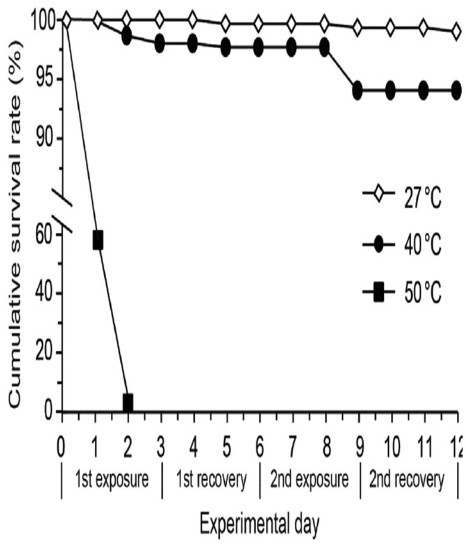
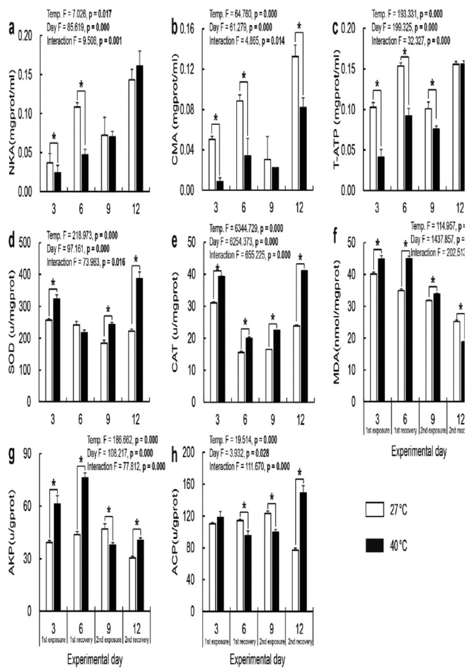
Heatwaves have become more frequent and intense in the last two decades, resulting in detrimental effects on marine bivalves and the ecosystems they sustain. Intertidal clams inhabit the most physiologically challenging habitats in coastal areas and live already near their thermal tolerance limits. However, whether and to what extent atmospheric heatwaves affect intertidal bivalves remain poorly understood. Here, we investigated the physiological responses of the Manila clam, Ruditapes philippinarum, to heatwaves at air temperature regimes of 40 °C and 50 °C occurring frequently and occasionally at the present day in the Beibu Gulf, South China Sea.

With the increasing intensity of heatwaves and following only two days of aerial exposure, Manila clams suffered 100 % mortality at 50 °C, indicating that they succumb to near future heatwaves, although they survived under various scenarios of moderate heatwaves. The latter is couched in energetic terms across levels of biological organization. Specifically, Manila clams acutely exposed to heatwaves enhanced their standard metabolic rate to fuel essential physiological maintenance, such as increasing activities of SOD, CAT, MDA, and AKP and expression of HSP70.

These strategies occur likely at the expense of fitness-related functions, as best exemplified by significant depressions in activities of enzymes (NKA, CMA, and T-ATP) and expression levels of genes (PT, KHK, CA, CAS, TYR, TNF-BP, and OSER). When heatwaves occur again, Manila clams can respond and acclimate to thermal stress by implementing a suite of more ATP-efficient and less energy-costly compensatory mechanisms at various levels of biological organization. It is consequently becoming imperative to uncover underlying mechanisms responsible for the such positive response and rapid acclimation to recurrent heatwaves.





THE CIRCULAR TRANSITION IN AQUACULTURE: A MULTICRITERIA ANALYSIS

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The circular economy (CE) approach can be a way to rebalance the intensification of the aquaculture sector and the depletion of the seas' resources, ensuring more efficient systems at the same time (FAO, 2022). The European Green Deal, the European Maritime, Fisheries, and Aquaculture Fund stressed the benefits of the Pauli's blue economy model (2010) since it promotes the resources' use imitating natural systems. The possibility to overcome a linear paradigm that has dominated production systems for decades is in doubt. Many studies (Ruiz-Salmón et al., 2020) offer circular aquaculture processes and products, but they are still experimental. Uncertainty exists, regarding the viability of this strategy and over the long-term success of this approach. As Masi al., (2023) showed, imagining what means becoming circular is not straightforward. The circular transition implies changes in product operations, in farm vision, but also producing new dynamics to dialogue with the external environment (Lacy et al., 2020). Circular practises need to grow community-based, requiring new models of integration between socio-technical systems and agro-ecosystems (Prost et al., 2023). Comprehending the perspectives of the producers, or in other words those directly acting around the aquatic resource—should be prioritised. Several studies highlight the existence of a gap between producers' and blue policies' perspectives about future challenges for the aquaculture sector (Krause et al., 2015, Vecchio et al., 2023). To build responsible blue policies, the knowledge should derive both from the aquaculture value chains and its institutional context.

This paper aims to deepen the perspective of the farmers acting for the aquaculture sector in Italy about CE. Following the Lacy et al., (2020) theoretical model (See Tab.1), a questionnaire with a list of circular practices have been proposed.

Initially, an initial qualitative analysis of the responses brings out a generally positive opinion of the CE concept, seen as an opportunity of self-sustaining aquaculture systems, efficient use of natural resources by reducing waste and environmental impact, and finally, an approach to innovate. Then, a multicriteria analysis has been carried out, the results emphasize a definite idea of the circular strategies they considered as prioritizing to be implemented in the future. These results can be useful: i) in understanding the dimensions in which circularity is most supported by different stakeholders, for example in operations, rather than in the organisation culture and ii) the "openness" of group of stakeholders. This study could provide insights for building responsible aquaculture policies in the face of future challenges.

Tab.1 – The multidimensional nature of circular economy

Dimensions	Circular strategies areas
Operations	Energy, Emissions, Waste, Water
Products and Services	Design, Use, Use extension, Use, End of use
Culture and Organisations	Vision, Innovation, People, Governance
Ecosystem	Sharing, Collaboration, Investment, Policy

WATER FOOTPRINT ASSESSMENT OF THE SHRIMP INDUSTRY IN BANGLADESH

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Water footprint assessments are increasingly used to improve sustainable water management. The approach consists of four distinct steps: setting the goal and scope, calculating the water footprint of a product or process, evaluating sustainability, and formulating the required response towards sustainability. This study executed all these steps in the shrimp industry in coastal Bangladesh, using multiple tools including monitoring and measurement of ponds, semi-structured questionnaire-based interviews, Focus Group Discussions (FGDs), and In-depth interviews for data collection. In first step, two research questions were formulated: what is the water footprint of the shrimp industry, and how does shrimp production and processing face water scarcity and pollution? Besides, only the shrimp farm and processing industry are considered as the research boundary. In the water footprint calculation, extensive farming with water exchange facilities had a blue water footprint of 1.87 m^3/kg, while closed ponds without water exchange facilities had a lower blue water footprint, averaging 1.52 m^3/kg. Semi-intensive farming used more blue water, around 8.640 m^3/kg, despite its high production. Additionally, processing industries also had a considerable blue water footprint of ~0.021 m^3/kg, which is entirely dependent on groundwater. Extensive farming also produced less grey water, ~0.008 m^3/kg and ~0.015 m^3/kg for ponds with water exchange facilities and closed ponds, respectively. The study used its own approach to explore the sustainability of the industry and found that farmers struggled with water availability and wastewater discharge, ultimately leading to increased groundwater usage. Increasing shrimp production with minimal use of feed and chemicals could potentially reduce the water footprint. This study has important implications for present and future policy interventions, particularly in decision-making regarding the sustainablewater supply without hampering groundwater resources and addressing challenges to transform the industry towards intensification.

COMPARISON OF THE IMMUNOLOGICAL RESPONSE TO WHITE SPOT DISEASE IN ZEBRAFISH AND RAINBOW TROUT

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Outbreaks of white spot disease (WSD) caused by the parasite *Ichthyophthirius multifiliis* results in high mortality and morbidity leading to significant economic losses for both the aquaculture and ornamental industries. Severe cases of WSD have been reported in various freshwater fish species including rainbow trout, carp and channel catfish. In contrast, zebrafish (*Danio rerio*) exhibit a high level of natural resistance to the parasite. The mechanisms underlying this increased protection in zebrafish are currently unknown. However, identifying the genes responsible for this resistance could be highly beneficial for mitigating the disease and for understanding protective immunity.

To investigate this, we conducted a comparative transcriptomic analysis between naturally resistant zebrafish and susceptible rainbow trout (*Oncorhynchus mykiss*). Both fish species were exposed to an equal number of parasites per unit surface area, and gills were sampled at 2, 24, 48 and 72 hours post infection (hpi). The differential gene expression analysis revealed an immediate response in zebrafish that peaked at around 24 hpi and normalized at 72 hpi (Table 1).

Additionally, there was a continuous decline in transcripts mapping to the *I. multifiliis* genome, indicating that the infection was being cleared (Figure 1). In contrast, the rainbow trout elicited minimal response (Table 1), with a continuous increase in transcription (Figure 1) suggesting ongoing growth and establishment of the parasite.

We observed an increase in the transcription of genes in zebrafish associated with the Toll-like receptor signaling pathway, MAPK signaling pathway and a cellular response. The implications of these findings are discussed and candidate genes responsible for resistance have been identified. To assess the functionality of these candidate genes, a knockout study using the CRISPR/Cas technology was performed. The knockout fish were then exposed to *I. multifiliis* to evaluate susceptibility.

Table 1. Number of differentially expressed genes in zebrafish and rainbow trout at 2, 24, 48 and 72 hours post infection.

Hours post infection	*Zebrafish*	*Rainbow trout*
2	67	11
24	1231	28
48	65	27
72	5	0

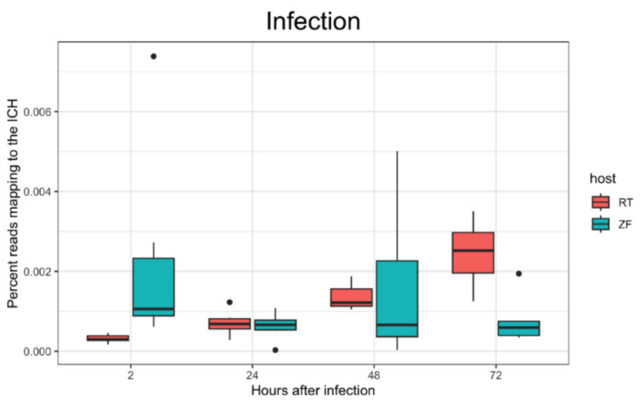

Figure 1. Percentage of transcripts mapping to the I. multifiliis genome in zebrafish and rainbow trout at 2, 24, 48 and 72 hours post infection.

SPATIAL AND TEMPORAL VARIABILITY OF THE REPRODUCTIVE ACTIVITY OF THE MUSSEL *Mytilus galloprovincialis* PRODUCED IN LONGLINES IN TWO AREAS OF SOUTH OF PORTUGAL

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Mussel commercial production depends on wild populations to obtain the seed. Therefore, any new farming initiative needs deep knowledge of the reproductive cycle of native mussel populations. The knowledge of the reproductive performance of *Mytilus galloprovincialis* is essential to increase the production of offshore companies since it allows the adjustment of ropes placement for maximization of larval recruitment. The present study focused on the reproductive cycle and nutrient storage, which is necessary to assess both sustainable exploitation rates for this species and the potential capacity for aquaculture production in the south of Portugal coast. The reproductive cycle of *M. galloprovincialis* from two sites (Olhão - 37°01,007'N; 07°44,499'W and Lagos - 37°04,200'N; 8°42,800'W) along the coast of the south of Portugal, with contrasting oceanic influences, was assessed using histological analysis of gonadal development, gonadal and condition index and gross biochemical composition over 1.5 consecutive years (2014 and 2015). Surface sea water temperature (SST) and chlorophyll a (Chl *a*) were recorded to evaluate the effect of these factors on the reproductive cycle. No specific spawning period was observed, with mussels under successively and continuously gonadal recovery and spawning during most of the studied period. The reproductive cycle of *M. galloprovincialis*, exhibited a year-to-year variability in both sites, revealing a significantly better condition in 2014 than in 2015 (Figure 1). Although it is widely accepted that these differences can be correlated with environmental conditions changes, in our study, it was not possible to correlate them with SST and Chl *a*. Storage and nutrient cycles showed that in both sampling sites, glycogen content, and gonadal index revealed opposite patterns, which allowed a rapid gonadal recovery after spawning, probably due to the constant food availability. The current study represents an important contribution to farms' profitability since it will allow better production management practices.

EFFECTS OF DIETARY FISH MEAL REPLACEMENT WITH PLANT PROTEIN ON LEUCOCYTE CYTOKINE RESPONSES IN YELLOWTAIL *Seriola quinqueradiata*

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Yellowtail, a carnivorous fish, is an important aquaculture species in Japan. In yellowtail farming, a reduction of dietary fish meal (FM) content is required to ensure sustainability. However, dietary FM replacement may alter the response of several inflammation-related genes in fish. This study aimed to evaluate the effect of dietary FM replacement with soybean meal (SBM) on the response of cytokine gene expression in yellowtail leucocytes.

<Leucocyte culture> Yellowtail leucocytes were isolated and cultured in a medium including one of two concentrations (50 and 100 µg/mL) of lipopolysaccharide (LPS) or water-soluble fraction of feed ingredient (FM, SBM, corn gluten meal: CGM, or soy protein concentrate: SPC). After 3 h and 9 h of incubation, the gene expression of four cytokines (IL-1**β**, IFN-γ, TNF-α, and IL-10) was measured by RT-qPCR. <Feeding trial and leucocyte stimulation> Yellowtail (initial average body weight = 175.2 g) were allocated to twelve 550 L tanks (8 fish/tank). FM in the basal diet was replaced to include 0% SBM (SBM0 diet), 15% SBM (SBM15 diet), 30% SBM (SBM30 diet), and 22.5% water-washed SBM (WSBM diet). The fish were hand-fed the experimental diets once daily until satiation. After 6 weeks of the feeding trial, fish were weighed, and blood was collected from four yellowtail per tank. Leucocytes isolated from the blood were stimulated with 50 µg/mL LPS or poly I:C, as described above. Cytokine gene expression levels were also measured.

<Leucocyte culture> After 3 h of stimulation, *il-1*β, *ifn*-γ, and *il-10* were significantly higher in the water-soluble fraction of feed ingredients, except CGM, than in the medium only (control). For *il-1*β, the SBM groups had significantly higher levels than the other groups after 9 h of stimulation.

<Feeding trial and leucocyte stimulation> Final average body weight was the lowest in the WSBM groups. For *il-1*β and *il-10*, LPS stimulation of the WSBM group was significantly higher than that of the other dietary groups after 3 h of incubation. For *ifn*-γ, the SBM15 and SBM30 groups with poly I:C stimulation were significantly higher than those in the SBM0 and WSBM groups of 9 h of incubation.

The water-soluble fractions of FM, SBM, and SPC were found to have leucocyte-stimulating effects. Replacing dietary FM with SBM or WSBM in yellowtail might change the response of leucocytes to LPS and Poly I:C.

This work was supported by JST Grant Number JPMJPF2117.

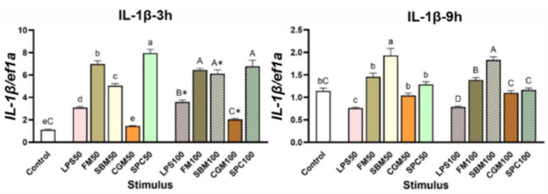

Fig.1 Relative expression of *il-1β* in leucocytes incubated with LPS or water-soluble of feed ingredient leucocyte by RT-qPCR.

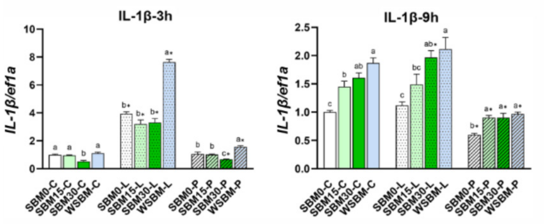

Fig.2 Effect of FM replacement by SBM or WSBM on the expression levels of *il-1β* at the end of the 6-week study, at the time of leucocyte stimulation.

INTEGRATED RESTORATION OF WATER BODIES USING MICROBIAL BIOTECHNOLOGY

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The war in Ukraine has significant environmental consequences, particularly for aquatic ecosystems. These ecosystems are essential for food security, as well as for Ukraine's economic and social development. The war can cause extensive harm to natural ecosystems, leading to a decline in biodiversity, destruction and degradation of habitats, and changes in ecosystem services. Aquatic ecosystems are one of the most vulnerable to warfare-related impacts because they provide many essential environmental resources and services to society (Lawrence et al., 2015).

During the war, various toxic substances, including metals, petroleum products, explosive residues, and related compounds, contaminated components of the ecosystem. Additionally, water bodies were contaminated with biogenic substances, such as biogenic amines and other substances derived from the decomposition and improper burial of large numbers of human and animal corpses, as well as the mass death of fish (Guo et al., 2022).

Ukraine receives significant amounts of fish from both natural water sources and the aquaculture sector. The infrastructure of the latter was damaged or destroyed during the war, making it even more vulnerable to the devastation of water resources caused by armed conflicts. This poses a threat to safety and social stability.

This requires rapid and effective solutions that take into account political, economic, environmental and social aspects. We are currently working on NATO Science of Peace and Security (SPS) Programme project "Creating a Strategy for Assessing and Restoring War-affected Aquatic Ecosystems" No. SPS G6085 envisages the use of microbial technologies (Fig. 1) that can become the basis for the restoration of natural aquatic ecosystems and aquaculture farms in Ukraine. The main research activities include rapid assessment of the state of aquatic ecosystems and aquaculture using low-cost methods, risk identification, restoration of bioresources and ensuring safe use of natural waters for fisheries and aquaculture in the future.

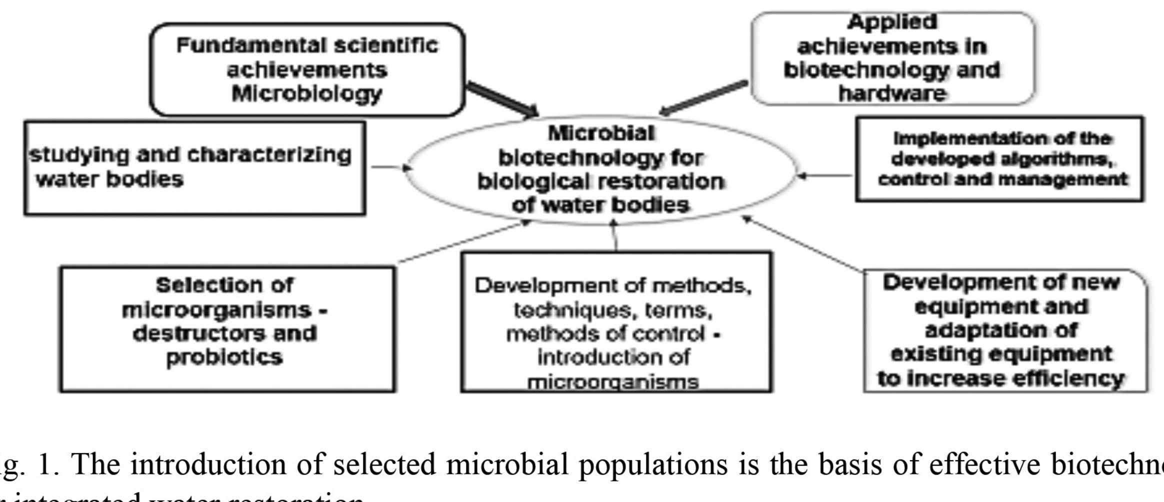

Fig. 1. The introduction of selected microbial populations is the basis of effective biotechnologies for integrated water restoration.

BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE MEAL IMPROVES GROWTH, FEED UTILIZATION, AND INTESTINAL HEALTH IN MIRROR CARP (*Cyprinus carpio*) JUVENILES

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Aquaculture is crucial for global food and nutrition security but relies on unsustainable inputs like fishmeal and soybean meal. Global fishmeal production is declining due to overexploitation and climate change impacts, while soybean meal faces food-feed competition, making the cost of these commodities increasingly high. Insect meals, especially from black soldier fly larvae, provide a sustainable alternative with high protein content and low environmental impact. This study investigated the effects of BSF meal on growth, feed utilization, and intestinal health of mirror carp (*Cyprinus carpio*) juveniles.

An 8-week feeding trial was conducted with *C. carpio* (initial body weight: 6.44 ± 0.05 g) in tanks (13 L, each) in a Recirculatory Aquaculture System (RAS). Three isonitrogenous and isolipidic diets were prepared based on the known nutritional requirement of carp to contain 0 % (Control: BSF0), 20 % (BSF20), and 40 % (BSF40) defatted black soldier fly larvae (BSF) meal (Table 1). The fish were divided into 3 groups of 20 juvenile fish in triplicate tanks and were fed the diets at 4 % biomass. After the trial, final body weight (FBW), weight gain (WG), specific growth rate (SGR), and protein efficiency ratio were improved while feed conversion ratio (FCR) was lowered significantly with increasing BSF meal in the diet compared with the control diet (Table 2).

Ongoing analyses include investigation of fish carcass composition, chitinase enzyme activity, intestinal histology applying both light and electron microscopy, expression of immune-related genes, and intestinal microbiota. These analyses aim to provide deeper insights into the effect of BSF meal on the fish.

Table 1: Composition of the experimental diets used in the study (g/100g)

Ingredient (g/100 g diet, DM)	Dietary administration		
	Control (BSF0)	BSF20	BSF40
Soybean meal	40.00	26.40	4.60
Sunflower meal	32.00	37.50	39.40
Black soldier fly meal	0.00	20.00	40.00
Wheat gluten meal	1.50	0.50	0.50
Wheat meal	0.00	0.00	5.00
Fishmeal	2.00	2.00	2.00
Soy protein concentrate	15.00	5.00	1.00
Sunflower oil	6.80	5.60	4.30
Fish oil	1.00	1.00	1.00
Lysine HCL	0.30	0.50	0.90
Carboxymethyl cellulose	0.50	0.50	0.50
Fish premix (0.3%)	0.50	0.50	0.50
DL Methionine	0.30	0.30	0.30
Total	100.00	100.00	100.00
Proximate composition (g/100 g diet, DM)			
Dry matter	90.89 ± 0.07	91.52 ± 0.25	91.46 ± 0.11
Crude protein	42.66 ± 0.24	43.10 ± 0.11	42.69 ± 0.31
Crude fat	7.54 ± 0.44	7.56 ± 0.18	7.67 ± 0.24
Ash content	5.54 ± 0.26	5.92 ± 0.35	6.11 ± 0.29

Table 2: Growth and feed utilization (Mean±SEM) of *Cyprinus carpio* juveniles fed experimental diets

	BSF meal inclusion levels in the diets (%)		
	BSF0 (Control)	BSF20	BSF40
Initial body weight (g)	6.47 ± 0.06	6.43 ± 0.06	6.43 ± 0.06
Final body weight (g)	17.57 ± 0.48^a	21.25 ± 0.94^b	22.99 ± 0.57^c
Weight gain (g)	11.10 ± 0.43^a	14.81 ± 0.88^b	16.55 ± 0.61^c
Specific growth rate (%/day)	1.78 ± 0.03^a	2.13 ± 0.06^b	2.27 ± 0.06^c
Feed conversion ratio	1.89 ± 0.04a	1.54 ± 0.05^b	1.43 ± 0.01^c
Survival Rate (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Condition Factor	2.19 ± 0.14	2.08 ± 0.18	2.17 ± 0.16

This study, therefore, suggests that BSF meal can be used as an alternative protein source in the diets of *C. carpio* juveniles to promote growth performance and feed utilization.

INTEGRATING INTERNATIONAL AQUACULTURE PRACTICES THROUGH ONLINE LEARNING

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Through Collaborative Online International Learning (COIL) participants have gained dynamic applicable skills, knowledge and new collaborations on aquaculture practices. Dalhousie University, Faculty of Agriculture will provide three examples where COIL opportunities have amplified learning experiences at both partnering institutions: Rhodes University, South Africa- Ecology in South Africa Course; PWANI and RIAT Universities, Kenya- Certificate in International Aquatic Rearing Practices; Topical Aquaculture in Philippines (MMSU) and Aquaculture in Atlantic Canada course. The focus lies on understanding the impact of COIL on enhancing students' knowledge, skills, and global perspectives in the field of aquaculture. By leveraging digital platforms and virtual collaboration tools, COIL initiatives facilitate interactions between students from different regions, fostering diverse perspectives and cross-cultural understanding. Innovative pedagogical approaches have allowed direct sharing of knowledge which in turn transpired to immediate applications within the classrooms and industry practices.

REGIONALISED GENE EXPRESSION WITHIN THE PEARL OYSTER MANTLE: IMPLICATIONS FOR PEARL FORMATION

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Pearls are highly-prized and structurally complex biominerals fabricated by a wide range of bivalve molluscs. Pearls that are composed of nacre (mother-of-pearl) are of commercial value and form the basis of a lucrative aquaculture industry. In cultured pearl formation a small section of the organ that secretes the shell (the mantle) is transplanted into the gonad of a receiving 'host' oyster, along with a spherical bead (the nucleus). The transplanted mantle cells proliferate and grow around the nucleus to form the pearl sac, which secretes the pearl material around the nucleus.

The mantle controls shell (and pearl) formation by secreting an 'organic matrix' composed of proteins, polysaccharides, and lipids. This organic matrix is ultimately responsible for dictating shell architecture, including the formation of different pearl layers (e.g., nacreous and prismatic layers, Fig. 1). Studies in pearl oysters and other molluscs have determined that different regions of the mantle secrete different proteins, and that this correlates with the proteins isolated from different shell layers. These mantle regions are presumably specified during the development of the mantle by a regulatory gene network.

The aim of this study was to investigate regionalization of gene expression within the pearl oyster (*Pinctada maxima*) mantle to better understand the regulation of nacre formation. To achieve this we microdissected the mantle of four oysters and generated transcriptomes for each region individually, using a low-input RNAseq method (CEL-Seq2). We then performed differential gene expression analysis to identify genes that are associated with particular mantle regions, and used bioinformatic methods to predict the gene regulatory networks that drive differential gene expression. Our results clearly demonstrate the existence of a nacre-producing region within the mantle and suggest how this may be regulated. These results not only reveal core regulatory processes governing biomineralisation, but may also have application in the improvement of pearl quality.

Figure 1. Shell and pearls produced by the silver-lip pearl oyster *Pinctada maxima*. The inner zone of the shell is the mother-of-pearl (nacreous) layer, and the outer, brown-coloured zone is the prismatic layer.

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

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The rapid growth of aquaculture must be made in a sustainable manner and the use of responsibly sourced raw materials adhering to the circular economy/zero waste concept is a priority. Therefore, the search continues for more environmentally friendly terrestrial plant alternatives, compared to traditionally feed constituents' fishmeal and fish oils, and current alternatives such as soy. Hempseed, derived from low cannabinoid (THC <0.3 %) industrial hemp, is one promising alternative as the crop is grown within the UK and the cultivation absorbs more CO_2 than other commercial cultivars (Viskovi□ et al., 2023). Moreover, hempseed is a secondary by-product, further reducing the carbon footprint, because the crops primary use is to produce materials for construction and energy using the plants stalks.

The present study investigated the suitability of a UK produced hemp seed by-product as a protein source for the Scottish Atlantic salmon industry in two stages. The first validated the use of hemp protein (HP) as an ingredient for aquafeeds and assessed its nutritional attributes and digestibility value. This trial included three experimental feeds (a commercial formulation control and two feeds included 30 % of either hempseed meal [HM] or soy protein concentrate [SPC]) containing yttrium as an inert marker, which were fed to Atlantic salmon. At the end of the trial all fish were euthanized, performance and welfare indicators were recorded, and faeces collected for digestibility. No difference in performance or welfare was found, regardless of diet, and hempseed protein digestibility proved to be good with protein and amino acid values close to 100%.

A second longer term trial is ongoing focussing on fish performance and filet quality for the consumer, in addition evaluating effects of the hempseed diet on the gut. Atlantic salmon are being fed either a commercial formulation control or an HM inclusion diet over a ten-week period before euthanasia and collection of samples for chemical, histological and gene expression analysis. Results from this trial will be presented to reveal whether hempseed meal is a viable alternative to SPC in Atlantic salmon diets.

Visković et al., 2023; https://doi.org/10.3390/agronomy13030931

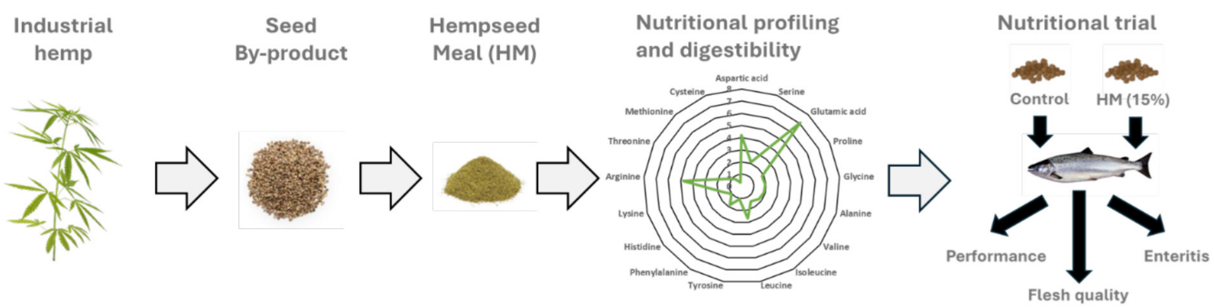

Figure 1. Schematic of hempseed protein production and nutritional assessment.

POTENTIAL LIVE FEEDS FOR LARVAL FISH CULTURE IN ETHIOPIA

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The artificial propagation of commercially important fish species in Ethiopia is constrained by high mortality rate at the early stage of larval rearing and a subsequent poor growth performance during later stages. This is mainly due to absence of live feeds suited to the requirements of the larvae of the species. Although not organized, several studies have indicated that microalgae, copepods, cladocerans and rotifers are the dominant inhabitants in several water bodies that are the precursors in the larviculture of commercially important finfishes in the world. However, apart from studying the abundance and diversity of these important planktonic organisms, efforts made to culture any of them for use in the larviculture of commercially important fish species in Ethiopia is minimal. This may be due to the lack of comprehensive and organized information on the distribution and abundance of these important species in the context of their potential in aquaculture of live feeds in Ethiopia. The objective of this review is therefore to compile the available information on the abundance and distribution of the major potential live feed organisms in the Ethiopian water bodies with a special emphasis on freshwater live feed organisms currently used in larviculture of commercially important freshwater finfishes. In line with this, four potential live feeds (i.e. microalgae, rotifers, copepods and cladocerans) were included in the review. It is hoped that the review will provide baseline information for future research in the culture of economically important larval live feeds.

DHEA PROFILE IN TELEOSTS: A FOCUS ON THE GONADS OF THE RAINBOW TROUT

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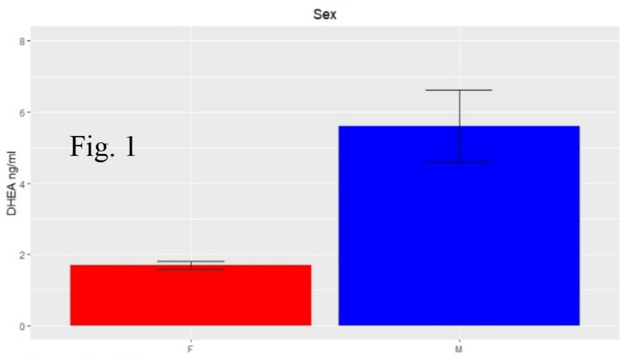
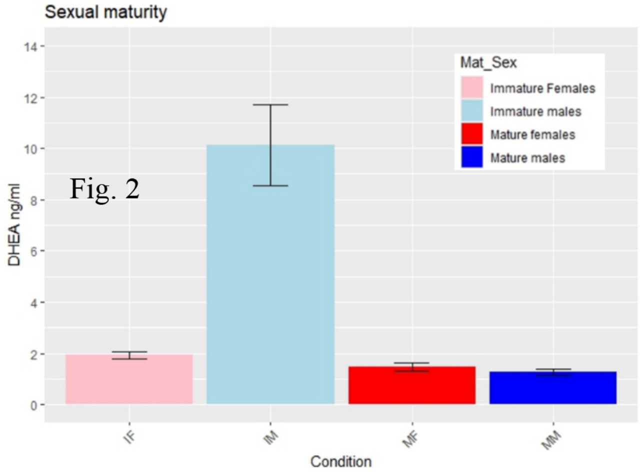
Recently, Dehydroepiandrosterone (DHEA), a steroid previously believed to function solely as a pro-hormone, has been utilized to measure chronic stress levels in humans. DHEA is abundant in primates but found in lower concentrations in rodents and other animals. In mammals, DHEA is produced in steroidogenic tissues and the nervous system from pregnenolone, the same precursor used to synthesize cortisol, through the $\Delta 5$ pathway. DHEA profile differs among species and varies according to the different physiological status, and although limited information is available regarding non-mammalian species, recent research has demonstrated that DHEA is present and detectable in teleost scales and appears to be correlated with chronic stress exposure. This study is focused on evaluating the gonad DHEA profile associated with a teleost species, Rainbow trout, as a potential synthesis site and the correlations with other biological matrices.

Sampling was carried out in a commercial freshwater fish farm and trout were sampled based on sex, and sexual maturity. Furthermore, half of the fish from each group were subjected to acute stress conditions through overcrowding for thirty minutes. Trout were thus divided into 8 different groups based on the combinations of physiological conditions: sex (male or female), sexual maturity (mature or immature), and stress application (control and stress), with 12 animals per group (Total n = 96; 1182.1 g ± 263.4).

For cortisol and DHEA analysis, an adapted specific microtiter radioimmunoassay (RIA) was used to measure the hormone levels.

Despite acute stress appeared to not cause a DHEA profile modification, an higher level has been observed in females than in males in all the matrices besides the gonads, where males showed higher levels than females (Fig. 1). Sexual differentiation has been shown, even differently, for other species such dogs, killer whales and rhesus monkeys and the European starling. Moreover, in our study, gonads level showed an interesting pattern (Fig. 2), with immature males presenting an higher level of DHEA if compared to all the other groups, suggesting a potential participation of this hormone in maturation processes and identifying testis of pre-puberty specimens as potential sites of its synthesis.





ADVANCING AQUACULTURE CAPACITIES: THE AQUAE STRENGTH PROJECT

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In recent years, Aquaculture has emerged as one of the fastest-growing sectors in zootechnical production, addressing this crucial need for sustainable protein sources. However, fish diseases are still a limiting factor and innovative control strategies are essential to maximise aquaculture productivity. In this context, the Aquae Strength project aims to improve the capabilities for managing fish diseases and build capacity in GIS techniques to support surveillance and disease response. The project encompasses many beneficiary institutions spanning various countries from the North Africa to Indochinese Peninsula.

The Aquae Strength project "*Strengthening Capacity on Aquatic Animal Health and Epidemiological Surveillance*" is driven by a comprehensive set of objectives aligned with WOHA priorities for the Aquatic Animal Health Strategy. The project is composed of a consortium that comprises seven Italian institutions "*Istituto Zooprofilattico Sperimentale*" (IZSVe, IZSSA, IZSPLV, IZSAM, IZSLT, IZSUM and IZSM) and three external advisory bodies from UK (Cefas), Denmark (DTU) and Norway (NVI). Capacity-building activities started with online webinars designed to provide stakeholders with the theoretical basis of the main project's topics. Subsequently, the project staff conducted on-site evaluations for each beneficiary country on their diagnostic capacity for fish pathogens. The assessments were performed using customized questionnaires, field visits to fish farms and laboratories and focused discussions with beneficiary's staff. These activities successfully identified gaps and areas to guide the project's efforts. The main intervention areas encompass improving diagnostic capabilities, ensuring the responsible use of Veterinary Medicinal Products (VMPs), involving veterinary services in disease outbreaks management, and establishing an official farm registry.

Activities performed so far highlighted several differences regarding the level of diagnostic capacities and management of VMPs among Beneficiary Countries. The role of veterinary services during disease outbreaks in these countries remains ambiguous. The project staff promoted the involvement of veterinary services and the adoption of antimicrobial susceptibility tests (AST) by diagnostic laboratories. The lack of an official fish farms registry and laboratory diagnostic capabilities makes epidemiological evaluation and product traceability challenging. These countries aim to establish trade with the EU, which requires compliance with animal health and food safety standards. The project staff delivered customised training sessions for each stakeholder based on their needs. Training activities were conducted both in the beneficiary countries and at the consortium's laboratories. Enhancing fish farms health status and disease management is essential for food safety, economies, and the environment. Assisting beneficiaries in improving their aquaculture industry will contribute to exploiting the full potential of aquatic resources. In this scenario, the Aquae Strength project represents an international effort in building a resilient and sustainable aquaculture industry, aligning with the One Health approach.

IMPACT OF ORGANIC-BASED AND CIRCULAR ECONOMY-DRIVEN FEEDS ON GLOBAL WARMING POTENTIAL, PERFORMANCE AND ROBUSTNESS OF JUVENILE GILTHEAD SEABREAM (*Sparus aurata*)

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To align with societal demands, aquaculture needs to enhance its environmental performance by improving the environmental sustainability of its feeds. Towards this objective, novel formulations could be based on organic or circular economy-driven ingredients. This study aimed to evaluate the global warming potential of such non-conventional feeds and their effects on growth performance and health status of juvenile gilthead seabream (*Sparus aurata*) in grow-out conditions and after exposure to a challenge event (overcrowding).

Three isonitrogenous and isoenergetic experimental diets were formulated: a control (CTRL) commercial-like feed; an organic (ORG) diet based on ingredients compatible with organic certification and with limited inclusion of animal proteins; and an eco-efficient (ECO) feed using circular economy-driven subproducts and with limited inclusion of fishmeal. The Global Warming Potential (GWP) of each feed was calculated using the Life Cycle Impact Assessment (LCA) methodology. Diets were tested in triplicate during stage 1 (growth) for 9 weeks with a fish density of 2.5 kg/m^3 and on stage 2 (challenge) with overcrowding for 2 weeks by increasing density to 12.5 kg/m^3. At the end of Stage 1, fish were sampled for whole-body composition, as well as dissected for liver and anterior intestine samples in both stages (growth and challenge) for assessment of lipid peroxidation (LPO) and molecular biomarkers of the gut immune condition, oxidative status and epithelium integrity.

At the end of Stage 1, final body weight was higher in fish fed diets CTRL and ECO, than those fed with ORG (p=0.002). The FCR was significantly higher for ORG (p=0.046). The digestibilities of nutrients and energy were higher in CTRL and ORG, but lower for ECO ($p < 0.03$). Whole body composition was not affected by the dietary treatments (p>0.05). Diet ORG showed higher GWP, followed by ECO and CTRL. There were no statistical differences (p>0.05) regarding the LPO among treatments in the liver and anterior intestine of Stage 1 fish or between fish from both stages. The feeds only affected the relative expression of one gene related with intestinal adaptive immunity (*igm*) (p<0.001) and another with epithelium protection (*muc13*) (p=0.001) at the end of the growth trial.

This study suggests that while the organic feed may slightly reduce fish performance, it still demonstrated positive results. In addition, the diets did not exhibit significant impacts on fish oxidative status. These novel feeds and particularly the organic have a higher GWP associated. Thus, more research is needed on the effects of such feed concepts and other environmental impacts (e.g., resource consumption) to provide a more comprehensive evaluation.

This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 956129 "EasyTRAIN". Additional funding was obtained from the Portuguese national funds from FCT – Foundation for Science and Technology through projects UIDB/04326/2020 (DOI:10.54499/UIDB/04326/2020), UIDP/04326/2020 (DOI:10.54499/UIDP/04326/2020) and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020), as well as from "Pacto da Bioeconomia Azul" (Project No. C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR).

AI CAPABILITIES IN UNSUPERVISED CATEGORIZATION AND COLOR QUANTIZATION OF TROPICAL FISH IMAGES

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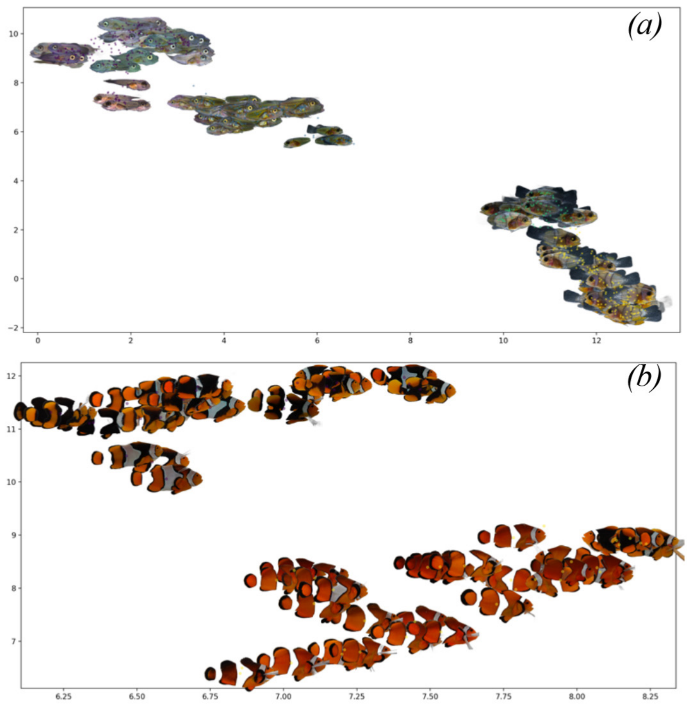
This study investigates the application of machine learning algorithms to explore the unsupervised categorization and color quantization processes of tropical fish images. Through the utilization of a neural network algorithm, the study aims to categorize images predominantly featuring *Amphiprion Ocellaris* by their developmental stage (Figure 1 *a*). Additionally, the study seeks to extend this classification capability to encompass images of various species or treatment groups, thereby expanding the applicability of the developed methodology. A crucial aspect of this research involves implementing a color quantization methodology to simplify the representation of intricate color patterns within the images. By employing these techniques, the study aims to not only characterize both qualitative and quantitative traits but also highlight the growing potential of AI in facilitating detailed analysis within the realm of tropical reef fishes. The experimental results demonstrate the efficacy of the machine learning algorithms employed in both the unsupervised categorization and color quantization tasks. Specifically, the neural network algorithm efficiently categorizes images of *Amphiprion Ocellaris* by their developmental stage and effectively distinguishes between different species such as *A. Percula* and *A. Ocellaris*. Moreover, the color quantization methodology proves to be successful in streamlining the representation of complex color schemes within the images, thereby contributing to the comprehensive characterization of qualitative and quantitative traits. These findings underscore the transformative impact of AI in facilitating precise and efficient analysis within the field of marine biology, paving the way for future advancements in understanding tropical reef fishes and their ecosystems.

Figure 1: The figure illustrates the performance of the clustering algorithm, showcasing its effectiveness in categorizing tropical fish images based on their developmental stage *(a)* and by species *(b)*. The embedding is done using a UMAP projection of the feature vectors calculated using the pre-trained neural network. This process is an unsupervised learning technique.



USING THERMAL PRIMING TO MITIGATE THE LETHAL EFFECTS OF MARINE HEATWAVES ON THE MANILA CLAM *Ruditapes philippinarum*

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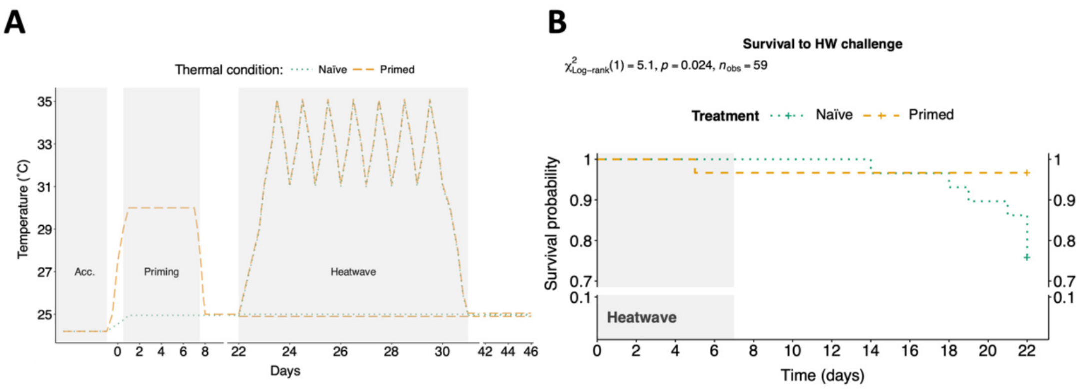
Bivalve aquaculture is an important activity able to provide healthy food and has a low carbon footprint. This activity is threatened by climate extreme events such as marine heatwaves (MHWs), which are increasing their frequency, duration and intensity every year. In shellfish farming the scope for contrasting the impact of MHWs is limited. Heat-priming may be an effective solution to mitigate the negative consequences of these events. Priming is a plastic response of the phenotype triggered by non-lethal stress stimuli, which might help reducing the impact of a subsequent lethal stress. In this work we assessed whether heat-priming in Manila clams for 7 days at 30 ˚ C could increase survival to a lethal HW even a relatively long time (two weeks) after the end of the priming period and show long term (> one month) protective effects.

Half of a hatchery-produced population was subjected to priming (Primed, P), while the remaining animals were kept at normal summer conditions (Naïve, N) for 7 days. After a recovery phase of 15 days, for each group half of the animals were exposed to a simulated HW (Heat wave challenged, H), while the remaining were not (Controls, C), in a 2x2 full factorial design (four groups: PH, PC, NH, NC; Fig. A). Mortality was recorded daily. Immediately after the HW, clam burrowing behaviour was tested, while 15 days after the end of the thermal challenge, the animal antioxidant activity, digestive gland transcriptome and microbiome were evaluated.

A higher survival rate in PH clams (compared to NH) was found after HW (Fig. B). At the behavioural level, 64% of PH clams were able to fully hide in the sand, while only 20% of NH clams did so. At the transcriptomic level we found upregulation of HSPs expression and metabolic pathways in PH clams. Beneficial bacterial taxa were more abundant in PH clams while families associated with detrimental effects were more abundant in NH clams. Signatures of putative protective changes were evident 38 days after priming in PC clams (primed, but not exposed to HW). Those changes included higher antioxidant activity and upregulation of metabolic pathways (in contrast with NC clams). Overall, all the evidence suggests long-term protective effects of priming and its potential as a mitigation strategy to alleviate the negative consequences of MHW on this important aquaculture species.



ENHANCING AQUACULTURE SKILLS AND TRAINING THROUGH A GAMIFICATION APPROACH AND DISTANCE LEARNING PLATFORM (BLUEAQUAEDU BLUE CAREER PROJECT)

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BlueAquaEdu is a project co-funded under the EMFAF-2023-BLUECAREERS, and aims to explore the potential of gamification in aquaculture education for teenagers (15-18) and students (18+) interested in aquaculture, post-harvesting value chain, and processing by-products sectors. By developing a serious game, BlueAquaEdu seeks to revolutionize aquaculture education, offering an immersive learning experience to engage and motivate learners. The integration of gamification tools into educational programs is envisioned to enhance the knowledge, skills and readiness of future professionals in the blue economy, enabling them to address the complexities of sustainable aquaculture practices effectively. As technology evolves, serious games present an innovative and efficient means of training the next generation of aquaculture experts. The creation of a virtual Aquaculture Educational Network (v- AquaEduN) is a central component of this endeavour. This network, comprising academics, aquaculture industry representatives, and public authorities, is dedicated to establishing and nurturing connections between education and the aquaculture industry. By fostering collaboration and knowledge exchange, v-AquaEduN aims to bridge the gap between theory and practice, ensuring that educational programs remain relevant and responsive to the needs of the aquaculture sector.

BlueAquaEdu capitalize on the results from previous relevant projects in the region, such as the MENTOR- “Blue Career Centre for Eastern Mediterranean & Black Sea” and “EU-CONEXUS RFS” to promote transferability of the results in other geographical areas. Special attention will be given to the knowledge triangle approach. Following this rationale, partners from France, Greece, Portugal and French Guiana join forces to identify and map all the accredited training programmes (both academic degrees and vocational education and training - VET) in the region, including the training programmes provided there-in.

A mapping of the provided training programmes for aquaculture in Europe identified Universities and VET providers for aquaculture. The survey focused on information in each BlueAquaEdu Country as well as in selected EU Countries (Belgium, Bulgaria, Croatia, Cyprus, Denmark, Finland, Germany, Ireland, Italy, Malta, Norway, Poland, Romania, Spain, and The Netherlands while the survey was made in the EU outermost region in the Atlantic (Guadeloupe, Martinique, Mayotte and Reunion). This mapping will create a network for dissemination purposes as well as for the creation of a wider Blue network that would support the creation of synergies with the e-platform. The BlueAquaEdu mobile game aims to educate the target audience on how fish farms function, the risk involved in such facilities and their environmental footprint on the planet. The game enhances and promotes the digital learning experience of the students with a special online learning content, to make it fun and engaging and encourage learners to explore and learn as they try to achieve a goal.

EFFECTS OF TIO_2-BASED PHOTOELCTROCATALYTIC (PEC) WATER PURIFICATION SYSTEM ON SKELETAL MORPHOLOGY AND GROWTH IN RAINBOW TROUT *Onchorhyncus mykiss* REARED IN A RECIRCULATING WATER SYSTEM

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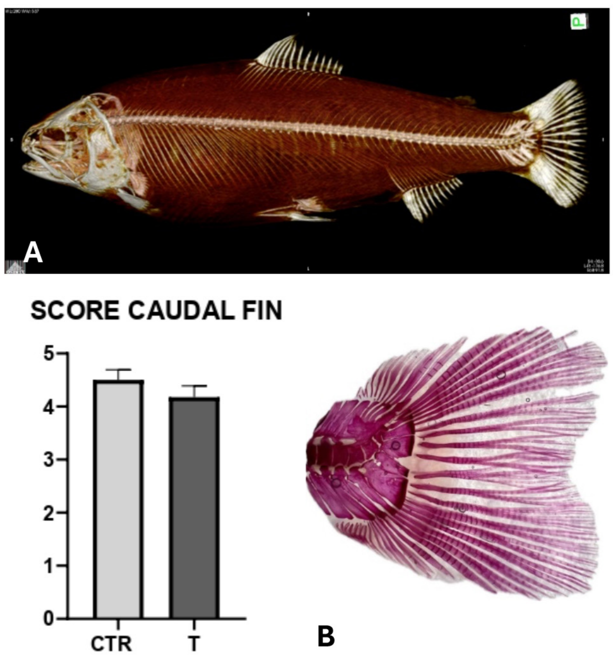
Investigating water purification systems is essential for enhancing the sustainability of recirculating aquaculture systems (RAS). This study wants to lead the way in developing an innovative tool that utilizes TiO_2-based photo-electrocatalysis (TiO_2PEC) to address this need. The advancement involves enhancing the conventional UV-filtration system integrated with biofiltration, thereby bolstering the purification of the water. In RAS, fish may be exposed to altered water quality parameters, including nitrate-nitrogen potentially associated with skeletal deformities or alterations. Our investigation aims to examine the skeletal assessment in Rainbow trout (*Oncorhynchus mykiss*) farming. Fish has been raised at a density of 15 kg/m^2 in both a control group (CTR) and a TiO_2PEC group (T) during the grow-out phase (100g-160g).

Firstly, water parameters were controlled to monitor quality indices. Moreover, a scoring was performed to evaluate the condition of the fins. As concerning skeletal assessment, it has been followed a multidisciplinary approach, with i) imaging analyses and ii) histochemical staining techniques, to achieve a comprehensive assessment of skeletal structures and potential deformities. Imaging: X-ray imaging (radiography) was employed to examine the skeletal structures for the detection of any abnormalities or irregularities in bone formation. Then, computed tomography (CT) scans were performed to generate detailed cross-sectional images. ii) Histochemical staining was applied to visualize fin structures (Alcian blue-alizarin red whole mount staining for bone and cartilage respectively). Moreover, bone tissue samples obtained from vertebrae were subjected to histological examination with: Picro Sirius Red for collagen distribution. Water parameters revealed that nitrates were lower in the T group (122.211 mg/L vs. 108.510 mg/L; $p < 0.001$). In skeletal analysis, no significant differences were observed between the groups: no anomalies, affecting the vertebral column and the cephalic region were observed. Similarly, no alterations of fin rays were found (Figs A and B). Histochemistry revealed that the vertebral bodies were mainly organized with collagen type 1 fibers. These findings suggest that TiO_2PEC did not introduce discernible variations in the assessed parameters between the two groups, but it is possible to appreciate an improvement in the quality of the water in the T group.



EMPOWERING WOMEN IN SRI LANKAN FISHERIES AND AQUACULTURE: UNLOCKING OPPORTUNITIES FOR SUSTAINABLE GROWTH

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The aquaculture industry in Sri Lanka is vital to the nation's economy and food security, yet little is known about the role that women play in this field. This study explores the complex dynamics of gender involvement in Sri Lankan fisheries and the aquaculture sector, emphasizing the opportunities, difficulties, and significant contributions made by women to the economy. Utilizing an all-encompassing examination of socio-economic variables, cultural standards, and legislative structures, it illuminates the diverse roles that women assume across the aquaculture value chain. Several case studies show the empowerment routes, barriers, and tactics used by women to be involved in this male-dominated sector. Moreover, the emphasis on stakeholder input in Sri Lanka's legal system may open opportunities for women to participate in decision-making. The contribution of Sri Lankan women in the labour force is 32.92% according to the statistics in 2022 and they make up about 7% of the 275,046 workers in the nation's marine fisheries industry. The fisherwomen demonstrated a higher tendency to gain a concrete understanding of value addition and novel post-harvest techniques by voluntary participation in training programmes conducted under the state organizations (National Aquaculture Development Authority of Sri Lanka (NAQDA)) and Non-Governmental Organizations (NGO). These outreaches added new skill sets like ornamental fish farming which encouraged them to become future entrepreneurs (Table 1).

The involvement of women in Sri Lankan fisheries and aquaculture can be enhanced through a comprehensive approach that addresses barriers and promotes equal opportunities. Strategies include training and skill development programs, equitable access to resources, technical support and extension services, promotion of women's groups and cooperatives, infrastructure development, legal and policy support, market access and value addition, empowerment and leadership development, research, and data collection, and raising awareness about gender equality and women's empowerment. These strategies can help women gain access to resources, improve their competitiveness and income, and contribute to their socio-economic empowerment and sustainable development. By implementing these strategies, Sri Lanka can create an enabling environment for women to actively participate and benefit from these sectors.

TABLE 1. Outreaches to encourage the women in fisheries and aquaculture sector.

Organization	Skill development
Sevalanka Foundation	Drying, preserving, and processing fish in communities
SEED initiative	Processing and postharvest production of fish
NAQDA	Ornamental fish breeding, ornamental fish farming, disease diagnosis and fish health management, ornamental fish feed and nutrition, propagation, and cultivation of ornamental aquatic plants

INACTIVATION OF *E. COLI* AND ANTIBIOTIC RESISTANCE BY UVC/CHLORINE FOR THE FEEDING OF AQUAPONIC SYSTEMS WITH REGENERATED WASTEWATER

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The problem of water scarcity and the increasing difficulty of access to water has led to the development of new technologies for the purification and reuse of wastewater. For this purpose, this work has evaluated the efficacy of photolysis process in bacterial inactivation with the goal of being applied as a regenerated wastewater treatment to be reused in the aquaponics system. In this installation, monochromatic UVC mercury lamps emitting at 254 nm have been used. UVC radiation has a very effective action against different pathogenic microorganisms because it directly attacks the genetic material. However, the use of other oxidizing agents are supposed to increase the efficacy of this process to be applied as a tertiary treatment in a sewage treatment plant. In this work use of the UV/Cl process is proposed. It is known that chlorine would generate toxicity for fish in the aquaponics system, so it may be necessary to install an active carbon filter before entering the aquaponic system in case there is chlorine remaining after treatment. We worked at 5 different intensities (from 3 W/m^2 to 82 W/m^2) inoculating *Escherichia coli* and resistant bacteria to the antibiotic vancomycin (ARB-Van) with an initial concentration of 10^6 CFU□mL^{-1}, in a matrix of sterile distilled water at 9% NaCl. In addition, the concentration of the oxidizing agent studied corresponded to 0.1 ppm and 0.25 ppm of chlorine. The kinetic constant obtained in the *E. coli* inactivation processes was calculated. As a result, the kinetic constant increases linearly as the irradiance increases. The same occurs in the oxidation processes with chlorine and acting in the same way for the combined UVC photooxidation process with chlorine. However, in the case of ARB-Van, no higher inactivation rate was observed with increasing chlorine concentration in the dark oxidation processes, showing the difficulty to degrade this type of pollutant when using only chemical oxidants. However, when combining UVC radiation and chlorine, the inactivation of ARB-Van increases with chlorine concentration up to a 4-log reduction.

Acknowledgement: Aquaponics from wastewater reclamation (AWARE). GA N. 101084245

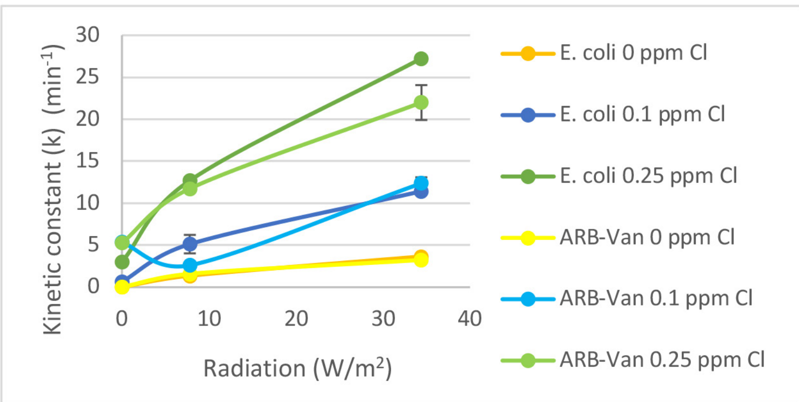

Figure 1: Inactivation kinetic constants for E. coli *and ARB-Van in UV/Cl process.*

PURIFIED BREWERS' YEAST–DERIVED FUNCTIONAL FEED ADDITIVES ALLEVIATE SOYBEAN MEAL-INDUCED ENTERITIS IN ATLANTIC SALMON PARR

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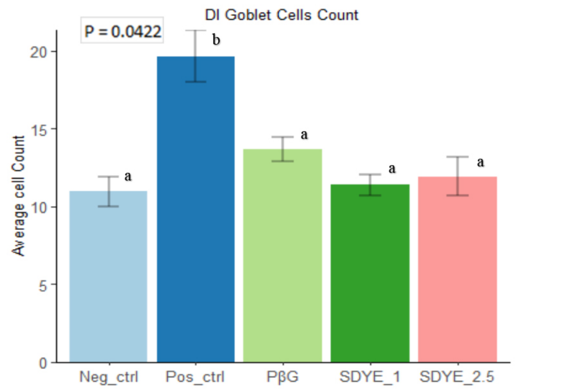
The inclusion of high levels of soybean meal and other plant ingredients have been shown to trigger inflammatory response and induce dysbiosis in the distal intestine of Atlantic salmon. Functional feed additives (FFAs) such as brewers' yeast (*Saccharomyces cerevisiae*) may confer immunomodulatory effects in fish. In this experiment, we investigated the ability of two brewers' yeast FFAs from Leiber GmbH to modulate the known pathological effects of high SBM in Atlantic salmon parr. Product A (Purified β-glucan) contains cell wall extracts rich in β-1,3 and -1,6-glucans while product B (Soluble Dried Yeast Extract) is made of soluble dried cell extracts rich in amino acids, glutamic acids, nucleotides and peptides.

A total of 450 salmon parr (ca. 24 g) were randomly assigned into 15 experimental units and fed one of 5 experimental diets: Neg_ctrl (0% SBM), Pos_ctrl (30% SBM), PβG (30% SBM + 0.02% β-glucan), SDYE_1 (30% SBM + 1% yeast extract) and SDYE_2.5 (30 % SBM + 2.5% yeast extract), with each treatment replicated three times. Fish were fed an average of 1.5% body weight per day with feeding rate adjusted following periodic batch weighing. At the end of the experiment, samples of skin and distal intestine were collected for light microscopy, scanning and transmission electron microscopy, gene expression, and microbiome analysis.

The results from this experiment clearly showed that the enteritis model (diet Pos_ctrl) with 30% SBM composition induced extensive signs of enteritis with significantly higher enteritis score (Figure 2) and higher density of goblet cells (Fig 1) in the epithelium than the Neg_ctrl diet (0% SBM). These negative physiological changes were not observed in the yeast treated groups, despite the high level of SBM. Thus, evidencing a protective role of the yeast FFAs against enteritis.

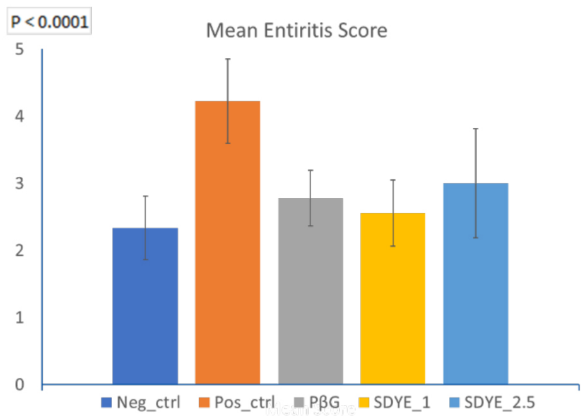
On-going analyses include 1] electron microscopy to establish the physical modulations induced by the treatments at the ultrastructural levels, 2] the transcriptional responses of enteritis biomarkers, immunological and barrier-regulating genes and, 3] metabarcoding analysis using full-length 16S sequencing (SMRT, PacBIO®) to obtain high resolution data on microbial diversity and taxa relative abundance.

Figure 1: Goblet cell density per 400 μm² of mucosal fold.



Different superscripts represent significantly different means of goblet cell count between the treatments.

Figure 2: Histological appraisal of the distal intestine



Bars represent the mean enteritis score of the different treatment groups, where 1 = Normal, 2 = Minor, 3= Moderate, 4 = Marked, and 5 = Severe.

PURIFIED BREWERS' YEAST (*Saccharomyces cerevisiae*) ADDITIVES MODULATE THE MUCOSAL HEALTH OF ATLANTIC SALMON PARR

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Cell wall components of *Saccharomyces cerevisiae* (rich in β-1,3 and -1,6-glucans and mannan oligosaccharides) in isolated or whole forms have been shown to confer immunomodulatory effects in fish. These benefits are at least partially induced by improvements of intestinal health. Despite the reported benefits, many knowledge gaps exist with regards to the optimal form and dosage.

An experiment was conducted to investigate the efficacy of highly purified β-glucans (PβG) and whole yeast cell walls (WYCW) to enhance the mucosal health of Atlantic Salmon (*Salmo salar*) parr. A total of 120 parr (ca. 21g) were randomly assigned into six experimental units (20 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 treatments were fed to the same % of biomass (between 1.5% and 2% per day). 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 (data not shown).

Histological appraisal 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.

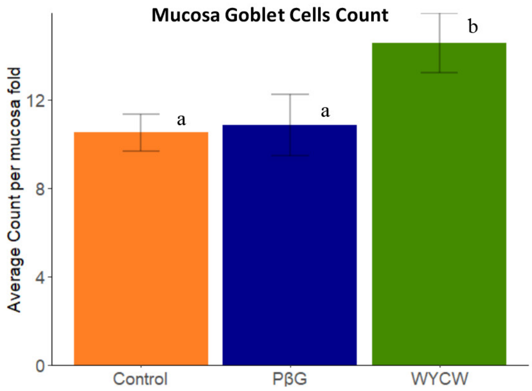

Figure 1: Goblet cells levels of the distal mucosal folds of Atlantic salmon parr after 4 weeks feeding on experimental diets. Treatments with different letters are significantly different ($P < 0.05$).

In addition, transmission electron microscopy (TEM) analysis of the distal intestine revealed significantly different microvilli morphometrics. Fish fed the PβG treatment had significantly longer ($P < 0.0001$) and more densely packed (P = 0.0001) microvilli than the control and the other yeast treatment. Whereas fish fed the WYCW treatment had significantly denser microvilli arrangement (P = 0.0056) than the control group.

Ongoing analysis includes gene expression profiling of immunomodulatory and barrier function genes.

In conclusion, both dietary products demonstrated the potential to enhance the epithelial barriers studied.

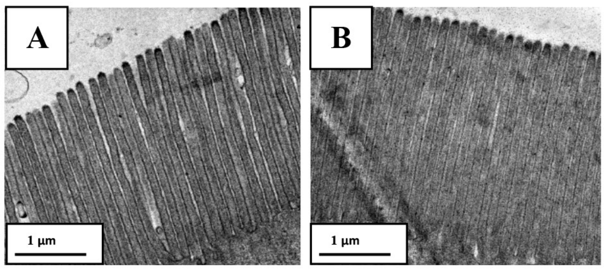

Figure 2: TEM electron micrographs of the microvilli from the distal intestine of Atlantic salmon parr subjected to (A) Control (B) PβG treatments. Scale bars = 1 µm.

POTENTIAL UNIDIRECTIONAL IMPACT OF CLIMATE CHANGE ON THE WORLD FISHERIES: CASE STUDY FOCUSING TWO MACKEREL SPECIES OF THE NORTH-WESTERN PACIFIC

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Given the potential for rising global temperatures and changing oceanic conditions to affect marine organism distribution, the implications of climate change on marine ecosystem are a major concern. Thus, this study used ensemble modelling and two "Representative Concentration Pathway" scenarios (2.6 and 8.5) to investigate the effects of expected climate change on Indo-Pacific King mackerel and narrow-barred Spanish mackerel distribution in the Taiwan Strait. The findings of our study indicated that the geographical range of both species in the Taiwan Strait currently spans from 22 to 25.5^{0}N and 118 to 121^0. Both species showed peak distribution in the regions where the sea surface temperature, sea surface chlorophyll, sea surface salinity, and sea surface height were 25–27 °C, 0.35-0.45 mg m^{-3}, 33.5–34.5 PSU, and 0.625–0.675 m, respectively. Both RCP 2.6 and 8.5 scenarios indicated a habitat gain of 50% to 80% by 2045–2050 for the Indo-Pacific king mackerel. RCP 8.5 predicted a greater loss of habitat for the Indo-Pacific King mackerel (-30% to -70%) than RCP 2.6 (-7% to -15%) by the end of 2095–2100. On the other hand, 30% to 90% of habitat gain was observed from 2045–2050 to 2095–2100 for the narrow-barred Spanish mackerel under RCP 2.6. However, according to the RCP 8.5 scenario, a significantly greater extent of habitat loss was projected, ranging from -60% to -80%, compared to the habitat loss projected under the RCP 2.6 scenario, which ranges from -2% to -10% for the narrow-barred Spanish mackerel. Present results indicated that both mackerel species will have a significant adverse impact by the end of the 21st century under RCP 8.5, whereas possible chances of habitat gain were anticipated under RCP 2.6. The populations of both mackerel species in the Taiwan Strait have already collapsed, and organisations responsible for fisheries management need to implement ecosystem-based management strategies to maintain biologically sustainable levels of species stock. By incorporating the present knowledge into managerial strategies, decision-makers can make well-informed decisions that may also safeguard the other fisheries of the Taiwan Strait and the people who depend on them under evolving climatic circumstances.

AQUAPONIC PRODUCTION SYSTEMS: EVOLUTION, CURRENT TRENDS AND FUTURE PERSPECTIVES

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Introduction

Aquaponic systems, the combination of aquaculture (mainly recirculating aquaculture systems) and hydroponics (soilless plant production) as well as the corresponding research, are already present for more than 40 years (Naegel 1977, Watten and Busch 1984). As integrated agricultural systems they offer a huge potential with regard to the improvement of resource efficiency and circularity in agriculture. Nevertheless, this technology has not yet achieved widespread success and one important scientific approach, next to e.g. the systemic research on specific mechanisms, bottlenecks and technical optimizations, is to foster commercial success through the provision of reliable data on production efficiencies, product safety, best practices as well as the holistic comparison of different production systems using e.g. life cycle assessments (LCA´s).

Material & methods

A literature search was conducted to collect and evaluate the diverse field of aquaponics research of the last decades. Additionally, a consultation of different colleagues from various aquaponic research groups as well as different professional networks has been done to supplement these findings. And finally, also personal observations and findings are used to complete this overview aiming at presenting a bigger picture of this complex research area.

Results

Aquaponic systems offer diverse opportunities to overcome major bottlenecks of conventional production practices in aquaculture and horticulture such as the eutrophication potential or excessive water and resource use. Additionally, the use of aquaponic systems could also support the reduction of greenhouse gas emissions in controlled hydroponic production systems through savings of water and synthetic fertilizers without losses in productivity (Monsees et al. 2019). But here, the choice of system design itself plays also an important role (e.g. coupled or decoupled aquaponic systems, Fig 1).

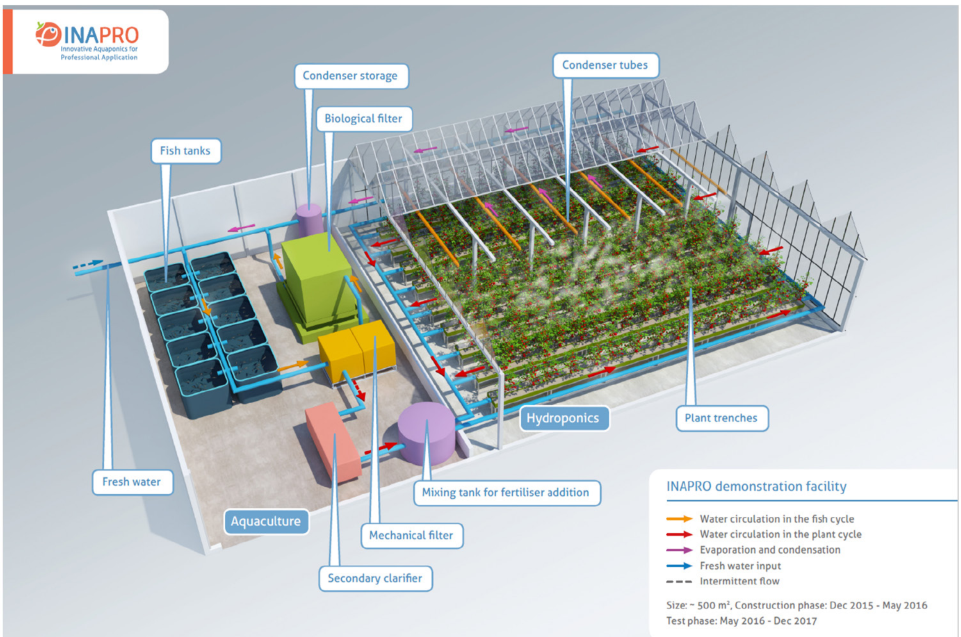

Fig. 1. Schematic drawing of a decoupled aquaponic facility (500 m^2) for the professional, commercial production of fish and plants(INAPRO, IGB).

(Continued on next page)

Discussion

Despite the innovative and sustainable approach of aquaponics, further research is still needed to promote this promising technology. One important reason for the missing commercial success is probably the lack of standardized scientific experiments as well as meaningful data on the production costs, product values and investment returns (Colt et al. 2022). Difficulties with regard to the labeling of aquaponic products as organic (Fruscella et al. 2021) might be another obstacle, but only a minor one.

Here, the lack of applied data paired with the higher financial risks of building two production systems instead of one (and being able to professionally manage them together) and often complicated authorization procedures are still seen as the biggest barriers for economic success. Targeted subsidies as well as advanced education and research programs for these integrated agricultural systems might help to foster professionalization in aquaponics, to lower the risks for potential investors and to finally pave the way for the commercial success of these production systems.

References

Colt, J., Schuur, A. M., Weaver, D., & Semmens, K. (2022). Engineering Design of Aquaponics Systems. Reviews in Fisheries Science & Aquaculture, 30(1), 33–80. https://doi.org/10.1080/23308249.2021.1886240

Fruscella, L., Kotzen, B. and Milliken, S. (2021), Organic aquaponics in the European Union: towards sustainable farming practices in the framework of the new EU regulation. Rev. Aquacult., 13: 1661-1682. https://doi.org/10.1111/raq.12539

Monsees H, Suhl J, Paul M, Kloas W, Dannehl D, & Wuertz, S. (2019) Lettuce (*Lactuca sativa*, variety Salanova) production in decoupled aquaponic systems: Same yield and similar quality as in conventional hydroponic systems but drastically reduced greenhouse gas emissions by saving inorganic fertilizer. PLOS ONE 14(6): e0218368. https://doi.org/10.1371/journal.pone.0218368

Naegel, L. C. (1977) Combined production of fish and plants in recirculating water. Aquaculture, 10, 17-24. https://doi.org/10.1016/0044-8486(77)90029-1

Watten, B. J. & Busch, R. L. (1984) Tropical production of tilapia (*Sarotherodon aurea*) and tomatoes (*Lycopersicon esculentum*) in a small-scale recirculating water system. Aquaculture, 41, 271-283. https://doi.org/10.1016/0044-8486(84)90290-4

MANILA PROJECT: TESTING INNOVATIVE STRATEGIES TO MITIGATE SHELLFISH VULNERABILITY TO CLIMATE CHANGE AND POLLUTANTS

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Sessile filter-feeders, such as bivalves, are increasingly exposed to multiple concurrent environmental stressors including marine heatwaves and chemical pollution. These challenges exacerbate the vulnerability of marine ecosystems improving biodiversity loss and carrying significant socio-economic consequences for aquaculture. A pertinent case study involves the Manila clam (*Ruditapes philippinarum*) in North Adriatic Sea. Here, several mass mortality events linked to ongoing harsh environmental conditions, have significantly caused a dramatic reduction of clams' stock availability in recent years. The project MANILA is designed to develop innovative strategies to improve Manila clam's performance under multifaceted stress conditions. By employing innovative tools, such as thermal and chemical priming, alongside microbiota manipulation, the project aims to lay the foundation for exploring biodiversity conservation and sustainable aquaculture management strategies (Figure 1). Priming refers to a process where exposure to mild or sub-lethal stress triggers a heightened response in the organism, enhancing its ability to withstand subsequent, more severe stressors. In this study, a wide panel of key molecular, biological and cellular processes are investigated in chemically- and thermally-primed Manila clams. While a protocol for heat-priming has been already validated, we investigated the possible application of hydrogen peroxide (H_2O_2) priming. Clams were subjected to low (10 µM) and high (50 µM) H_2O_2 doses over seven days, alongside a control group. Then transcriptomic and biochemical analyses were applied focusing on two critical tissues: the digestive gland and gills. Preliminary transcriptome results indicate that distinct tissue-specific responses were observed upon exposure to low and high doses to oxidative stress, suggesting that the digestive gland may prioritize lipid metabolism and reduce cellular maintenance functions in response to low-dose stress, while gill tissues adapt through modifications in protein synthesis and heightened signal transduction under chemical stress. The integration of our findings with biochemical analyses initiates the beginning of establishing chemical priming protocols for the MANILA project. This foundational step is crucial for developing strategies to enhance the Manila clams' resistance to environmental stressors. Furthermore, it opens up new scenarios for research in ecology, ecotoxicology, biology, and sustainable bivalve aquaculture management.

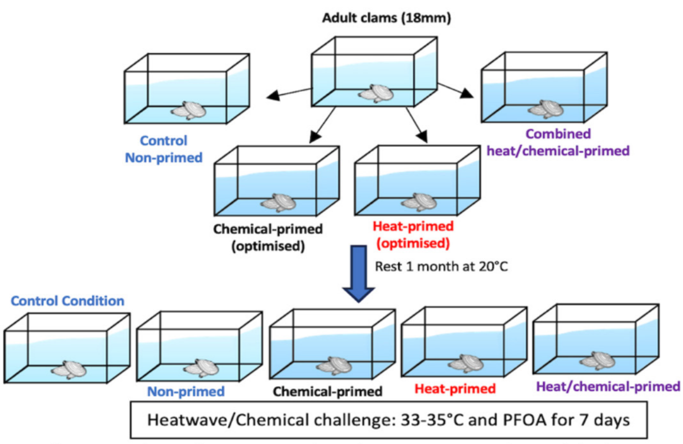

Figure 1. Schematic representation of MANILA experimental design.

MATHEMATICAL MODELLING FOR OPTIMIZING ALKALINITY CONTROL IN RECIRCULATING AQUACULTURE SYSTEM (RAS)

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Ensuring water quality in recirculating aquaculture systems (RAS) is complex due to numerous interdependent factors that can influence water quality. Mathematical models have proven to be powerful tools for assessing the interactions between RAS design, operations, and system dynamics. However, few models include pH and carbonate species as dynamic variables.

This study introduces a dynamic model for RAS (dynRAS) that integrates fish growth, CO_2 and Total Ammonia Nitrogen (TAN) excretion and removal rates with a pH and carbonate system reaction model formulated according to the law of mass action. Additionally, the model incorporates a dosing system for OH^-OH^- and $HCO_3^-HCO_3^-$ which framework is based on a structure similar to a Hill function. We use empirical data recorded during the experimental trial of Jafari et al., (2024) as a benchmark to validate our model.

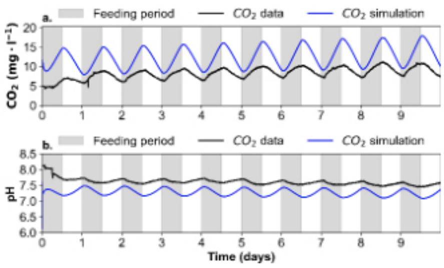

***Fig.1**. Comparison of simulation results (blue lines) with empirical data (black lines) for CO2 (panel a) and pH (panel b) over 10 days. A grey background indicates a feeding period of 12h.)*

Model simulations were consistent with empirical data, reproducing observed patterns and interdependencies between pH, alkalinity, and CO_2 (Fig.1). Despite some quantitative disparities, we could use the model to explore scenarios where we simulate the addition of NaOH or $HCO_3^-HCO_3^-$ to control different alkalinity levels and investigate the changes in the dynamic of the system over long periods (Fig. 2). The simulation results highlight that optimal alkalinity control is not immutable and should be adjusted in accordance with the system state. With future refinements, the dynRAS model presented here could act as a potent resource for the development of tools to work toward an adaptive control system for alkalinity and pH.

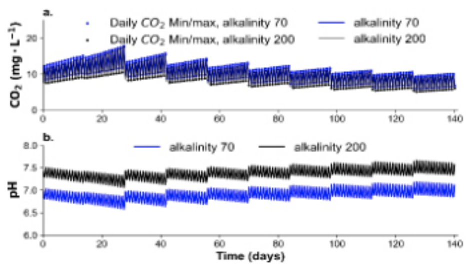

***Fig. 2.** Simulation results for pH (panel a) and CO2 (panel b) at alkalinity 70 (blue lines) and 200 (black lines) mg.L⁻¹ as CaCO₃.*

Reference

Jafari, L., Montjouridès, M.A., Hosfeld, C.D., Attramadal, K., Fivelstad, S., Dahle, H., 2024. Biofilter and degasser performance at different alkalinity levels in a brackish water pilot scale recirculating aquaculture system (RAS) for post-smolt Atlantic salmon. *Aquaculture engineering* 102407

EFFECTS OF EXPOSURE OF ZEBRAFISH *Danio rerio* TO MICROFIBERS ON ADULT FISH AND FERTILIZED EGGS DURING THE SPAWNING SEASON

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This study investigated the effects on the reproduction, spawning, and development of zebrafish using microfibers, which have recently been pointed out as a major cause of environmental pollution. First, we investigated the effects on fish reproduction, spawning, and fertilized egg production when male and female zebrafish are exposed to microfibers. In addition, we investigated the effects on hatching, survival, and development of fertilized eggs spawned from healthy male and female zebrafish when exposed to microfibers in the early stages of development.

First, zebrafish *Danio rerio* were divided into a control group (CON) and a group to which microplastic fibers were added (MF), and male and female fish were raised separately. Additionally, 1000/L of microplastic fibers in the MF group were added. Water changes were performed once a day, and feed was provided twice a day. Afterwards, males and females were produced fertilized eggs, and observed every 4 hours after fertilization. The hatching rate and survival rate of fertilized eggs were measured, and the egg size and body length of the larvae were also measured. In the next experiment, fertilized eggs were produced from healthy male and female zebrafish *Danio rerio* and exposed to microfibers until hatching. Developmental stages were observed under a microscope, and survival rate and egg size were measured. After being exposed to microfibers for 5 days, the fish were monitored during the experiment to measure their survival rate, and the possibility of ingestion of microfibers into the body was observed under a stereomicroscope.

As a result of the experiment, HSI of female MF adult fish and GSI and HSI of male fish increased. There was no difference in the total protein content of the gonads, but the egg size, hatching rate, survival rate, and body length of fertilized eggs and larvae produced from MF adults were decreased. In the case of fertilized eggs, there was no significant difference in survival rate. However, microfibers were observed in the body of fish exposed to MF, and motility was observed to be reduced compared to CON. Based on these results, it is predicted that microfibers can accumulate in the body from the hatching period and cause problems in growth and development. In the future, additional research is needed on the effects of endocrine disruptors caused by microfibers on reproduction and the environmental toxicological effects on juvenile fish.

NILE TILAPIA FARMING INTEGRATED WITH BOTTOM-FEEDER SPECIES

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Integrated aquaculture systems can boost the final biomass and align with sustainability principles and the circular economy concepts. Thus, we investigated the integrated culture of Nile tilapia (*Oreochromis niloticus*) with curimbatá (*Prochilodus lineatus*), an iliophagus bottom-feeder, and giant river prawn (*Macrobrachium rosenbergii*), an omnivorous bottom-feeder. An experiment that lasted 176 days was developed at UNESP - Aquaculture Center, in Jaboticabal - SP, Brazil (21º15'22''S; 48º18'48''O). Twelve earthen ponds with an area of ~0.015 ha and a depth of 1 m were used. The experimental design was completely randomized, consisting of four treatments and three replications (ponds): MT - monoculture of tilapia (3 ind.m^{-2}); ITC - integrated culture of tilapia (3 ind.m^{-2}) and curimbatá (5 ind.m^{-2}); ITP - integrated culture of tilapia (3 ind.m^{-2}) and prawn (5 ind.m^{-2}); ITCP - integrated culture of tilapia (3 ind.m^{-2}), curimbatá (5 ind.m^{-2}) and prawn (5 ind.m^{-2}). Only tilapia was fed twice a day with an extruded commercial diet. At harvest, all animals were counted and weighed. Final mean mass, survival, productivity, and feed conversion ratio (FCR) were calculated for all treatments (Table 1). The co-culture of Nile tilapia, curimbatá and giant river prawn did not lead to the expected increase in productivity and reduction in FCR compared to tilapia monoculture. Curimbatá, stocked at a density of 5 ind.m^{-2}, negatively affected the development of Nile tilapia and giant river prawn. The integrated culture of Nile tilapia at a density of 3 ind.m^{-2} with giant river prawn at 5 ind.m^{-2} increased productivity and reduced FCR. Further research should be performed to define the best stocking density of each species to optimize their integrated culture.

Table 1. Production performance parameters for each treatment. Initial size: *O. niloticus* = 2.80 ± 1.19 g; *P. lineatus* = 15.54 ± 7.79 g; *M. rosembergii* = 0.03 ± 0.01 g.

	MT	ITC	ITP	ITCP
Total Yield (t.ha^{-1})	11.0 ± 0.4	10.2 ± 1.5	12.9 ± 1.5	11.4 ± 0.9
Feed Conversion Ratio (FCR)	2.1 ± 0.4	2.0 ± 0.2	1.8 ± 0.1	2.0 ± 0.05
Oreochromis niloticus				
Final mean mass (g)	475 ± 144^{a}	380 ± 96^{c}	471 ± 83^{a}	434 ± 87^{b}
Survival (%)	78.9 ± 15.1	76.9 ± 3.8	86.7 ± 8.3	72.0 ± 0.9
Yield (t.ha^{-1})	11.0 ± 0.3^{ab}	8.8 ± 1.3^{b}	12.3 ± 0.9^{a}	9.4 ± 0.4^{b}
Feed Conversion Ratio (FCR)	2.1 ± 0.3	2.0 ± 0.2	1.9 ± 0.1	2.2 ± 0.02
Prochilodus lineatus				
Final mean mass (g)	-	31.0 ± 15.4^{b}		39.2 ± 14.4^{a}
Survival (%)	-	88.5 ± 7.4		74.2 ± 7.9
Yield (t.ha^{-1})	-	1.4 ± 0.2		1.5 ± 0.2
Macrobrachium rosenbergii				
Final mean mass (g)	-		22.0 ± 8.9^{a}	16.6 ± 7.2^{b}
Survival (%)	-		51.6 ± 25.8	65.1 ± 19.9
Yield (t.ha^{-1})	-		0.6 ± 0.3	0.6 ± 0.3

Means followed by different letters in the same line indicate significant differences between treatments by ANOVA followed by the Tukey-Kramer test or *t-test* (P <0.05).

OLIVE BIOACTIVES DERIVED FROM CIRCULAR BIOECONOMY AS A FEED ADDITIVE FOR COMMON CARP *Cyprinus carpio* L. ENHANCING GROWTH, STRESS RESISTANCE AND IMMUNITY

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In the process of olive oil production, of which Spain is by far the biggest producer in the world, large volumes of olive pomace "waste" are generated, which still contain important amounts of olive bioactive compounds, mainly triterpenoids. This side stream is a highly valuable resource that can be converted into feed additives to improve aquaculture productivity and animal well-being following sustainable circularity principles. The present study was developed to test the efficacy of an olive pomace extract (OPE) in common carp *Cyprinus carpio* L. farming.

A trial was performed at the Fisheries Research Station of the University of Agriculture, Krakow (Poland), in six flow-through earth ponds (3 per treatment) stocked with fifty 11-month-old sexually undifferentiated carps (start weight: 122.3±1.03 g). Two diets were tested: a standard commercial pelleted feed (control diet) containing 34% CP and 7% CF, and a similar diet supplemented with 0.2% of an additive containing OPE (Luctactive® AQUA). Carps were fed at 2.5% to 3.5% body weight, depending on water temperature, from early May until the end of September (5 months duration). Every month, all surviving fish (>78% at 5 months) from each pond were weighed and counted. Additionally, blood and tissue samples were collected from 10 fish at the start of the trial and from 5 fish per pond (15 per treatment) at 3 and 5 months.

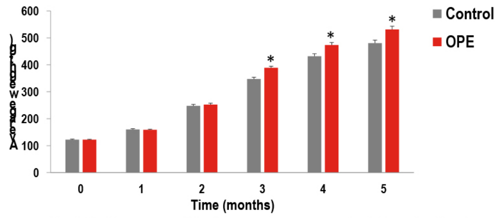

Figure 1. Fish growth (average weight, g) during the trial. Mean ± SEM, n=94-150.

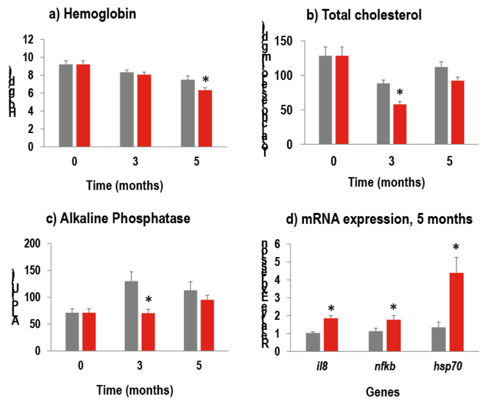

*Figure 2. Representative changes in plasma haematological (a) and biochemical (b, c), or hepatic gene expression (d) indicators. Mean ± SEM, n=15. *Indicates significant differences between control and OPE (P<0.05).*

Growth was significantly improved (*P<0.05) by the additive, with 10-12% higher weight in fish fed the OPE treatment from 3 months onwards (Fig. 1). Several haematological parameters were also significantly affected at either 3 or 5 months (Fig. 2a): haemoglobin, mean corpuscular haemoglobin (MCH), MCH concentration and white blood cells count were all increased in the control, indicating a higher oxygen transport rate and energy expenditure, potentially associated to a higher stress response. Plasma biochemical parameters (Fig. 2b) revealed significantly higher levels of glucose, triglycerides, total cholesterol and HDL-cholesterol in the control. An improved liver function was indicated in the OPE treatment by significant reductions in plasma levels of alkaline phosphatase (ALP) and bilirubin (Fig. 2c). Finally, gene expression (qPCR) analysis at 5 months revealed a significantly elevated expression of *il8*, *nfkb* and *hsp70* in the liver of carp fed the OPE additive (Fig. 2d). In head kidney, only *hsp70* was significantly elevated in OPE, but a similar trend (P=0.065) was observed for *nfkb* and *infg*. These results indicate a potential immunostimulatory and immunoprotective effect of the OPE additive, which has also been suggested by previous studies testing similar olive extracts in fish.

DIETARY SUBSTITUTION OF SOYPROTEIN CONCENTRATE BY BLACK SOLDIER FLY LARVAE MEAL IMPROVED SENSORY QUALITY OF ATLANTIC SALMON FARMED IN COMMERCIAL SEA-CAGES

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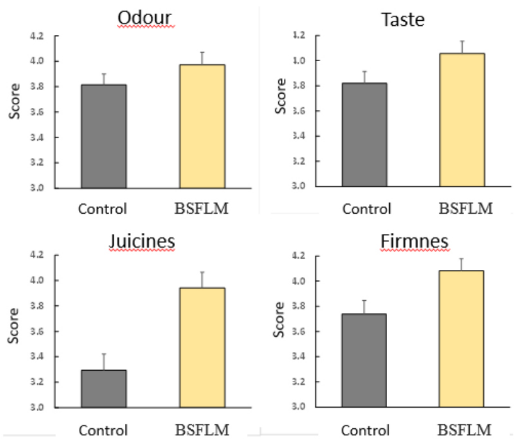
A feeding trial accounting the whole seawater phase was conducted in commercial sized sea-cages using either a standard commercial feed (Control) or the same feed supplemented with black soldier larvae meal (BSFLM) as a replacement for 4% soy protein concentrate. Each feed was fed to triplicate sea-cages. No adverse welfare issues were observed when the fish were harvested at an average body weight of 4.5kg. The fish fed BSFLM had higher fillet yield and less visceral fat. Raw fillets of salmon fed the BSFLM diet had firmer fillets with less gaping, coinciding with increased level of cysteine and glycine of the connective tissue. Sensory evaluation of baked fillets likewise revealed improved firmness of the salmon fed the BSFLM diet, in addition to higher scores for juiciness, odor, taste and general acceptance.

Atlantic salmon (*Salmo salar* L.) were farmed in seawater cages on the Norwegian West Coast and fed either a commercial feed (36.5% protein and 33% fat) or the same feed where 4% SPC was replaced by black soldier fly (*Hermetia illucens*) larvae meal. Each diet was fed in triplicate during the seawater phase. At harvesting, fish were scored for external welfare indicators before they were filleted and analyzed for firmness (TA-AX2, SMS) and fillet gaping (score 0-5). Fillets were prepared for sensory testing by baking fillets at 175°C for 12 min (50 participants, hedonic analyses). Skeletal muscle (NQC) was analyzed for content of fat and astaxanthin. Connective tissue (CT) was isolated from the muscle and analyzed for amino acid (AA) composition and by Differential Scanning Calorimetry (physical, chemical methods are described in Mørkøre et al. 2020).

Salmon fed BSFLM had higher slaughter yield (90.6 vs. 88.3%) and higher fillet yield (61.6 vs. 61%), but fillet fat and pigment content were similar for the fish groups. However, AA composition of the CT showed higher content of cysteine (5.2 vs. 4.4, P=0.05) and glycine (296.3 vs. 276.3, P=0.03) (residues/1000 AA residues), both of which are associated with collagen strength. Results from DSC analyses indicated higher thermal stability of the salmon fed BSFLM compared with the Control (P=0.07). Fish fed the BSFLM achieved higher (positive) scores for odor, taste, juiciness, and firmness as well as general acceptance (Figure 1).



Reference: Mørkøre et al. 2020. Dietary inclusion of Antarctic krill meal during the finishing feed period improves health and fillet quality of Atlantic salmon (*Salmo salar* L.). B.J.Nutr 124, 418-431.

BEYOND THE MICROBIAL TAXONOMY WITH SAMBA. DISCLOSING THE FUNCTIONALITY AND CORE GUT MICROBIOTA OF GILTHEAD SEA BREAM FROM A CAUSAL BAYESIAN NETWORK PERSPECTIVE

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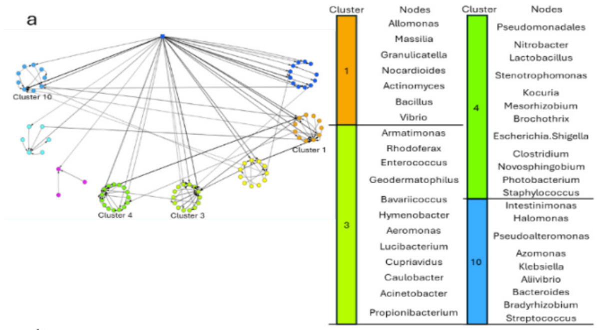
One of the most documented features about microbiomes is its great taxonomic variability, which is described in animals, like fish, as well as in humans. Despite this enormous inter and intra-species heterogeneity, at larger scale, microbial populations seem to exhibit a redundant functionality, in which phylogenetically related or unrelated taxa can share similar genes that accomplish for analogous general functions. At lower scale, biotic and abiotic factors such as those occurring in aquaculture production systems can actually disturb this balance, defining advantages and/or disadvantages in terms of performance. However, the bacterial specific functional contributions are difficult to be defined and measured.

Thus, the present investigation aimed to reinterpret the apparent functional redundancy of the microbiota from an applicative point of view using a Bayesian network (BN) approach. The bioinformatic analyses were performed using three experiments, which investigated the effects of different dietary fishmeal substitutions on anterior and posterior intestinal microbiota of gilthead sea bream. The construction of the BN was conducting using the updated version (V2) of SAMBA platform (Soriano et al., 2023; https://doi.org/10.3390/genes14081650), implemented with community detection method (Leiden method) with which the microbial population can be re-organized for the identification of strictly connected bacterial clusters. The specific functional profile of each cluster is then performed using KEGG pathways annotation and directly included in the BN. Our results allow the identification of key bacterial genera in the clusters (**Fig. 1a**), which trough the dietary modulation can lead to metabolic changes, such as those related to metabolites biosynthesis and lipid metabolism (**Fig. 1b**), that can also be part of the interaction with the host. These findings represent a specific and targeted approach of SAMBA. However, the platform can also be used to obtain a global and general organization of the microbial network associated with a compartment (e.g., anterior intestine, posterior intestine). Hence, the second aim of this study was to perform a meta-analysis, building a BN with SAMBA, merging different experiments, to identify the most representative connections between bacteria. Our results shown how the constant and variable fractions of microbial populations, including the CORE microbiota, are distributed in the network and interact with each other. All these outcomes represent the starting point to define a new reference of microbial organization, which can serve also as material for comparison between species or distinct body compartments.



b

Cluster	Description	pvalue	p.adjust
Cluster 1	Description	pvalue	p.adjust
	Flavone and flavonol biosynthesis	8.86E-05	0.003101
Cluster 3	Description	pvalue	p.adjust
	Flavone and flavonol biosynthesis	0.000589	0.021141
	Bile secretion	0.000604	0.021141
	Endocrine and other factor-regulated calcium reabsorption	0.001037	0.024192
	Parathyroid hormone synthesis, secretion and action	0.002425	0.042435
Cluster 4	Description	pvalue	p.adjust
	Toll and Imd signaling pathway	0.00022	0.009675
	Flavone and flavonol biosynthesis	0.000845	0.012708
	Bile secretion	0.000866	0.012708
	Steroid biosynthesis	0.00411	0.045208
Cluster 10	Description	pvalue	p.adjust
	Biosynthesis of various other secondary metabolites	5.64E-05	0.002088
	Toll and Imd signaling pathway	0.00232	0.042926

Fig. 1a. BN of one of the three experiment considered.
b. Cluster- associated metabolic functions.

This work was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (THINKINAZUL/2021/024).

PREVALENCE AND SEVERITY OF CORONARY ARTERIOSCLEROSIS IN FAROESE FARMED ATLANTIC SALMON (*Salmo salar*)

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The primary function of the coronary circulation is to supply oxygen and nutrients to the cardiac muscle. Blockages, such as those that can result from coronary arteriosclerosis, can lead to myocardial ischemia, causing cardiac damage and potentially death. One-third of teleost species have a coronary circulation, predominantly in highly active species like Atlantic salmon, facilitating an active aerobic lifestyle and demanding swimming behaviors.[1] Despite the necessity of the additional energy resource provided by the coronary circulation, coronary arteriosclerosis is highly prevalent in Atlantic salmon, with ubiquitous lesions in sexually mature individuals.[1–3]

Even though coronary arteriosclerosis is considered an inevitable aspect of their physiology, its severity may impact their health, welfare, and performance. Experimental studies have demonstrated that salmonids with surgically occluded main coronaries exhibit reduced tolerance to warming, hypoxia, impaired aerobic performance, and increased susceptibility to crowding stress.[4-7] These findings have significant implications for aquaculture practices involving stressful handling such as crowding, pumping, transport, and different treatments.

The pathogenesis of coronary arteriosclerosis in salmonids is not completely clear, but studies have shown that farmed salmonids bred at elevated temperatures, resulting in increased growth rate exhibit a higher prevalence and severity of arteriosclerosis compared to wild and farmed fish with slower growth rates.[1,3,4] The most recent comprehensive study on the prevalence and severity of coronary arteriosclerosis in wild and farmed Atlantic salmon was conducted 30 years ago.[3] Since then, smolt breeding programs have intensified, potentially exacerbating the issue due to increased growth rates. Therefore, we assessed the prevalence and severity of coronary arteriosclerosis in commercially farmed Atlantic salmon to assess its status in relation to today's farming practices.

Hearts from Faroese farmed Atlantic salmon were sampled at a range of different farming sites to ensure comprehensive representation. We sampled one month after sea transfer, and again ~10 months later, just prior to slaughter. From each fish, 18 serial sections of the main coronary artery were examined, and lesions were scored from 0-5 according to their severity, with 0 representing no lesions and 5 representing severe lesions.
One month after sea transfer, lesions were found in 23-37.7% of the all the fish examined at the different sites. One site, however, had a much higher prevalence where 66.6 % of all the fish contained lesions. This same site also exhibited more severe lesions, with a mean lesion score of 0.80, compared to the other sites, which had a mean lesion score between 0.12-0.46.

Just prior to slaughter the lesion prevalence was significantly higher with lesions found in 89.5% to 100% of all the fish examined. The lesions were also more severe with a mean lesion score between 3.54 and 4.14. Variations in lesion prevalence and severity may be attributed to differences in size and growth rates among sites, as fish that where larger or had a higher growth rate displayed higher prevalence and severity. Our findings highlight significant concerns regarding both the prevalence and severity of coronary arteriosclerosis in commercially farmed Atlantic salmon. The observed variability among farming sites underscores the importance of critically evaluating current aquaculture practices. As smolt breeding programs intensify and farming techniques aimed at maximizing growth are implemented, it is crucial to also consider the cardiovascular health and welfare of the fish.

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References
1. Farrell, A. P. Coronary arteriosclerosis in salmon: Growing old or growing fast? *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **132**, 723–735 (2002).
2. Farrell, A. P., Johansen, J. A. & Saunders, R. L. Coronary lesions in Pacific salmonids. *J. Fish Dis.* **13**, 97–100 (1990).
3. Saunders, R. L., Farrell, A. P. & Knox, D. E. Progression of Coronary Arterial Lesions in Atlantic Salmon (Salmo salar) as a Function of Growth Rate . *Can. J. Fish. Aquat. Sci.* **49**, 878–884 (1992).
4. Brijs, J. *et al.* Prevalence and severity of cardiac abnormalities and arteriosclerosis in farmed rainbow trout (Oncorhynchus mykiss). *Aquaculture* **526**, 735417 (2020).
5. Ekström, A., Axelsson, M., Gräns, A., Brijs, J. & Sandblom, E. Influence of the coronary circulation on thermal tolerance and cardiac performance during warming in rainbow trout. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **312**, R549–R558 (2017).
6. Ekstrom, A., Axelsson, M., Grans, A., Brijs, J. & Sandblom, E. Importance of the coronary circulation for cardiac and metabolic performance in rainbow trout (Oncorhynchus mykiss). *Biol. Lett.* **14**, (2018).
7. Morgenroth, D. *et al.* Coronary blood flow influences tolerance to environmental extremes in fish. *J. Exp. Biol.* **224**, (2021).

GENETIC PARAMETER ESTIMATION OF GROWTH AND QUALITY TRAITS IN THE OSO FARMING POPULATION OF MADAGASCAR TIGER SHRIMP *Penaeus monodon*

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Introduction

Several breeding programs and genetic parameters studies have assessed the heritability of production and survival traits in *Penaeus monodon*. Very few have investigated sensory quality traits such as flesh texture or color, and their correlation with production traits. Heritabilities related to weight in tiger shrimp are high and range between 0.39 and 0.48 (Hasan et al., 2020). To our knowledge, no genetic parameters are available for texture measurement, but high heritability of color, particularly in *L. vannamei*, was reported (Giang et al., 2019).

Material and Methods

A cohort of shrimp from the OSO Farming selection program was phenotyped and genotyped. One thousand animals were weighed before and after freezing, as well as after cooking. Texture measurements were obtained for each individual before and after cooking using a texturometer. Color was evaluated on the L*a*b* space through image analysis from a photo capture under standard condition. The same animals were genotyped using a SNP panel of 1500 SNPs. The pedigree was reconstructed from the genotyping data, and the heritabilities and the genetic correlations were assessed using genetic mixed models.

Results & Discussion

Results are presented in the table below (heritabilites on the diagonal, genetic correlation above diagonal). The estimated heritabilities were found to be very high for all growth traits, with genetic correlations among them close to unity, suggesting that a selection program focused on growth could be effective. Tail yield had low heritability and was poorly correlated with growth. Firmness appeared to be moderately heritable and correlated with growth parameters, implying that selection for growth would also result in the selection of animals with firmer flesh. Regarding color, redness and yellowness parameters were heritable but not correlated with other measured traits. Thus, a growth-based selection would not impact the color of raw and cooked shrimp. However, specific selection for color could be considered if a change to a different shade is desired.

Conclusion

Overall, the results of this study are extremely encouraging for OSO Farming as they confirm the effectiveness of their breeding techniques and growth selection plan. The investigation of texture and color provides valuable insights into the interactions between various parameters and opens up opportunities for future selection.

	Cooked Weight	**Length**	**Tail yield**	**Raw flesh firmness**	**Cooked flesh firmness**	**Raw b* color**	**Cooked a* color**
Cooked Weight	**0.50±0.09**	0.99±0.01	0.16±0.31	0.74±0.16	0.95±0.05	0.17±0.17	0.22±0.23
Length		**0.53±0.09**	0.11±0.27	0.72±0.17	0.95±0.05	0.17±0.17	0.20±0.21
Tail yield			**0.14±0.05**	-0.02±0.38	0.20±0.32	0.15±0.28	0.13±0.34
Raw flesh texture				**0.16±0.05**	0.84±0.14	-0.23±0.27	0.25±0.29
Cooked flesh texture					**0.28±0.09**	-0.25±0.20	0.24±0.26
Raw b* color						**0.38±0.08**	-0.13±0.37
Cooked a* color							**0.19±0.05**

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References

Giang, C. T., Knibb, W., Muu, T. T., Ninh, N. H., & Nguyen, N. H. (2019). Prospects for genetic improvement in objective measurements of body colour in Pacific Whiteleg shrimp (Litopenaeus vannamei). *Journal of Marine Science and Engineering*, *7*(12), 460

Hasan, M. M., Tulloch, R. L., Thomson, P. C., Raadsma, H. W., & Khatkar, M. S. (2020). Meta□analysis of genetic parameters of production traits in cultured shrimp species. *Fish and Fisheries*, *21*(6), 1150-1174.

MIC-RAS: NOVEL INTENSIVE RECIRCULATING AQUACULTURE SYSTEM INTEGRATING A PHOTO-MEMBRANE-BIOREACTOR

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Traditional Recirculating Aquaculture Systems (RAS) requires nitrification and water exchange to remove solids and accumulated nitrate, resulting in the waste of valuable nutrients and contributing to environmental pollution. Microalgae represent a renewable source of nutrients and valuable bioactive compounds, including polyunsaturated fatty acids (PUFAs), antioxidants, pigments, and more. Microalgae require a steady supply of nutrients for biomass production, primarily carbon, nitrogen, and phosphorus, all of which are found in fish excretions. Additionally, microalgae offer sustainability benefits by absorbing carbon dioxide (CO_2) and releasing oxygen during their growth. The microalga chosen for this investigation is a novel freshwater species belonging to the Eustigmatophytes group, known for its high levels of omega-3 PUFA, specifically EPA (eicosapentaenoic acid).

Our aim is to develop a novel microalgae-integrated RAS (mic-RAS) where microalgae are used to treat fish wastewater (containing soluble and solid excretions) while producing valuable biomass.

Methods: A recirculating system was developed, incorporating a fish aquarium and a microalgal photo-membrane-bioreactor. The microfiltration/ultrafiltration membrane facilitates the circulation of clean permeate from the microalgae-containing photo-bioreactor back to the fish aquarium, while retaining the microalgal biomass. This system was compared with a conventional nitrification bioreactor (c-RAS), with water subjected to analysis for nitrogenous compounds, elemental composition, and total organic carbon content. Microalgae assessment involved cell counts, dry biomass determination, chlorophyll concentration measurement, optical density analysis, and evaluation of fatty acid and C:N contents. Over the course of several months, the system was operated, gradually increasing fish stocking densities and correspondingly augmenting the applied feed (2% of fish body weight).

Results: In the mic-RAS, total ammonia nitrogen (TAN), nitrite, and nitrate levels were nearly undetectable. In contrast, the c-RAS exhibited a significant rise in nitrate levels, and the control group showed an increase in TAN levels (see Fig. 1a). Microalgal concentrations demonstrated an upward trend alongside the escalation in fish stocking densities and the corresponding rise in the quantity of feed applied (see Fig. 1b).

Conclusions: The study highlights the potential of a novel RAS, wherein nutrients originating from fish excretions are efficiently assimilated into valuable, sustainable microalgal biomass.

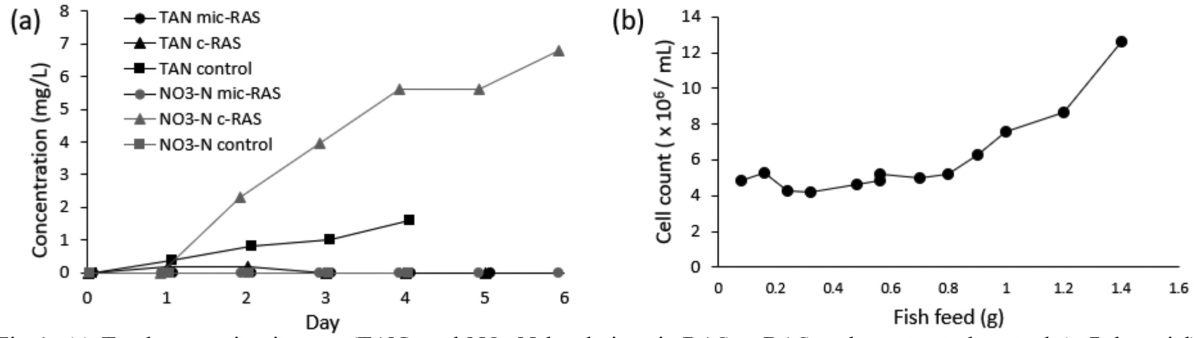

Fig 1: (a) Total ammonia nitrogen (TAN) and NO_3-N levels in mic-RAS, c-RAS and non-treated control (a 7-day trial). Experimental aquaria (7.5 L) were stocked with 6 grams of guppies, *Poecilia reticulata*, fed at 2% of their body weight. (b) Increase in microalgal densities associated with increased feed application in the mic-RAS (a trial conducted over several months with gradual increase in fish stocking densities and feeding).

ENVIRONMENTAL BENEFTIS OF AQUACULTURE AND CHALLENGES AND OPPORTUNITIES IN PROMOTING THOSE BENEFITS

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Background

The Strategic guidelines for a more sustainable and competitive EU aquaculture[1] outline the steps required to drive the development of aquaculture in a manner that aligns with goals of the European Green Deal. Low trophic aquaculture, in particular the farming of marine bivalves and low-trophic finfish (in ponds, lagoons, estuaries, reservoirs and wetlands) presents a viable option for a resilient, competitive and enviro-compatible aquaculture sector in the EU due to the favourable ecosystem conditions, markets for and historical experience in farming such species. Despite indications that these types of aquaculture can have minimal impact on the environment (and in some cases be beneficial to the environment), there are no defined measures for, and standardized techniques to assess the sustainability and eco-compatibility of these types of aquaculture. Negative consumer perceptions and a lack of awareness of the potential benefits, coupled with environmental, regulatory and economic challenges, present significant barriers for the growth of this sector.

Methodology & Aims

The present study aims to assess the environmental benefits of these types of aquaculture through a semi-quantitative analysis of the available scientific evidence and through engagement with key stakeholders. The study seeks to highlight if and how these types of aquaculture can and are being promoted across the Member States, as well as providing recommendations to overcome barriers. The study looks to provide an overview of how these types of aquaculture, and their positive environmental impacts, can be successfully promoted, with the aim that aquaculture in the EU can become a global reference for sustainability.

Conclusion

This study falls under the EU Commission project *Assessing the environmental benefits of aquaculture and challenges and opportunities in promoting those benefits.* The abstract and presentation will focus on the contextual background and project methodological approach, setting the context for elaboration on future project results.

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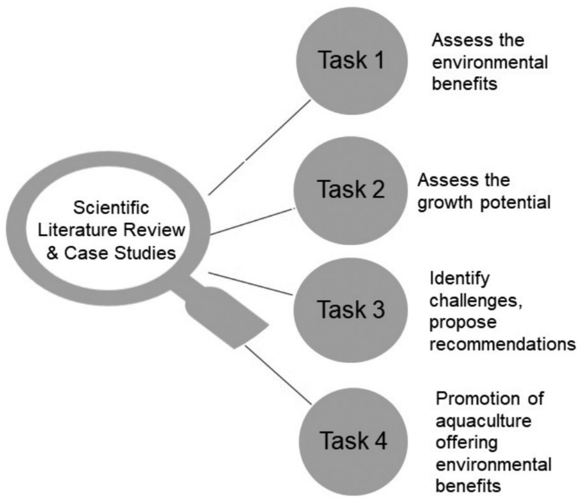

INVESTIGATING LOW SALINITY PERFORMANCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* USING A GENOMIC APPROACH

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Global aquaculture production of shrimp has increased drastically over the last two decades with production increasing from ~1.3 million MT in 2001 to ~7.5 million MT by 2021. Despite the global increase in farmed shrimp production, US shrimp aquaculture production has remained relatively low over this same period (<3,000 MT in most years with a maximum of ~6,000 MT in 2003). The lack of growth in US shrimp aquaculture can be attributed to many factors, including low costs of imported products relative to US products, permitting issues and regulations, and land use conflicts. However, there is one sector of the industry that has shown signs of growth: inland farms. It is estimated that there are ~100 inland shrimp farms in operation in the US (including startups and "backyard" farms) and these farms produce >400 MT/year. Some of these use outdoor ponds/tanks and have access to low-saline groundwater, but a majority of inland farms are indoors, utilize recirculating aquaculture systems (RAS), and rely on seawater made from natural or synthetic sea salts.

Inland farming is attractive for several reasons, including increased biosecurity, potential for year-round production, farms can be closer to major markets allowing for direct marketing to consumers and food service industry, and locating farms away from sensitive coastal areas. Inland farming does have inherent challenges though, most notably high capital and operational costs. Reducing capital costs industry-wide will be difficult, as most inland farms will require costly infrastructure, and solutions for capital reduction may be region specific. Thus, to support the continued growth of inland shrimp farming, efforts are needed to reduce production/operational costs.

This project focused on the genetics of *Litopenaeus vannamei* growth and survival under normal and reduced salinity conditions. The impacts of reducing salinity on the performance of selectively bred *L. vannamei* were determined, so that inland farmers can weigh the economic benefits of reducing salinity (i.e. reducing input costs) against the impacts on shrimp performance. In addition, genetic correlations were estimated and these estimates will allow breeders to determine trade-offs in genetic gain if selection and farming occur at different salinities and, ultimately, determine if developing separate shrimp lines for high and low/reduced salinity farming is warranted. Lastly, the use of genomic data was found to provide improvements in model fit and genetic parameter estimates (heritability and genetic correlations), as well as increases in selection accuracy for growth (≥6.5%). The benefits for survival were less clear due to a large genotype × environment effects for salinities tested across two generations.

A DHA-RICH MICROALGAE DIET AFFECTS GROWTH AND THE RESPONSE TO OXIDATIVE STRESS OF ATLANTIC SALMON RAISED IN RAS

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Modern salmon diets are low in finite marine resources and dominated by plant-based ingredients. This results in reduced availability of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) such as EPA and DHA for the fish. To maintain health-promoting aspects of aquafeeds, enriching feeds with essential fatty acids of non-marine origin becomes increasingly important. Here, we investigated whether diets supplemented with the DHA-rich microalgae *Schizochytrium* can improve production performance and the response to a commonly used disinfectant that causes oxidative stress. Atlantic salmon post smolts (~126 g) held in triplicate tanks were fed diets enriched with *Schizochytrium limacinuum* at 2% (SL2) or 14% (SL14) inclusion or a control diet (CD). Following eight weeks of restricted feeding at 1% of their biomass, the fish were exposed to peracetic acid (Wofasteril classic, Kesla, Germany, at 2.5 μl/l), a commonly used disinfectant in RAS. Fish were sampled before exposure, 1 hour, and 18 hours after exposure, reflecting an acute response.

Including *Schizochytrium* in the diet improved feed conversion, growth, and protein retention in Atlantic salmon, most notably in fish receiving the SL 14 diet (Figure 1). A trend of increasing lipid content with SL inclusion was apparent in whole-body and muscle samples. Fatty acids in whole-body and muscle samples reflected dietary levels, with enrichment of DHA but decreasing concentrations of the pro-inflammatory C20:3n6 in fish fed SL diets. The diet significantly influenced bacterial ß-diversity in the digesta but not in the mucosa of the hindgut. Most abundant taxa in the digesta were *Floricoccus*, *Vibrio* and *Lactococcus* and additionally *Streptococcus* in the mucosa. Overall exposure to PAA resulted in a classic stress response with an increase in plasma glucose and cortisol levels. However, glucose levels in fish fed SL14 did not increase following PAA exposure. Catalase protein levels in the liver increased following exposure to PAA by 2-fold except for fish fed SL2, while levels of SOD1 remained unchanged. Feeding SL2 reduced lipid peroxidation (MDA concentration) in the liver, while exposure to the stressor had no effect on lipid peroxidation. Overall, our results highlight that including *Schizochytrium* in the diet can improve growth performance and stress resilience of Atlantic salmon. This is an important step towards developing health promoting aquafeeds for Atlantic salmon.

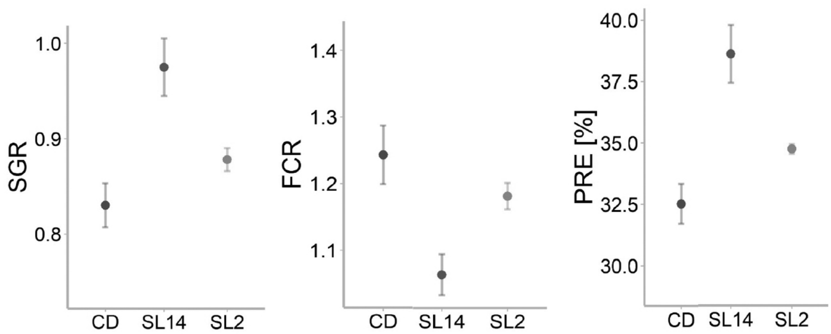

Figure 1 Growth (SGR), feed conversion (FCR) and protein retention (PRE) of Atlantic salmon fed diets enriched with *Schizochytrium* at 2% (SL2) or 14% (SL14) inclusion or a control diet (CD) for eight weeks; mean ± SD, n = 3.

CHRONICALLY ELEVATED CORTISOL LEVELS AND ITS INTERACTION WITH A FUNCTIONAL FEED IN ATLANTIC SALMON

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Chronic stress negatively affects growth, health, and welfare of fish and is characterized by elevated plasma cortisol levels over time. While the short-term action of cortisol in fish is well described, less is known about the health effects of chronically elevated cortisol levels, in particular under intensive production. Furthermore, it is unclear how cortisol affects the intestinal microbiota. Here, we investigated how elevated cortisol levels affect the health of Atlantic salmon in recirculating aquaculture systems and whether these interact with feeding a functional diet containing the DHA-rich microalgae *Schizochytrium*. Atlantic salmon post-smolts (~557 g) were reared in six single RAS units of 1.5 m^3, and three experimental groups in duplicate were established: a control group not receiving cortisol (CD-), a group fed cortisol at 15 mg/kg fish (CD+), and a group receiving cortisol in addition to a DHA-rich algae feed (SL+). Following four weeks of restricted feeding at 1.2% of the biomass, the fish were sampled to investigate effects on growth performance, plasma and organ health indicators, as well as the bacterial composition of the digesta using 16S metabarcoding.

Fish receiving cortisol showed reduced growth and increased feed conversion, along with a significant increase in plasma cortisol and glucose levels. Fish fed the algae feed showed intermediate growth performance. Cortisol levels also increased strongly in the RAS rearing water. Fish receiving cortisol had reduced condition and hepatosomatic index, as a consequence of increased energy mobilization. Histological analysis of the liver, heart, and intestine will give further insights into the health effects of chronically elevated cortisol levels and its potential interaction with a DHA-rich diet. The ongoing analysis of the bacterial communities in the digesta as well as the rearing environment will reveal the effects of cortisol and the functional DHA-rich feed on the microbiota.

OCCURRENCE OF MYCOTOXINS IN AQUACULTURE FEED AND HOW THEY AFFECT AQUACULTURE SPECIES

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Mycotoxins are common contaminants in animal feed and pose a risk to animal health and business profitability. Although knowledge of the negative impact of mycotoxins on aquaculture species is lacking behind terrestrial animal species, the number of scientific publications is increasing which allows insights into the toxins' effects on fish and shrimp.

As these secondary fungal metabolites are mostly produced during crop growth in the field, prevention of contamination is limited. Additionally, during storage, mycotoxin levels can increase with fungal growth if storage conditions are not fully optimized. To get a better understanding of their occurrence in aquafeed, we dedicated a specific survey program collecting aquafeed samples from around the world and analysing these samples for the presence of (EU) regulated mycotoxins as well as the non-regulated mycotoxins so-called emerging mycotoxins.

In 2023 and 2024 over 140 samples were analysed with two different LC-MS/MS-based multi-mycotoxin analysis methods, Spectrum Top®50 performed by Romer Labs Austria and Singapore, and the Spectrum 380® method, developed and performed at the University of Natural Resources and Life Sciences (BOKU) at the IFA Tulln.

Results of this aquafeed survey will be presented with a focus on the current knowledge on effects on important European aquaculture production species such as gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), and salmonids (family Salmonidae).

DEVELOPING AN INNOVATIVE RAPID RISK ASSESSMENT FRAMEWORK TO ADDRESS THREATS FROM AQUATIC DISEASES IN A CHANGING WORLD

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Climate change can impact aquatic animal health by influencing pathogen expression, virulence and survival, and by modifying the distribution and interaction between pathogens and susceptible hosts. To effectively anticipate and mitigate risks under future climate conditions, the specific nature of these impacts need to be captured.

A rapid risk assessment (RRA) tool was developed specifically for aquatic pathogens and explores its practical application to risk communication. It gathers essential knowledge about pathogen characteristics, host specificities, feasible routes of introduction, conditions for establishment, potential consequences, and mitigation measures. Additional information on diagnostic methods is also provided when available. Based on the best scientific evidence available, supported by the literature and expert opinion, the main objective of the RRA is to estimate likelihoods of the introduction, establishment and potential consequences of a pathogen under different climate change scenarios. The tool therefore provides evidence to 1) inform disease risk profiles 2) predict ecological, economic and social impacts of disease, 3) inform and underpin communication of risk, and 4) direct future research to address knowledge gaps.

The RRA approach provides a robust and systematic framework for the ranking of aquatic disease risks under climate change, allowing for an adaptive and effective mitigation management. The tool supports risk communication with stakeholders and provides evidence to support the industry in addressing future climate challenges and identifying opportunities for aquaculture diversification.

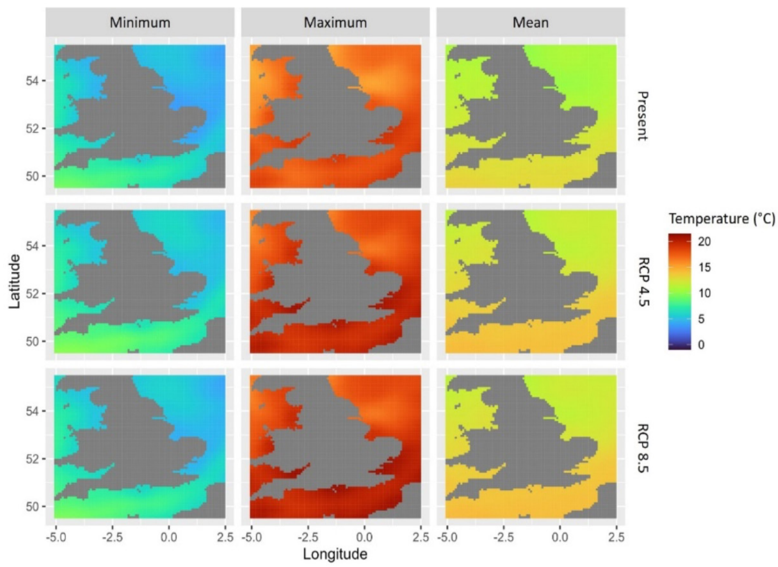

Figure 1 Minimum (left), maximum (centre) and mean (right) marine temperatures in England and Wales per 50km grid cell, both in current conditions (top) and under two climate change scenarios, namely RCP 4.5 (middle) and RCP 8.5 (bottom).

THE SCOPE EXTENSION PROCESS OF THE AQUACULTURE STEWARDSHIP COUNCIL

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The Aquaculture Stewardship Council (ASC) is the world's leading certification scheme for farmed seafood. In 2023, the total global production of ASC certified seafood amounted to more than 2.04 million tonnes, which corresponds to 2.4% of the global aquaculture production, while certified fish accounted for 2.3% 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.

The ASC is integrating various species-specific standards into a single ASC Farm Standard to normalize the assessment of common aquaculture impacts. This unique standard will cover all impact areas and currently certifiable species, but it will also facilitate species expansion efforts and metric updates. It includes core indicators for monitoring legal, social, and environmental impacts uniformly, while establishing species-specific limits as necessary (e.g., mortality levels, feed conversion). In this context, the ASC Species Scope Extension Project aims to tackle unaddressed aquaculture opportunities thought the expansion of the Farm Standard's scope.

All the submitted new species requests undergo initial evaluation, including species scoring against ASC requirements and data availability checks. ASC also conducts a market analysis for market insights on the species. Upon ASC Board approval, the project Terms of Reference are developed and released for stakeholder consultation. Feedback from these consultations shape white papers, which are documents on various aspects of the species including production insights and challenges. Eventually these species are integrated into ASC Farm Standard, maintaining Principles 1 and 3, which cover (mostly) general common aquaculture requirements. Additional species-specific metrics will be added via the Metrics Methodology, if necessary. ASC considers social and environmental concerns when adding species to the standard, aiming for balanced regulation without hindering aquaculture development or market access. Finally, within this process, ASC further addresses stakeholder's barriers to engagement in standard-setting, applicable to new species additions. Active participation of stakeholders throughout the entire process, particularly during the white paper development phase, is essential to ensure the transparent and evidence-based inclusion of new species into our standards.

CARBON AND NITROGEN STABLE ISOTOPES: IS A GOOD PROXY OF INDIVIDUAL FEED EFFICIENCY IN SEABASS (*Dicentrarchus labrax*)?

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Improving fish feed efficiency is of major interest to reduce feed use in aquaculture and improve its sustainability through reduced costs and environmental impacts. Fish efficiency is defined as the ability of fish to convert feed intake into body weight gain over a given time period. The main challenge to improve feed efficiency in breeding programs is our capacity to accurately measure feed intake at the individual level on a large number of fish. In previous studies, individual feed efficiency has been evaluated on fish reared in isolation. Each fish was given a known amount of feed, and two hours after feeding, uneaten pellets were manually collected and counted to determine the exact feed intake. However, this method is really tedious and difficult to use in aquaculture production outside laboratory conditions. Another drawback of this method is that fish are isolated in individual aquaria with no interactions with congeners. The measurement of natural abundance of carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of organisms could reflect feed efficiency as the difference between isotopic values of an organism and its feed is related to protein retention. This method would have the advantages of being rapid, low cost and a non-destructive for fish.

To test the correlation between individual fish efficiency and stable isotope values, 178 individuals of seabass were raised in individual aquariums during six weeks. The quantity of food ingested by each fish was recorded every day. Every two weeks, seabass were individually weighed. Feed conversion ratio (FCR) of each individual was then calculated as the ratio between the quantity of food ingested and body weight gain. In parallel to the weight measurements every two weeks, few scales and part of caudal fin were sampled from each fish. Following the six initial weeks in individual aquaria, fish were reared for an additional 9 weeks in a collective tank. At the end of this growing period, scales, fin, muscle and blood were sampled on each fish. $\delta^{13}C$ and $\delta^{15}N$ values of scales, fin, muscle and blood at each sampling periods were measured by EA-IRMS.

Our results show a very strong negative correlation between individual FCR and body weight gain ($R = -0.95$, $p < 0.001$). $\delta^{15}N$ values of muscle sampled after 15 weeks of experimentation showed a negative correlation with individual FCR ($R = -0.2$, $p < 0.05$). At the all times of sampling, $\delta^{13}C$ and $\delta^{15}N$ values of scales, fin and blood were not correlated to individual feed efficiency.

In conclusion, carbon or nitrogen stable isotopes cannot be used as a reliable proxy to evaluate feed efficiency of the seabass *D. labrax*. This study does not corroborate previous studies on rainbow trout (*Oncorhynchus mykiss*) showing that $\delta^{13}C$ of muscle and $\delta^{15}N$ of liver were potential good biomarkers to evaluate individual feed efficiency in fish (Dvergedal et al., 2023). Further studies are necessary to better understand the metabolic processes driving isotopic incorporation and such differences between fish species.

Dvergedal H., Ødegård J., Galloway T.F., Klemetsdal G. 2023. Isotope fractionation in juvenile and large rainbow trout (*Oncorhynchus mykiss*): Repeatability of stable isotope measures and their relationship to growth rate. Aquaculture, **569**, (739380).

Bonamia ostreae PREVALENCE AND ITS EFFECTS ON EUROPEAN FLAT OYSTERS (*Ostrea edulis*) UNDER A SIMULATED ICE WINTER

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European flat oysters (*Ostrea edulis*) are a commercially valuable species that have a 90% mortality rate when infected by the parasite *Bonamia ostreae*. Oysters infected with *B. ostreae* were relayed briefly in the Limfjorden in Denmark, which was followed by a series of ice winters between 1980 and 1990 and subsequently by an increase in oyster populations. Survey programs conducted in the Limfjorden declared it free of *B. ostreae* from 1996 to 2014. Similar phenomena were observed in USA and Norway, where a low prevalence of the parasite was seen following below zero temperatures.

To understand how extreme low temperatures affect *B. ostreae*, a temperature challenge experiment was conducted. This study aimed to assess the efficacy of non-invasive environmental DNA (eDNA) sampling for parasite detection in close water systems and to measure the amount of *B. ostreae* released at ice winter temperatures.

Sets of Bonamia-positive and Bonamia-negative oysters were individually incubated at either -1°C or 12°C for a period of one, two or three months. Each set was then incubated in groups at 20°C for two months to stimulate larvae production. Hemolymph, water samples and passive eDNA sensors were collected for the quantification of *B. ostreae* with qPCR.

5.56% (two individuals) of the infected oysters from the -1°C treatment survived, in contrast to 0% in the infected 12°C treatment groups. These surviving oysters also showed no detectable levels of *B. ostreae* at the end of the experiment. *B. ostreae* was detectable in water samples and passive sensors deployed in the tanks of the oysters. Uninfected oysters in the 1-month cold treatment group produced the highest number of larvae amongst all the groups.

These findings offer an insight to the host-parasite dynamics between flat oysters and *B. ostreae* in the context of a warming climate. The eDNA quantification techniques can be applied in other hatcheries and be further tested on open sea sites.

GENOME RESOURCE-BASED MULTI-OMICS APPROACH IN NON-MODEL AQUATIC ANIMALS FOR ENVIRONMENTAL HEALTH ASSESSMENT

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In the field of aquatic ecotoxicology, toxicity assessment at molecular level through multi-omics approaches has been primarily conducted in model organisms such as zebrafish and water fleas in laboratory rather than on native species in real-world environments. In this study, we aimed to use a multi-omics platform with non-model domestic species for health assessment by analyzing molecular and biochemical responses. To facilitate omics applications, we established genome database for the swamp shrimp *Neocaridina denticulata* and the pale chub *Zacco platypus*. For the toxicity assessment of triclosan (TCS) as an aquatic pollutant, *N. denticulata* and *Z. platypus* were exposed to TCS for acute toxicity evaluation. A range of TCS concentrations below LC50 values was determined to reduce mortality effects. We analyzed molecular and biochemical responses at the transcriptomic, proteomic, and metabolomic levels in response to TCS exposure. Finally, we integrated the three omics dataset such as transcriptome, proteome, and metabolome, obtained from the two species exposed to TCS. We found that glutathione metabolism was commonly changed in both species in response to higher concentrations of TCS. Furthermore, *N. denticulata* exhibited differential expression in carbohydrate metabolism such as glycolysis or starch metabolism, whereas *Z. platypus* showed significant changes in oxidative phosphorylation. Through the integration of multi-omics datasets, we identified several biomarkers with potential applications in health assessment and further validated their applicability in actual field environments.

SEASONAL REPRODUCTIVE PHYSIOLOGICAL CHANGE OF LARGEHEAD HAIRTAIL

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Largehead Hairtail (*Trichiurus japonicus*) is a very important commercial fish species in East Asia. In Korea, *T. japonicus* is a highly valuable commercial fish species. However, their reproductive ecology has been not much research yet. In this study, we investigated the reproductive physiology of *T. japonicus* targeting cutlassfish with a preanal length of 25-35cm, which are the most used commercially, from April to December.

As for the experimental method, Gonadosomatic index (GSI), Hepatosomatic index (HSI), average preanal length and total weight were investigated for female fish. Additionally, the ovary was analyzed using histological methods. The absolute fecundity was investigated through microscopic observation of the tissue.

As a result, the average preanal length and total weight significantly increased from July to December. GSI, HSI and absolute fecundity were significantly higher from July to September. Histological results also showed that the proportion of maturity oocytes was higher from July to September than in other months. Therefore, *T. japonicus* with a preanal length of 25-35cm grow rapidly from summer to fall and are considered to have a high possibility of spawning between July and September. Based on these results, we hope that our understanding of the reproductive physiology of *T. japonicus* will increase and that it will be helpful in recovering the resources.

LIVELIHOOD ASSET OWNERSHIP AND VULNERABILITY TO CLIMATE CHANGE OF SHRIMP FARMERS: EVIDENCE FROM SOUTHWEST COASTAL REGIONS OF BANGLADESH

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In the southwest coastal regions of Bangladesh, shrimp farming serves as a vital source of livelihood, yet it faces increasing vulnerability due to climate change. This study utilizes the Sustainable Livelihood Framework (SLF) to assess the existing livelihood assets of shrimp farmers and analyze the livelihood vulnerability across three distinct shrimp production systems, namely extensive, semi-intensive and intensive. Primary data were gathered from 150 randomly selected shrimp farmers in Khulna, Satkhira and Bagerhat districts. Three methods, Climate Vulnerability Index (CVI), the Livelihood Effect Index (LEI), and the Livelihood Vulnerability Index (LVI) were employed for assessing livelihood vulnerability. Shrimp cultivation in the country is done under mostly extensive farming methods where the traditional farming system is used.

Analysis reveals significant vulnerability variations among shrimp farmers practicing different production systems. Tables 1 and 2 present the results using a score-based approach, where each aspect (livelihood asset or vulnerability index) is assigned a numerical score ranging from 0 to 1, with 1 representing the highest level or vulnerability. Extensive farming exhibits greater resilience to climate change, indicated by lower vulnerability scores across all three assessment methods. In contrast, semi-intensive and intensive shrimp farming show heightened susceptibility to climate-related stressors, attributed to increased dependence on external inputs and infrastructure (Table 2).

These findings underscore the critical need for promoting sustainable and resilient shrimp farming practices in the face of escalating climate risks. Policy interventions and adaptive strategies should prioritize enhancing farmers' adaptive capacity and diversifying livelihood assets.

Table 1: Livelihood Assets of Shrimp Farmers

Livelihood Asset	Methods of farming		
	Extensive	Semi-intensive	Intensive
Financial capital	Low (0.22)	High (0.77)	High (0.82)
Physical capital	Low (0.35)	Medium (0.56)	High (0.76)
Social Capital	Medium (0.64)	Medium (0.66)	Low (0.30)
Human Capital	Medium (0.68)	High (0.85)	High (0.80)
Natural Capital	High (0.88)	Medium (0.52)	Low (0.35)

Table 2: Livelihood Vulnerability of Shrimp Farmers

Production System	CVI	LEI	LVI
Extensive	Low	Medium	Low
Semi-intensive	Medium	Low	Medium
Intensive	High	High	High

EXPLORING DIETARY PROTEIN-ENERGY RATIOS FOR THE FLATHEAD GREY MULLET *Mugil cephalus* REARED AT TWO DIFFERENT TEMPERATURES

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Flathead grey mullet (Mugil cephalus) is a catadromous omnivorous fish species that has recently come into prominence among researchers because of its potential as a sustainable low-trophic species. In particular, it is interesting to evaluate the interaction between environmental factors (temperature), growth and feed. Understanding the role of temperature is particularly relevant in a scenario of global warming. Feeding strategy optimization related to environmental conditions is necessary to pursue more efficient aquaculture production. Temperature is the key environmental factor, playing a crucial role on metabolism, nutrient utilization and gut health. The objective of this trial is to explore the effect of two different protein/energy ratio requirement (diet A: 30% proteins; 10% lipids; diet B: 30% proteins, 15% lipids) on growth of grey mullet juveniles under two different water temperature condition (22 °C and 28°C). Diets were formulated using circular ingredients (fisheries and aquaculture by-product, poultry and agriculture sector).

The experiment took place at the the Laboratory of Aquaculture, Department of Veterinary Medical Sciences of the University of Bologna (Cesenatico, Italy). Flathead grey mullet specimens were obtained from IMC (Torre Grande, Italy) and adapted to the laboratory facilities for two weeks at 25 °C. Thereafter, 50 fish per tank were randomly distributed into twelve 450 L flat bottom tanks connected to a recirculating system. Each fish was weighed (initial average weight: 42,37 ± 10,87 g) and tagged using a PIT tag to follow individual performance throughout the trial. Water quality was maintained constant (O_2 8 mg L^{-1}, salinity 7 ‰, pH 8.0). Feed (diet A and diet B) was provided to apparent satiation during 6 hours meal once a day over a period of 120 days across two temperatures (22°C or 28°C). Growth and feed utilization were monitored throughout the experiment.

After 40 days from the beginning of the trial, middle body weight (MBW), specific growth rate (SGR), feed intake (FI), feed conversion rate (FCR) and condition factor (CF) were calculated. Similarly, at the end of the trial final body weight (FBW), SGR, FI, FCR and CF were calculated. This study investigated the optimal temperature for flathead grey mullet growth. These findings highlight the importance of understanding protein and energy requirements at different temperatures for flathead grey mullet. This knowledge is a fundamental step towards establishing successful aquaculture practices for this emerging species.

PRIN: progetti di ricerca di rilevante interesse nazionale – bando 2022; prot. 2022snlr4y. *Mugil cephalus* (linneus, 1758): the flathead grey mullet as a model for sex determination in fish with implications for aquaculture (acronym mugilsex).

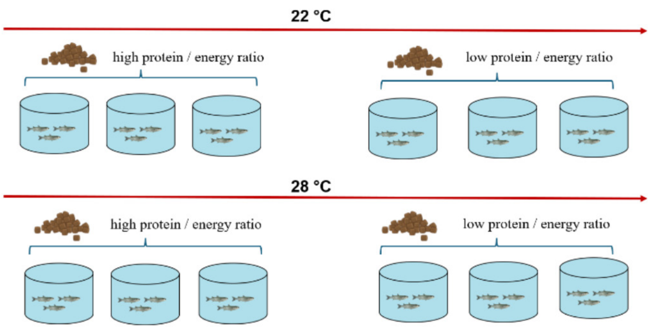

Figure 1 Experimental design

THE CASE OF NOVEL INGREDIENTS IN THE FEED AND FOOD INDUSTRY: HOW TO APPROACH NOVELTY

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Sustainability is currently a strong driver for replacing animal proteins in the feed and food industries and some activists claim large-scale animal protein production is an unproductive use of natural resources when compared to the environmental impact of plant-based protein production. Indeed, feed production for animal consumption and food preferences in humans are changing as there is greater moral and nutritional interest in the vegan lifestyle. The complexity of animal products and the physicochemical principles you need in order to assemble novel plant-based ingredients, each with their own physical, functional, nutritional, and sensory attributes is a major challenge. In addition, sustainability must also include the entire ecosystem from clean energy sources to feedstock. On the other hand, fermentation has enormous potential to positively transform the food system, but feedstocks are the major cost driver for most fermentation processes. Fermentation processes can take advantage of a variety of host organisms, feedstock sources and cultivation processes, offering an unparalleled opportunity to decouple protein production from limited resources such as land, freshwater and other inputs. Traditionally used to improve food functionalities such as preservation, taste, and texture, novel fermentation solutions are enjoying a resurgence with new, innovative technologies being developed across various segments of the feed & food industries. New generation genetic modification technologies can be applied to increase the functionality of microorganisms including novel ones for fermentation and benefits. The use of precision fermentation for the production of major food components (proteins, lipids, and carbohydrates) is emerging as an attractive option for the transformation of food systems. In this presentation, we will highlight a leading-edge fungal framework being used for precision fermentation, and we will briefly overview our groundbreaking technology to create novel ingredients that can accelerate the growth of the feed and food industries in a more sustainable way.

IDENTIFYING MACROPLASTIC PATHOBIOMES AND ANTIBIOTIC RESISTANCE IN A FISH FARM

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Through its great characteristics, plastic has spread through human society. It is a light, solid, and inexpensive material, making it ideal for long-term purposes as well as for single uses. All industrial sectors rely on plastics, and aquaculture make no exception since plastics are ubiquitous in aquaculture structures. This ubiquity raises the concern of the threat they could represent for reared species and human consumers. Plastics has deleterious effects through direct mechanicals actions, but also through indirect chemical intoxication or biological contamination, subsequent to ingestion of microplastics or cutaneous contact with plastic equipment.

Plastics carry a bacterial biofilm on their surface, called Plastisphere, that is composed of various micro-organisms. The presence of potential human pathogenic bacteria (PHPB) and antibiotic resistant bacteria (ARB) in the plastisphere has been described.

In this study, we used a combination of metabarcoding and standard antibiotic susceptibility testing to study the pathobiome and resistome of macroplastics, fish guts and the environment in a marine aquaculture farm. Plastics were found to be higher in PHPB from the *Vibrionaceae* family (Fig. 1) compared with environmental samples. Moreover, isolates from aquaculture plastics showed higher significant multiple antibiotic resistance (MAR) compared to non-plastic samples of seawater, sediment and fish guts (Fig. 2). These results suggest that plastics act as a reservoir and fomite of PHPB and ARB in aquaculture, potentially threatening the health of farmed fish and human consumers.

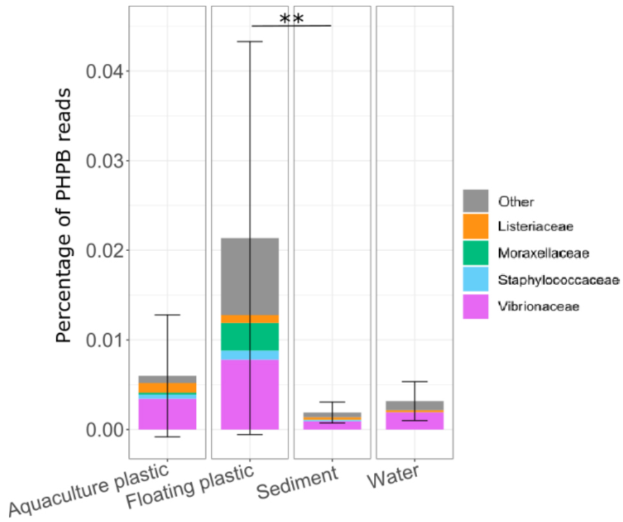

Figure 1: Percentage of pathogenic reads according to the origin of the sample.

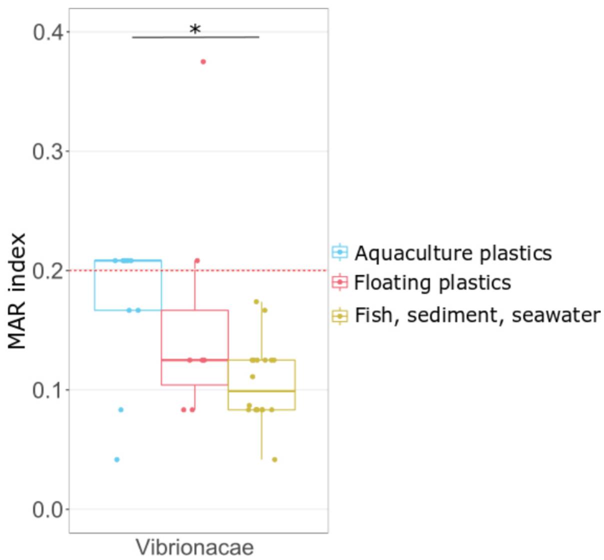

Figure 2: MAR index of isolated Vibrionaceae strains according to the sample type.

BIOACTIVES FROM *Euglena gracilis* TO BOOST IMMUNITY AND DISEASE RESISTANCE IN FISH

Muhammad Nauman*, Francisca Silva-Brito, Márcia Saraiva, Paulo Santos, Pedro Cunha, Helena Cardoso, Hugo Pereira, Luis E.C. Conceição, Ana T. Gonçalves, and Benjamin Costas

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The food sector is witnessing aquaculture emerging as a sustainable and reliable source of animal protein. However, farming intensification may translate into immunosuppression and poor growth. Therefore, sustainable and cost-effective ways to implement prophylactic measures alternative to antimicrobials are urgently needed. Microalgal species have the potential to serve as source of immunomodulating bioactives that can be added to fish feeds. This study aimed to investigate the potential of dietary bioactive compounds derived from *Euglena gracilis* to enhance the immunological responses and disease resistance of European seabass (*Dicentrarchus labrax)*.

European seabass juveniles were fed five isoproteic and isolipidic experimental diets for two weeks. G-H and G-L: *E. gracilis* G strain broken cells at high or low concentrations, respectively; and J-H and J-L: *E. gracilis* J strain extract in combination with a carbohydrate-rich residue at high or low concentrations, respectively. Fish were submitted to a bacterial challenge by inoculating *Photobacterium damselae* subsp. *piscicida* (*Phdp*) into the peritoneal cavity. At the end of the trial and following 24 hours post-infection, tissue samples were collected. Several haematological, oxidative and innate immune parameters were evaluated.

In terms of mortality data following bacterial challenge, G-H and J-L fed fish showed a clear trend to be more resilient to the pathogen compared to those fed the other diets. Regarding plasma peroxidase, fish fed G-L and J-L diets were significantly different, with lower values in fish fed J-L. In oxidative stress biomarkers, lipids peroxidation (LPO) and ratio of reduced (GSH) to oxidised (GSSG) glutathione (GSH:GSSG) did not presented any significant different among diets. Catalase activity revealed a significant difference with fish fed G-H presenting higher values than those fed J-L diet before infection, whereas hepatic SOD activity in fish fed G-L was lower than those fed G-H dietary treatment.

Preliminary data suggests that dietary supplementation *E. gracilis* bioactive compounds modulate immune and oxidative stress responses which could translate into a protective effect against a bacterial pathogen after a short feeding period. Further studies are required to investigate the impact of increased microalgae concentrations on fish health and their ability to withstand disease.

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MEASUREMENT OF EUROPEAN SEABASS *Dicentrarchus labrax* IN THE REARING ENVIRONMENT BY IMAGES TAKEN BY CAMERAS ABOVE RAS TANKS

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This abstract describes an innovative method for monitoring the growth of European seabass (Dicentrarchus labrax) using stereo imaging over recirculating aquaculture system (RAS) tanks. This method involves a series of processes that begin with the rectification and contrast enhancement of images captured by binocularly arranged cameras. Instance segmentation is performed using the YOLO-v8 deep learning model (AI model) trained on seabass images to recognize fish silhouettes in the captured images. The centerline of the contour is then calculated using the Voronoi algorithm, segmenting the fish into five parts and marking a skeleton of ten points of interest (PoI) along the image boundary. Stereo matching then identifies these silhouettes in all images and allows the real-world coordinates (x, y, z) for these points to be accurately determined, taking into account the refraction effects of the water.

Fish images where the PoI do not meet specific consistency criteria are not considered. An allometric regression model, trained with distances from these points on top-view images of European seabass, estimates the weight of each fish. Preliminary results from trials in three experimental RAS tanks at CIIMAR facilities indicated the effectiveness of the method with a Mean Absolute Percentage Error (MAPE) of less than 10% for weight estimation. This cost-effective and accurate method underlines its potential to improve fish farming management. European seabass has significant value in the European food market, with the success of aquaculture highly dependent on optimal breeding and rearing conditions, water quality and animal welfare. Conventional growth monitoring methods involving physical sampling cause fish stress, increase disease susceptibility and incur high labor costs. Conversely, this non manipulation imaging technique allows continuous, stress-free monitoring that reduces operational costs and enables early detection of diseases through visual inspection and behavioral analysis.

Despite challenges such as fish movement, water refraction and variable image quality factors (luminosity, reflection, turbidity), the proposed method integrates advanced deep learning (AI), instance segmentation, filtering and computer vision stereo techniques that surpass those problems. It enables aquaculture producers to efficiently monitor fish stock growth, predict biomass and weight distribution at harvest, improve planning and sales negotiations, increase economic results and support animal welfare, leading to higher product quality standards.

Example of left and right images with fish PoI stereo matching.

TOWARDS A WIDE-GENOME TISSUE-SPECIFIC EPIGENOMIC PROFILING FOR A TAILORED IMPROVEMENT OF GROWTH PERFORMANCE WITH GLOBAL WARMING IN GILTHEAD SEA BREAM

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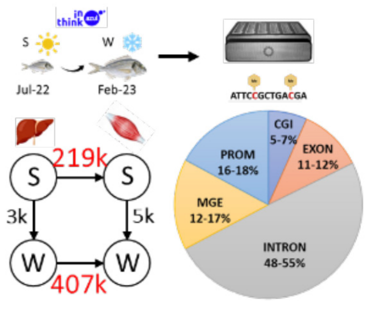
Global warming is inducing frequent and severe heatwaves, especially in the Mediterranean area, and changing the neutral range of ambient temperature of farmed fish. Evidence of maladaptation is increasing with every increment of warming, although current research with a selected fast growing fish strain of gilthead sea bream (*Sparus aurata*) reports increased thermal tolerance and growth rates at the IATS aquaculture infrastructure during the extreme climate period from 2022-2023. We wanted to deepen our understanding of the epigenetic mechanisms of this fish population which enabled them to overcome the record-breaking temperatures which generated a water surface temperature of 30 ºC during two consecutive days. More precisely we were interested in exploring tissue- and seasonal-specific DNA methylation changes that can today be identified at a genomic-scale with third-generation sequencing. The specific aim of this study was to map seasonal changes of the epigenome from two metabolically active tissues (liver, white skeletal muscle), and explore whether this is a key driver of different tissue-specific metabolic capabilities of a gilthead sea bream strain.

Fish were fed a commercial-based diet formulation at the IATS flow-through system under natural day-length and temperature conditions. Liver and skeletal muscle tissue portions were taken at two different sampling points (summer (S), July-2022; winter (W), February-2023; 5-6 fish/tissue/season). Sequencing libraries were generated with Oxford Nanopore Technologies (ONT) PCR-cDNA Barcoding Kit (SQK-PCB111.24) and sequenced on a PromethION 24 (R10.4.1 chemistry flow cells). FAST5 files were modification-aware basecalled using Guppy and CSIC reference genome. Resulting files were interrogated for methylation (5mC) using ModKit. Differentially modified (DM) 5mC were calculated with the MethylKit package over those CpG sites with a minimum mapping coverage of 10 reads in at least 5 individuals of each experimental group, and considered significant at a $q < 0.05$. A total of 99.5 million reads were basecalled with a median Phred score of 16, and an average N50 of 16 kb. Methylation calling rendered ~2.9M 5mC passing the prevalence filters. From them, a total of ~735k DM5mC were found between all the pairwise comparisons, denoting a less-variable epigenome with changing season (3-5k DM5mC) than tissue in both summer and winter (219-407k DM5mC). In any case, CpG sites followed the same distribution pattern regardless of the comparison, being predominant in introns (~51%), followed by promoters (~17%), transposable elements (~15%), exons (~11%) and CpG islands (~6%). The crossing of epigenetic marks and host transcriptome expression remains to be analysed to translate on a functional basis the changing tissue and seasonal epigenetic landscape. Altogether, this work will offer novel genome-scale methylation marks that would contribute to improve selective breeding and environmental priming for producing tailored climate resilient gilthead sea bream in a challenging scenario.

This study was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (THINKINAZUL/2021/024).



UNRAVELLING QUEENSLAND UNKNOWN DISEASE: TRANSCRIPTOMIC AND GENOMIC INVESTIGATIONS OF THE OYSTER PARASITE *Marteilia sydneyi*

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Australia's *Saccostrea glomerata* (Sydney rock oyster) aquaculture industry annually contributes $107 million AUD to the economy. In 1910, the industry thrived, however, the 1960s saw to an outbreak of Queensland Unknown (QX) disease in Queensland. QX disease, caused by *Marteilia sydneyi*, leads to up to 90% mortality in cultivated and wild *S. glomerata* stock. This parasite remains the most limiting factor in Australian *S. glomerata* aquaculture, continually threatening its commercial viability.

M. sydneyi is an ascetosporean parasite, and several species within the *Marteilia* genus parasitize bivalve molluscs relied upon for aquaculture worldwide. *Ostrea edulis* (European flat oyster) and *Mytilus edulis* (European mussel) are two aquaculture species farmed extensively that have suffered recurrent outbreaks of disease and mass mortality due to another Ascetosporean parasite, *Marteilia refringens.* Despite the World Organisation for Animal Health listing *M. sydneyi* as a notifiable pathogen and its significant economic impact, there is minimal information available about the parasite. Currently, only two genomes are published for parasites within Ascetosporea, neither of which belongs to Paramyxida, to which *M. sydneyi* belongs. Despite the substantial economic impact *M. sydneyi* has on *S. glomerata* aquaculture in Queensland and New South Wales, very little is known about its lifecycle, evolutionary history, metabolism, and its interactions with its environment.

In efforts to fill this gap, we identified infected and non-infected oysters, extracted RNA from the digestive glands, and sequenced their transcriptomes. Sequences likely originating from *M. sydneyi* were identified by subtracting transcripts that originated from the host, followed by homology-based bioinformatic analyses. To better understand the parasite, we are also working on generating a draft genome for *M. sydneyi* by utilizing long-read Nanopore DNA sequencing and PacBio HiFi sequencing. Generating cutting-edge genomic resources will allow us to identify candidate genes for the development of much-needed diagnostic assays.

As well as generating transcriptomic and genomic data for *M. sydneyi,* we have compared gene expression between infected and non-infected *S. glomerata*, enabling us to understand the molecular responses of the host oyster to infection. Overall, this research allows us to address the many unknowns surrounding the parasite, leading to new avenues for mitigation of the negative impacts it causes.

Figure 1: Cultivated *S. glomerata* stock

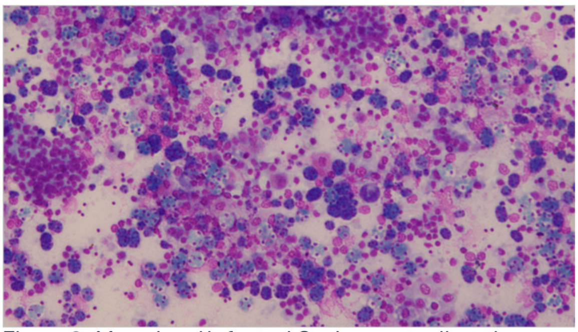

Figure 2: *M. sydneyi* infected *S. glomerata* digestive gland smear. Blue cells indicate *M. sydneyi* infection.

UNRAVELING QUEENSLAND UNKNOWN DISEASE: INSIGHTS INTO *Marteilia sydneyi's* IMPACT ON *Saccostrea glomerata*

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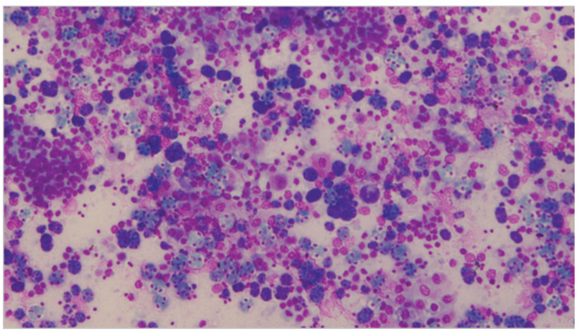

DEVELOPING AN INDICATOR TO MEASURE AND IMPROVE NUTRITIONAL RETENTION OF MARINE INGREDIENTS IN AQUACULTURE

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One of the heaviest criticisms of (especially carnivorous) aquaculture, has been its use of marine ingredients. There has been a narrative that aquaculture has increased the pressure on marine resources. Although this has largely been debunked, and the global supply of marine ingredients from forage fish has declined since the 1990s, the efficiency of their use is still paramount. Fish In Fish Out ratios (FIFOs) and various derivatives have been developed over the last twenty years as an indicator of the efficient use of marine ingredients in aquaculture. Several drawbacks of the methods have been highlighted over that time, including focusing on the limiting ingredient (meal or oil) in the diet rather than the overall efficiency of their combined use, and the quality and application of both the "fish in" and "fish out". The eFIFO method was developed that used an economic weighting to try to represent the qualitative value of the inputs (forage fish vs trimmings and meal vs oil), however it did not represent the nutritional content of either the fish input or output. Small, oily fish that have been the mainstay of the marine ingredients industry are extremely valuable as sources of micro-ingredients such as long-chain omega-3 fatty acids (LC n-3 FAs). It has been shown that retention of LC n-3 FAs from feed to whole salmon is around 50% but there may also be losses during the marine ingredient rendering process. This study sought to develop a simple metric based on the eFIFO principle that could measure the efficiency of nutrient transfer from marine raw materials (including forage fish and seafood processing by-products). A nutritional FIFO (nFIFO) was developed using a series of equations that followed the transfer of nutrients through materials linked to economic flows, so that the most limiting ingredients were identified, and that by-product utilisation was encouraged, meeting circular economy principles of zero waste and aligning closely with the guidelines set out in the EU Product Environmental Footprint Category Rules for Feed. To demonstrate the functionality of the tool, we applied the equations to literature data on LC n-3 FA content from forage fish species and derived marine ingredients as declared by the Norwegian salmon industry and for whole salmon from academic literature. We found that the tool worked well to highlight nutrient loss through the supply chain. We found losses at the rendering stage and at the feeding stage but the data on nutrient content was very variable within species and robust primary data should be used where possible. Using economic allocation much improved the nFIFO scores, serving the purpose of encouraging use of by-products from fisheries for marine ingredients over forage fish resources. The nFIFO tool can also be used for other key nutrients such as Vitamin D, B12, calcium, iron etc if the data is available for all relevant points in the production chain.

BLACK SOILDIER FLY UTILISAITON STRATEGIES FOR AFFORDABLE TILAPIA PRODUCTION IN EAST AFRICA

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Feed costs are one of the most prohibitive challenges to production of tilapia in Sub-Saharan Africa., making them unaffordable for the rural poor. This project aimed to reduce production costs of tilapia by reducing the reliance on formulated feed, by partial substitution with live black soldier fly (BSF) larvae produced locally BSF production was set up in a polytunnel in Rukungiri, Uganda. Flies bred freely and eggs were collected and incubated in maize flour where they were left until 2 days post hatch. At this point, the young larvae were inoculated into fruit and vegetable waste, collected from a local market. All waste was mashed using a hammer mill, mixed and stored in drums until time of use. Concrete bays of 1m2, 40cm depth and a 30° slope situated in a covered shed were used to grow larvae for 20 days. A feed trial was conducted on tilapia in fertilised ponds to assess the feasibility of partially substituting feed for BSF larvae. Larvae were substituted for feed at 33% and 66% on a live wet weight basis and tested against a commercial feed only diet and fertilisation only treatment. 100 acclimated tilapia of 42g were randomly stocked in hapas within a fertilised pond in three replicates of each treatment. Fish were fed at 3.5% body weight per day split equally between 3 rations during the day (apart from the fertilisation only treatment). Total weight of fish were taken on a weekly basis to calculate feeding rates for each individual hapa and individual weights were taken every three weeks. Fish were harvested after 15 weeks. Total feed, weight gain, Specific Growth Rates and Feed Conversion Ratios were recorded for each replicate. Economic data was collected on labour effort and costs to set up and manage BSF larvae production, the cost of feed and the income from fish, larvae and associated frass production.

Results showed that there was no difference in the growth rate or survival between all the fed treatments but the treatment that received no feed had reduced growth overall and lost weight in the initial period of the trial. The economic data showed that there was a marked decrease in costs required to produce fish supplemented with BSF larvae and that this was a promising method to produce affordable fish for food insecure rural poor in Uganda.

DEVELOPING ONE HEALTH SUSTAINABILITY INDICATORS FOR AQUACULTURE

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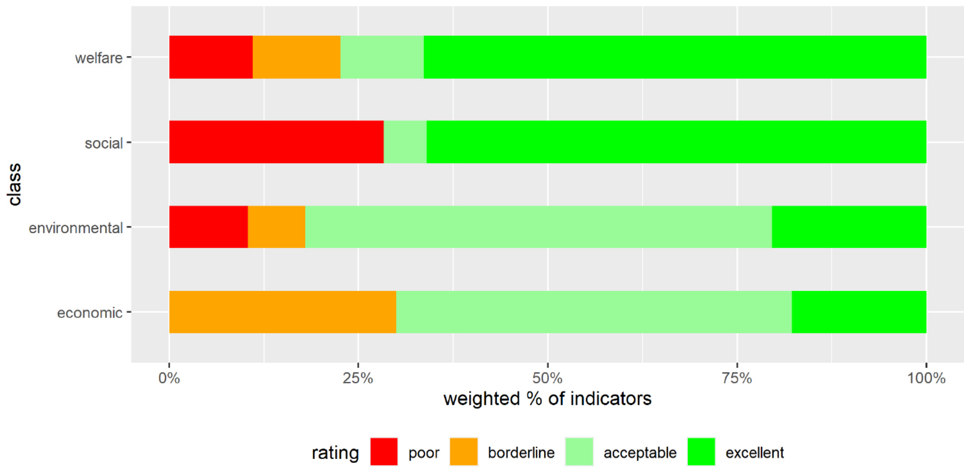
Sustainability of aquaculture is increasingly under the spotlight for various issues of concern to consumers, academia, NGOs and the industry. There are many trade-offs to consider between the impact on the environment, economic opportunities, social benefits or impacts and the welfare of the animals being cultured. The One Health framework considers people, animals and the environment, but as yet there have been few advances in developing sustainability indices that can comprehensively cover the range of different issues across the One Health spectrum. Environmental indicators may be at the local level such as in Environmental Impact Assessments, as a requisite for aquaculture facilities, or from a broader supply chain perspective such as in Life Cycle Assessments (LCA). Welfare tends to be assessed continuously using Operational Welfare Indicators at the farm. Socio-economic assessments tend to be highly varied, depending on the location and context, of production and it is targeted at local, urban or export markets and if production is smallholder, corporate or some intermediate scale. These different approaches highlight the lack of consistent focus when it comes to sustainability of the aquaculture sector and any comprehensive approach to assessing the full range of sustainability criteria. Challenges remain as to the relevance and importance (weighting) of different indicators the ability to apply them to a consistent framework. i.e. from a local production or supply chain perspective and the ease to which data can be collected and analysed which would satisfy a unifying framework across the sustainability spectrum. In this study we present the approach to applying a comprehensive sustainability index to the Norwegian salmon industry, using LCA as an initial framework along with stakeholders, co-created indicators covering global and local sustainability credential across the environment, welfare and socio-economic considerations. We piloted the index using a mixture of primary and secondary data from the Norwegian salmon industry. We found that the industry performed well in most categories, especially economic as would be expected for a mature industry. However, there were remaining concerns across a spectrum of social, welfare and environmental issues.



DEVELOPING OPTIMISED FEEDS FOR JUVENILE MALABAR SNAPPER (*Lutjanus malabaricus*)

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Malabar snapper (*Lutjanus malabaricus*) is an economically important marine fish throughout the Indo-Pacific region, commonly farmed in Malaysia and Singapore. Despite its economic importance, the nutritional requirements of snappers (*L. spp.*) are currently sparse and variable, and no species-specific feed is available. This project, comprising four interconnected studies, was conceived to (1) evaluate the energy utilisation efficiencies of digestible macronutrients, (2) determine the digestible protein and energy requirements, (3) assess the nutritional values of ten protein ingredients, including fish meal (FM), and (4) determine the cost-effectiveness of optimised feed formulations.

In Study 1, juvenile Malabar snapper's maintenance energy requirement (DEm) was estimated to be 76.7 kJ $kg^{-0.8}$ d^{-1}. The utilisation efficiencies of digestible protein and fat for growth were estimated to be 73.6% and 68.3%, respectively. A protein-sparing effect from lipids was observed when energy intake levels were below DE_m. Above DE_m, protein retention efficiency was lower when compared to fat, indicating a high demand for digestible protein to support growth. Findings from Study 1 were reaffirmed in Study 2 when the digestible protein level and digestible protein-to-energy ratio (DP/DE) were estimated to be 511.8 g kg^{-1} and 25.5 g MJ^{-1}, respectively, for optimum growth and protein deposition. In Study 3, ten alternative protein ingredients (i.e., plant-based proteins, animal by-products and insect meals) were evaluated against a 70% protein FM for their digestibility, nutrient availability and cost-effectiveness. Single-cell protein (SCP) from bacteria meal contained a profile of digestible amino acids most similar to FM, followed by black soldier fly larvae (BSFL) meal. However, the fish fed with SCP diet containing 30% SPC had significantly slower growth and lower feed intake ($P < 0.05$) than those fed on BSFL diet with the same inclusion rate. This indicates that SCP needs to be dose-optimised or balanced with nutrients other than amino acids for optimal performance. Study 4 compiled the results from Studies 1-3 to develop a series of optimised diets tailored to the essential nutrient requirements of Malabar snapper. Compared to a commercial control diet, the best-performing diets exhibited improved growth performance, feed conversion ratio (FCR) and cost efficiency by at least 50%, 25% and 20%, respectively (Figure 1). Although species-specific diets are more expensive, using a nutritionally optimised and cost-effective diet can ultimately reduce overall production costs and enhance the productivity of Malabar snapper aquaculture.

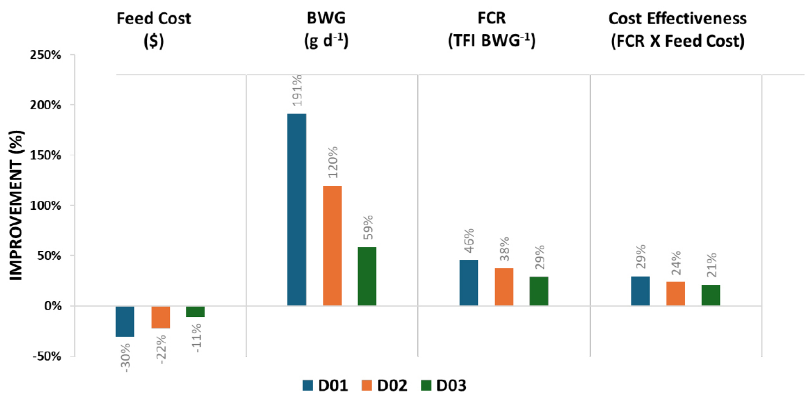

Figure 1. Bar graph comparing optimised diets (D01 – D03) against commercial diet (Ctrl). Values indicated are the mean values of each diet divided by the mean value of the Ctrl diet (n=4).

CIRCULAR INTENSIVE SHRIMP AQUACULTURE: ZERO DISCHARGE – REDUCED INPUT AND ENVIRONMENTAL IMPACT

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Whiteleg shrimp is the top aquaculture product, reaching nearly 7 million tons in 2023 and produced mainly in intensive farms. In addition to the big contribution to income, livelihood and global seafood trade, this also creates environmental concern as intensification often comes with discharge of wastewater into the open waters of farming landscape. A practical integrated multitrophic aquaculture (IMTA) system was designed to maintain intensive shrimp farming with "ZERO" water discharge for a consecutive seven production cycles at industrial scale indicating not only environmental benefit but also resilience of the system (no disease over all these cycles) by shared and interactive ecosystem role of different trophic component. The pilot was established in 2023 in Ca Mau province in 3 farms over 6 ha area. Shrimp waste was pumped to fish (tilapia, mullet and catfish) followed to green and red seaweed and then back to the shrimp system. The yield from the pilot was respectively shrimp 60 tons/ha/cycle, fish (all) 2 tons/ha/year and seaweed (all) tons/ha/year. This system used 1200% less water (calculation per a crop, much more efficiency of water utilization), 15% less feed and 5% less energy (electricity) compared to the conventional intensive system in the locality, and 50% increased shrimp production area within the farm area (shrimp: non-shrimp area ratio) compared to the existing zero discharge models in the region of leading to higher economic gain and environmental benefit.

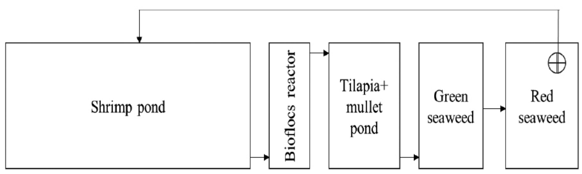

Figure 1. A schematic of shrimp farming

AN ANALYSIS OF THE EUROPEAN AQUACULTURE INDUSTRY USING THE AQUACULTURE PERFORMANCE INDICATORS

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The consumption of aquatic foods in Europe is high. In recent decades, aquaculture has gained significant importance as a domestic source of seafood, primarily due to the dwindling availability of wild fish stocks and the escalating demand for seafood. This demand is currently being met not only through domestic aquaculture but also through imports. This study investigates the environmental, economic, and social sustainability of 14 European aquaculture industries in 12 countries representing about 69% of total European aquaculture production using the Aquaculture Performance Indicators (API). We find that the average scores in Europe are higher than global average scores in all dimensions, as should be expected given that European countries are developed economies with comprehensive environmental and food regulation in place. There are significant differences between industry segments (marine cage culture, shellfish farming and land based pond farming). Our analysis also reveals that the sustainability pillars in Europe have positive (but not significant) correlations for the impacts on communities and environment; communities and economics; but negative (but not significant) correlations for the impacts on environment and economics when mussels are considered, and positive correlations when they are not. It is also of interest that the correlations are much weaker than for the global averages, suggesting that good governance is less beneficial with respect to sustainability performance. This may of course be due to the high scores in all pillars.

FUTURE PROSPECT OF mRNA VACCINES ENCODING BACTERIAL ANTIGENS IN AQUACULTURE

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mRNA vaccine technology received great concern in worldwide after COVID-2019 pandemic due to high efficacy, quick manufacturing process, safe (non-infectious and non-integration), and cost effectiveness. Since then significant amount of pre-clinical and clinical data have been reported in the mRNA vaccine industry, emphasizing the potential of mRNA vaccines to control various diseases in humans and other animals. In Korea, around 30 fish vaccines have introduced commercially for different pathogens; however, most of them are inactivated vaccines. Moreover, there is no commercialized mRNA vaccines have been developed for the fish until now.

Though the stability and storage are main limitations for the mRNA vaccine preparation, concerning with their advantages we believe mRNA vaccines strategy especially the development for bacteria mRNA vaccines may new step towards to improve the health and productivity of aquatic animals specially the farmed fish. However, selecting a suitable antigen, which elicit humoral and cellular responses, biocompatibility, efficient delivery and cellular uptake, would be crucial for bacterial mRNA vaccine preparation and may be challenging.

Edwardsiella piscicida (Gram-negative bacteria) is an important multi-drug resistant fish pathogen, which causes the disease Edwardsiellosis to broader hosts range. Various virulence factors, such as secretion systems, toxins, and adhesion molecules assist to cause the disease by invading and damage host tissues. The high mortality due to infected fish greatly impacts to economy of the aquaculture industry, thus control and mitigation strategies needs to develop continuously. We proposed that the mRNA vaccine encoding *E. piscicida* antigen/s that encapsulated with lipid nanoparticles would give great platform for controlling fast spreading of bacteria in fish especially to protect olive flounder (*Paralicthys olivaceus*) from the Edwardsiellosis. If the proposed study is succeed, it will be a great contribution to the advancement of the fish field in the future in response to new pathogenic MDR bacterial pandemics.

IMMUNO PROTECTIVE EFFICACY OF A DNA VACCINE ENCODING THE *Edwardsiella piscicida* FLAGELLIN ENCAPSULATED CHITOSAN NANOPARTICLES

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Edwardsiella piscicida (Gram negative bacteria) is one of the significant pathogens with a broad host range including aquatic animals causing the disease, Edwardsiellosis. DNA vaccination is one of the promising alternatives over traditional vaccines for controlling many pathogenic infections due to its ability to elicit both humoral and cellular responses. Though it is stable in *in vitro* when DNA plasmids delivered as a naked plasmids, it could undergo degradation by nucleases, thus cause inefficient delivery to immune cells. Hence, for efficient DNA vaccine delivery to fish, novel delivery strategies are required. Chitosan nanoparticles (CNPs) as nano-scaled carriers showed excellent value due to its biodegradable and biocompatibility and efficient gene delivery capacity.

In this study, we constructed *E. piscicida* flagellin DNA encapsulated chitosan nanoparticles based DNA vaccine, named pDNA-flagellin-CNPs. The encapsulated pDNA-flagellin-CNPs were nano sized and had high stability and encapsulation efficiency. *In vitro* transfection results demonstrated that pDNA- flagellin-CNPs expressed in human cells (HEK293T) and maintained its bioactivity. *In vitro* cell cytotoxicity analysis confirmed the safety of using pDNA-flagellin-CNPs. When zebrafish were treated with pDNA-flagellin-CNPs, the genes involve in pattern recognition (toll like receptors; *tlr5a*, *tlr5b*, and *myd88*), inflammatory (*tnf*□, *il1*□, *il6*, *il8,* and *il12*), and transcription factor (*nf-*□*b*,) responses were significantly upregulated (day 1 and 3 post treatment; dpt) compared to rest of the groups (negative control, naked pDNA, and pDNA-CNPs). Moreover, when zebrafish challenged with *E. piscicida* at 7 dpt, higher cumulative survival (>50%) was observed in the pDNA-flagellin-CNPs compared to the pDNA-CNPs and the naive group. In conclusion, the constructed pDNA-flagellin-CNPs are safe to use and produce efficient innate immune responses, which responsible for activating the humoral responses. It further suggests that CNPs are promising carriers for plasmid DNA encapsulation and efficient DNA vaccine delivery system for vaccinate the fish against *E. piscicida*.

IN SILICO IMMUNOINFORMATIC APPROACH FOR VACCINE DESIGNING AGAINST *Edwardsiella piscicida*

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An antigenic site (epitope) is a portion of pathogen, which, recognized by the immune system. Upon antibodies are specifically binding to the antigenic site, it neutralizes the pathogen or directed to destroy by other immune cells. Hence, in vaccine designing, identifying the precise antigenic epitopes is important in eliciting a protective immune response. Currently, in silico computational immunoinformatics approaches are widely used to map antigenic sites. *Edwardsiella piscicida* (Gram negative bacteria) virulence factors are significantly influences bacterial survival and pathogenesis in the host. Here we focused to find epitopes from *E. piscicida,* which could develop as safe and effective vaccine candidates in future.

In this study, proteomic sequence data of the previously generated *E. piscicida* extracellular vesicles were used for antigen selection. The outer membrane protein assembly factor BamA, which involve in assembly and insertion of □-barrel proteins to outer membrane was selected for predicting epitopes. First, the basic characteristics (potentiality of vaccine candidate, domains and characteristic motifs, physicochemical analysis) of the BamA sequence was performed using available in silico open source software. BamA sequence contained polypeptide-transport-associated (POTRA) domain that hypothesized involving in beta strand formation in OMPs and have chaperon like activity. The antigenic sites of the BamA OMP was predicted using Kolaskar and Tongaonkar antigenicity tool with the accuracy of 75%. We observed 27 antigenic determinants with average antigenic propensity of 1.014 for the BamA protein (795 AA). The Emini surface accessibility prediction showed 18 epitopes. The CTL epitopes were predicted by NetCTL 1.2 server (integrated with TAP transport efficiency, MHC class I binding, and proteasomal C-terminal cleavage prediction) with selected human leukocyte antigen (HLA) alleles MHC supertype A1. Moreover, we identified 31 epitopes which having higher than prediction score threshold (0.75000) as CTL epitopes. By BCPREDS Server 1.0, fbcpred prediction showed 4 epitopes (14 AA) having prediction score of >0.95 whereas FBCPRED predicted 4 epitopes with the prediction score of 1. Using AllerTOP and VaxiJen the both non-allergenic and antigenic epitopes were determined. After further evaluation of epitope feasibility, the selected epitopes could be compile to design a multi-epitope vaccine candidate for boosting immune system and protect the fish from *E. piscicida* infection in future.

OptiFeeSH PROJECT FOR OPTIMIZING FISH FEEDING AND COMPREHENSIVE PERFORMANCE ANALYSIS

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Fish feeding optimization solutions are critical to reduce on-farm trial-and-error iterations, further expedite production performance analysis and benchmarking is key to empower aquaculture companies to make informed decisions. Developing successfully such solutions represents a challenge: beyond expertise in data analytics techniques, it is also important, when developing tools targeted to feeding optimization, to integrate knowledge in fish nutrition, which is something that some tech teams will not be able to do easily. The OptiFeeSH project, driven by real fish farmer needs and requirements, aims to address this challenge and will enable fish farmers to 1) select best combination of feeds and feeding rates for their specific farm conditions and optimization targets (Figure 1); and 2) to monitor production performance as it unfolds and to identify the reasons for deviations. Herein, the generic OptiFeeSH approach and initial work is presented.

OptiFeeSH solution development is guided by expertise on fish production and industrial R&D, and combines fish nutrition and technology, all embedded in a user-friendly interface. The key technical methodologies applied at OptiFeeSH are:

1. State-of-the-art Nutritional-based fish simulation models, to support definition of optimal feeding plans and monitor production performance.
2. Data enrichment, to leverage data analytics and support the identification of key factors influencing production performance.
3. Optimization algorithms, to automate selection of optimal feeding plans.

This project receives funding from the Eurostars 3 (Call 3) joint programme under grant agreement E!2498. The Portuguese National Authorities or the European Union cannot be held liable for any use made of this publication. The project partners include: SPAROS, Blue Analytics, Integrated Information Systems and Flatlantic.

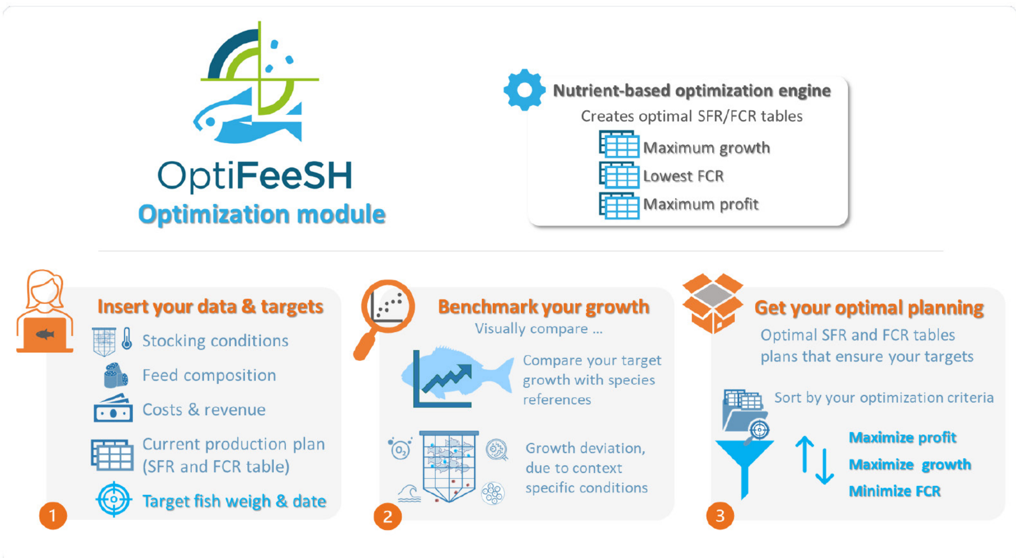

Figure 1. Optimization module conceptual framework.

ENVIRONMENTAL SUSTAINABILITY ASSESSMENT OF TWO CONTRASTED PILOT AQUAPONICS PRODUCTION (TILAPIA VS. PIKEPERCH) BY LIFE CYCLE ASSESSMENT

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Aquaponics is an integrated food production technique, often promoted as a sustainable method for growing both fish and plants, the integration of recirculating aquaculture system (RAS) and hydroponics increase water and nutrient efficiency[1,2]. However, information on the global environmental impact of aquaponics is scarce.

The aim of this work is to compare the sustainability of two contrasted fish farming scenarios in a coupled aquaponic pilot production system. The aquaponic pilot system (total volume of 18 m³) is an indoor system which has a fish production volume of 8 m³ and 50 m² of crop production on raft and NFT, equipped with LED lighting.

The two fish production models explored were tilapia (*Oreochromis niloticus*), a tropical, omnivorous and highly productive species with a low market price, and pikeperch (*Sander lucioperca*), a temperate, carnivorous, less productive species, with higher market price expectations. Vegetable production included lettuce, parsley, basil, coriander and arugula.

The sustainability of the two production scenarios was assessed by Life Cycle Assessment (LCA). An extensive data collection started at the construction of the aquaponic system and continued throughout the different productions periods. Only primary data were used and no assumptions were needed for the life cycle inventory. In order to compare the two production models, a one-year time span was considered. The system boundaries were set as the aquaponic pilot system and considered a cradle to gate approach.

Results show that the two aquaponics productions have similar profiles regarding the contribution of the processes (production, energy consumption, water consumption, transportation, aquaponic system, infrastructures) to the overall environmental impact of the aquaponic system. For the two production scenarios, energy consumption is the main environmental burden and strongly contributes to abiotic depletion (76-84%), global warming potential (73-78%) and acidification (64-60%). In terms of carbon footprint, tilapia production performs better than pikeperch. These results will help in identifying key environmental burdens and needs for improvement in aquaponic systems under temperate climate and help stakeholders decision-making concerning fish species options.

References

1. Lennard, W. & Goddek, S. Aquaponics: The Basics. in *Aquaponics Food Production Systems* (eds. Goddek, S., Joyce, A., Kotzen, B. & Burnell, G. M.) 113–143 (Springer International Publishing, Cham, 2019). doi:10.1007/978-3-030-15943-6_5.
2. Elnour, M., Haller, H. & Martin, M. Life cycle assessment of a retail store aquaponic system in a cold-weather region. *Front. Sustain.* **3**, 1051091 (2023).

CLOSING THE LIFE CYCLE OF EUROPEAN EEL (*A. anguilla)* IN CAPTIVITY: AN OVERVIEW OF LEPTOCEPHALUS LARVAE REARING

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Background
Glasaal Volendam is a private research company that aims to supply captive-bred eel fingerlings to the aquaculture industry, hereby replacing wild glass eels. The company was founded in 2012 in Volendam, a former eel-fishing city in the north of the Netherlands. The the European eel is a catadromous fish species that is considered a severely threatened species by IUCN. When the used eel is domesticated, there is no need to catch eel for human consumption.

All European eels (*Anguilla anguilla*) hatch in the Sargasso Sea, roughly 5000km away from Europe. Larvae grow out into leptocephalus larvae and eventually glass eel, as they migrate towards the inland waters of Europe. After arriving in coastal waters as glass eel, they migrate to fresh water, where they grow into elvers and develop pigmentation. Over several years, they reach adult sizes and undergo a transformation into silver eels before migrating back to the Sargasso Sea where spawning occurs.

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, Glasaal Volendam BV made considerable progress in recent years towards closing the life cycle of European eel in captivity.
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Materials and methods
To conduct our research, we rely on wild female broodstock and farmed male eel. Eels are housed in seawater Recirculation Systems. To induce vitellogenesis, females receive weekly injections of Carp Pituitary Extract (CPE) for 15-20 weeks (Kagawa et al., 2005) and biopsies are taken to evaluate oocyte development (Palstra et al., 2005). To induce ovulation, females are injected 12 hours before spawning with DHP (7□,20ß-dihydroxy4-pregnen-3-one; 2 mg per/kg body weight) according to Ohta et al. (1996). Male broodstock receives weekly injections of human chorionic gonadotropin (Perez et al. 2005). Both males and females are *stripped*, milt is mixed with eggs at a fixed ratio before activating the cells by exposing them to seawater. Fertilized eggs are incubated for 48 hours, hatching larvae are moved to our larvae 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. Weekly pictures of larvae of all ages (Fig. 1) are taken to allow for length and height measurements, and to assess developmental changes in larval morphology. Ingestion rates are evaluated by direct observation after each feeding session.

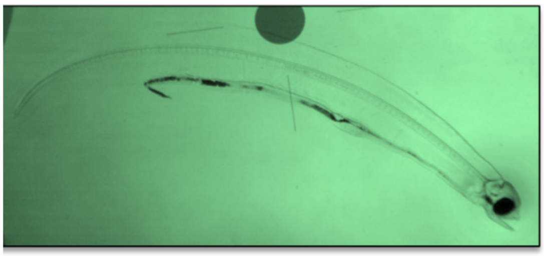

Fig 1: Microscope picture of an Anguilla anguilla leptocephalus larva of 159 days (2 cm length) with visible gut filling.

(Continued on next page)

Results and future steps

Currently we can produce large numbers of fertilized eggs weekly. We improved larval survival rates at first feeding from an average of 0.3% in 2018 to 3.8% in 2021, 21.7% in 2022 and to a stable 15% in 2023. We had maximal survival rates up to 60% of the larval batch surviving until first feeding. These improved survival rates allowed us to focus on the larval feeding stage. Feeding larvae are used for experiments from day 14 to 42 after hatching. This time interval is chosen to evaluate the effects of different diets or parameters on survival and growth rates. Increasing these parameters is fundamental to reach the glass eel in a reasonable number of days. In 2022 we successfully grew our first batch of larvae up to 1.4 cm. In 2023 larvae reached the 2 cm of length and more than 230 days of age. In the second part of 2023 and during 2024 we are focusing on the composition of the larval diets, to enhance growth and survival rates in pre-leptocephalus and leptocephalus larvae.

At the end of the experimental period from day 14 to 42, larvae are reared to transform into leptocephalus larvae. The primary and most compelling goal for the next future is to increase growth and survival rates to reach the glass eel stage. This presentation aims to give an overview of the rearing of eel larvae in the advanced leptocephalus stage.

References

Kagawa, H., Tanaka, H., Ohta, H., Unuma, T., Nomura, K., 2005. The first success of glass eel production in the world: basic biology on fish reproduction advances new applied technology in aquaculture. Fish Physiol. Biochem. 31, 193.

Ohta, H., Kagawa, H., Tanaka, H., Okuzawa, K., & Hirose, K. (1996). Changes in fertilization and hatching rates with time after ovulation induced by 17, 20P101 dihydroxy-4-pregnen-3-one in the Japanese eel, Anguilla japonica. Aquaculture, 291–301.

Palstra AP, Cohen E.G.H., Niemantsverdriet P.R.W., Van Ginneken V.J.T., Van den Thillart G.E.E.J.M. (2005) Artificial maturation and reproduction of European silver eel: development of oocytes during final maturation. Aquaculture 249(1–4):533–547.

Pérez L, Asturiano J.F., Tomás A., Zegrari S., Barrera R., Espinós F.J., Navarro J.C., Jover (2000) Induction of maturation and spermiation in the male European eel: assessment of sperm quality throughout treatment. J. Fish. Biol. 57(6):1488–1504.

Sørensen S.R., Tomkiewicz J., Munk P., Butts I.A., Nielsen A., Lauesen P., Graver C. (2016b) Ontogeny and growth of early life stages of captive-bred European eel. Aquaculture 456:50–61.

Tanaka, H., Kagawa, H., Ohta, H., Unuma, T., Nomura, K., 2003. The first production of glass eel in captivity: fish reproductive physiology facilitates great progress in aquaculture. Fish Physiol. Biochem. 28, 493–497.

NUTRITIONAL EFFECTS OF ETHYL ESTER OILS IN ATLANTIC SALMON (*Salmo salar*): A SHORT-TERM TRIAL

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The Norwegian Pelagic sector, known for producing pelagic fish products, including concentrated marine omega-3 fatty acids (FA) for dietary supplements, seeks to maximize fish source utilization by exploring valuable side stream products. One such product is ethyl ester (EE) oil, derived from concentrated omega-3 FA production. The EE oil synthesis involves reacting crude fish oil with ethanol, followed by heat distillation to produce concentrated omega-3 EE oil. An enzymatic reaction then reattaches FAs to a glycerol backbone, forming triacylglycerols (TAG), and leaving a small fraction of EE remaining.

Norway produces approximately 10,000 tons of this EE oil side stream annually, yet there is still limited knowledge on its suitability as fish feed ingredients. In this regard, the present study aims to evaluate the effects of different inclusion levels of EE oils on fish performance and health of Atlantic salmon parr in freshwater, as well as to identify tolerance levels and potential negative effects.

Lipid ingredients play a crucial role in aquafeeds, providing the necessary energy and essential FAs for fish growth and development. However, the oxidative stability of aquaculture feeds can significantly differ depending on the type and quality of the lipid ingredients used. Therefore, it was important to first assess the potential impact of the inclusion of EE oil on feed quality by evaluating the stability of the EE oil. The fresh oil used in the present study was of high quality. Results from an accelerated storage trial (100 ml of oil in vials with open lid, in the darkness, and at 40°C) showed that the oil was relatively stable compared to other marine oils indicating its suitability as a lipid ingredient in our experimental feeds.

Small rapidly growing salmon parr (5-10 g) will be reared in small freshwater tanks and fed a control diet (a commercial diet for this stage) and 5 experimental diets (in triplicates, 100 fish per tank) containing increasing EE oil levels (4, 12, 20, 30, and 35% of the total oil fraction in feed) for 5-6 weeks. We consider salmon parr as an optimal model for short-term exposure studies to screen and identify the potential positive and/or negative effects of EE oil at different feed inclusion levels. Fish performance, intestinal lipid uptake and overall health will be analyzed.

Overall, understanding the effects of EE oil on salmon performance and health will maximize the use of side stream products from omega-3 concentration processes, promoting sustainable marine resource utilization and at the same time address one of the major challenges of the Norwegian aquaculture sector, namely the need for locally produced sustainable ingredients for fish feed with low CO_2 footprint.

INVESTIGATION INTO THE IMPACTS OF REPLACING FISH MEAL IN WHITELEG SHRIMP (*Litopenaeus vannamei*) DIETS WITH INCREASING LEVELS OF PEKILO®Aqua

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The impacts of replacing fish meal in whiteleg shrimp (*Litopenaeus vannamei*) diets with increasing levels of PEKILO®Aqua were investigated in an eight-week nutrition study. PEKILO®Aqua mycoprotein, with high crude protein content and elevated levels of beta-glucans and nucleotides, is a sustainable alternative to fishmeal for feed formulations.

The trial was conducted at the AquaBioTech Group research facility using 20 growing units with 500L capacity, initially stocked with 60 juvenile *L. vannamei* with an average body weight of 3.7g. Five different diets were formulated with varying inclusion levels of PEKILO®Aqua, as shown in Table 1. The feeding and growth performance of the whiteleg shrimp were monitored over a period of eight weeks to determine various parameters including body weight gain, feed intake, specific growth rate (SGR), feed conversion ratio (FCR). Samples were taken for the determination of shrimp composition, and faecal material for the determination of apparent protein and amino acid digestibility coefficients (ADCs).

The results indicated significant differences in growth rates (but not FCR and survival, although differences were apparent) (Table 2) and the crude protein, moisture, and dry matter contents of shrimp carcasses (Figure 1). Amino acid ADCs also showed significant improvements, such as seen in the ADC of histidine, leucine, lysine, and methionine (Table 3).

In conclusion, this study clearly demonstrates the potential of PEKILO®Aqua as a sustainable alternative to fishmeal in whiteleg shrimp diets, with the addition of PEKILO®Aqua clearly increasing the average body weight and improving feed utilisation performance, survival and nutrient digestibility.

Table 1. Inclusions of fish meal and PEKILO®Aqua in the experimental feeds.

Ingredient (g/Kg)	Diet				
	0P	7.5P	15P	22.5P	30P
65% protein fish meal	240	180	120	60	0
PEKILO®Aqua	0	75	150	225	300

Table 2. Performance results for the whole trial. Results are presented as means ± SD, significance level $p < 0.05$. Different letters denote significative differences.

Diet	Final weight	SGR (%)	Feed intake (g)	FCR	Survival (%)
0P	19.64^{a} ±0.82	3.27^{a} ±0.06	1438.75 ±73.20	1.22 ±0.09	74.16 ±6.29
7.5P	19.90^{ab} ±0.33	3.29^{ab} ±0.04	1375.75 ±161.06	1.15 ±0.14	76.25 ±8.69
15P	21.65^{c} ±0.37	3.44^{c} ±0.02	1383.84 ±118.12	1.06 ±0.09	87.08 ±6.60
22.5P	21.05^{bc} ±0.54	3.39^{bc} ±0.03	1430.81 ±166.25	1.13 ±0.12	87.91 ±8.19
30P	22.09^{c} ±0.52	3.47^{c} ±0.04	1457.46 ±59.56	1.10 ±0.06	85.83 ±3.43
p-value	0.000	0.000	0.905	0.494	0.072

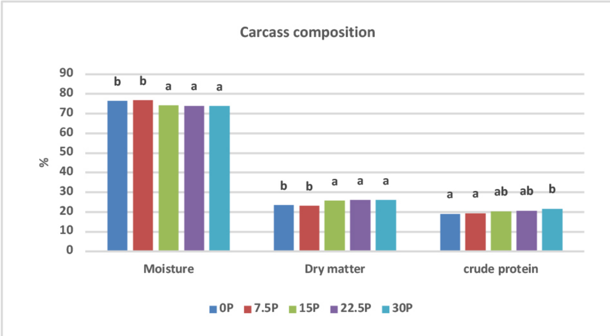

Figure 1. Final shrimp carcass composition.

Table 3. Amino acid ADCs. Results are presented as means ± SD, significance level $p < 0.05$.

	Histidine	Methionine	Leucine	Lysine
0P	81.23 ±1.69	84.67 ±1.19	79.64 ±1.40	80.46 ±1.40
30P	85.94 ±1.70	88.19 ±1.78	84.87 ±2.38	86.64 ±2.40
p-value	0.043	0.010	0.017	0.008

EFFECTS OF FOUR TRADITIONAL CHINESE MEDICINES ON THE GROWTH OF *Heterosigma akashiwo*

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Aquaculture has become an integral part of the global efforts to meet the growing demands for food security and sustainable resource management. In 2023, marine fish culture accounted for approximately 30% of the total aquaculture production in Hong Kong, representing about 57% of the total value of aquaculture. However, according to Sakamoto et al. (2021), due to the harmful algal blooms (HABs), a significant number of fish died, resulting in approximately USD 50.8 million in economic losses recorded in 1998 in the area around Hong Kong and over USD 290 million economic losses was recorded in Chinese coastal waters in 2012.

Heterosigma akashiwo, is a unicellular phytoflagellate with toxin-producing and bloom-forming features. It is a kind of representative species of harmful algal bloom that is widespread in Hong Kong marine water, as well as Greater Bay Area, China. It has caused significant economic losses due to the fish killed in many coastal regions.

With the increasing reliance on aquaculture comes the need for innovative approaches to enhance productivity, improve aquaculture health, and minimize environmental impacts. In comparison to other methods, such as the use of algicides, traditional Chinese medicines (TCMs) offer a potentially safer alternative with fewer negative effects on the environment and human health. Four TCMs, namely *Astragali Radix* (AR)*, Coptidis Rhizom*a (CR)*, Lonicera Japonica Flos* (LJF)*,* and *Poria* (P)*,* were selected to explore their effects on the growth of harmful algae, *Heterosigma akashiwo* (strain GY-H24). Each species of the TCM powder was soaked with di-water at concentration of 100 mg/mL stock solution.

When the algal cell density reached about 10,000 cells/mL, the cells were treated with either 0, 1, or 10 mg/mL of each of the selected TCMs for 6 hours or 24 hours, respectively. Three TCMs, included AR, CR, and LJF, showed prominent inhibitory effects. In contrast, P appeared to have a growth stimulating effect (Figure 1). Further studies on the underlying mechanisms are suggested.

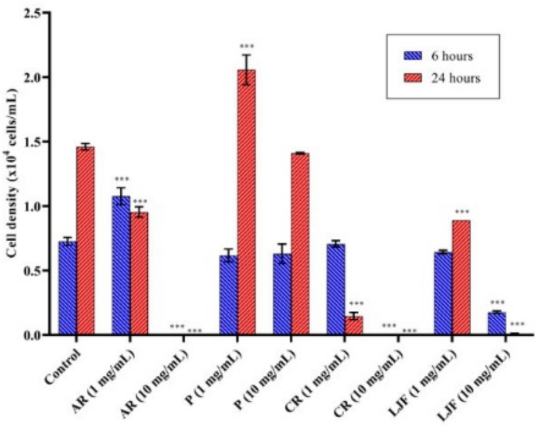

Figure 1 Effect of the four TCM extracts on the growth of *Heterosigma akashiwo* at 0, 1 and 10 mg/mL with different treatment durations, i.e. 6 hours or 24 hours. Mean with SEM of three replicates were shown above. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ indicate the significant difference compare with the control.

A NEW PLAN TO BOLSTER THE UNITED STATES AQUACULTURE ECONOMY

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The United States has substantial untapped potential to increase sustainable aquaculture production. To help reach this potential, for the first time since 1983 the United States is releasing an updated National Aquaculture Development Plan (NADP). The NADP includes a new Strategic Plan for Aquaculture Economic Development, which includes goals related to accessing capital, infrastructure and workforce development, market access, and literacy. Together with two existing plans - the Strategic Plan to Enhance Regulatory Efficiency in Aquaculture and the National Strategic Plan for Aquaculture Research - these documents comprise a holistic framework describing how U.S. federal agencies can better collaborate to advance sustainable aquaculture development to support resilient communities, a strong economy, and a healthy planet.

The NADP is a product of the Subcommittee on Aquaculture (SCA), a statutory group that operates under the Executive Office of the President. The SCA serves as the federal interagency coordinating group to increase the overall effectiveness and productivity of federal aquaculture research, regulation, technology transfer, and assistance programs. While the National Aquaculture Act of 1980 called for periodic updates of the NADP, a comprehensive update has not been completed until now. The original NADP drafted four decades ago does not capture the progress the U.S. aquaculture community has made to develop and adopt scientific advancements and sustainable aquaculture practices.

A MANUAL FOR MARINE IMTA PRODUCTION, HUSBANDRY, BIOSECURITY AND WELFARE

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Integrated Multi-Trophic Aquaculture (IMTA) is defined as the farming of aquaculture species from different trophic levels, and with complementary ecosystem functions in the same area or at the same site. This cultivation approach allows one species' uneaten feed, waste, and nutrients to be recaptured and converted into fertilizer and feed for the other aquaculture species.

The EU H2020 ASTRAL project developed new, sustainable, profitable and resilient value chains for IMTA production in the marine environment, within the framework of existing, emerging and potential Atlantic markets.

The 'IMTA labs' form the heart of ASTRAL and refer to the locations of the IMTA production sites (Figure 1). The project gathered four IMTA labs including open-water (Ireland and Scotland), land-based pump-ashore with partial (50%) recirculation (South Africa) and recirculation 'Biofloc' (Brazil) systems and one prospective IMTA lab (Argentina), focusing on a regional challenge-based perspective, and including fish, mollusc, echinoderm, crustacean and seaweed species.

Through 4-years of research and trials the IMTA labs were able to compile best practices to optimise growth for IMTA species. Three production manuals (open water, land-based pump ashore and Recirculating Aquaculture System Biofloc) have been produced, which contain detailed descriptions of the methods employed to produce the 23 species trialled in the ASTRAL research project. These manuals provide guidance on how to grow each species and the parameters needed for their successful production, including husbandry, welfare, and biosecurity. This contribution will provide a summary of the key findings and provide links to the production manuals compiled for each IMTA lab.

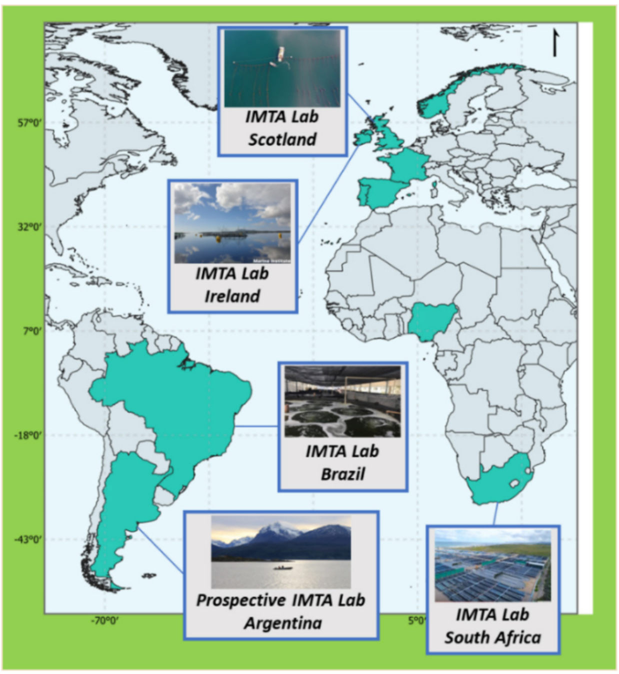

Figure 1: Location of IMTA labs around the Atlantic Ocean.

EVALUATION OF THE MUCOSAL HEALTH OF ATLANTIC SALMON *Salmo salar* FED DIETS SUPPLEMENTED WITH BREWER'S YEAST ADDITIVES

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The Atlantic salmon (*Salmo salar*) is a prominent species in aquaculture, and optimizing its growth and health is of paramount importance to the aquaculture industry. Brewer's yeast is a known source of essential nutrients and bioactive substances such as □-glucans, mannan-oligosaccharides, and nucleotides which can potentially enhance the growth and overall health of aquatic species. This study investigates the impact of incorporating two distinct commercial brewer's yeast additives, an autolysed brewer's yeast (ABY) and a soluble dried yeast extract (SDYE), manufactured by Leiber GmbH into the diet of Atlantic salmon juveniles on their performance and mucosal health.

A 9-week feeding trial was conducted in a cold freshwater recirculatory system with Atlantic salmon juveniles (37.08g) at the University of Plymouth. Three isonitrogenous and isocaloric diets were formulated to meet the known nutrient requirements of Atlantic salmon (Table 1). The control diet (T1) had no brewer's yeast additive while the other two diets, T2 and T3, were supplemented with 0.25g/100g of ABY or SDYE, respectively. The fish (20 fish/70L tank) were fed one of the three diets (n = 3 tanks) at 1% of biomass per day. All treatments grew well with no significant difference among the treatments (data not shown).

At the end of the feeding trial, skin and intestinal samples were taken for intestinal assessments using light and electron microscopy, expression of immunoregulatory genes, haematology, and 16S rRNA metabarcoding.

The result of the intestinal and skin histology revealed that the brewer's yeast diets significantly enhance (p<0.05) the intestinal physiology, and the goblet cell counts in the intestine as well as in the skin (Table 2).

Haematology result indicated that all treatments exhibited normal blood parameters with no significant difference in blood cell count and haemoglobin concentrations (data not shown).

Table 1: Experimental diets (g/100g)

Ingredients (g/100g)	T1	T2	T3
Corn gluten meal	22.00	22.00	22.00
Sunflower meal	15.94	15.78	15.78
Fishmeal	7.00	7.00	7.00
Soybean meal	15.00	15.00	15.00
Wheat gluten meal	13.82	13.82	13.82
Vegetable oil	12.08	12.08	12.08
Fish oil	9.00	9.00	9.00
Lysine	2.00	2.00	2.00
Threonine	0.17	0.17	0.17
DL methionine	0.32	0.32	0.32
Arginine	1.36	1.36	1.36
CMC	0.50	0.50	0.50
Mineral premix	0.50	0.50	0.50
Histidine	0.31	0.31	0.31
ABY	-	0.25	-
SDYE	-	-	0.25

Table 2: Histological appraisal of the intestine and skin

	T1	T2	T3
MFL (µm)	203.74±35^{a}	307.41±66^{b}	375.98±66^{b}
LPW Log10 (µm)	1.23±0.20^{a}	1.07±0.07^{b}	1.11±0.10^{ab}
MT (µm)	56.77±13.73	72.97±15.73	69.89±13.11
GC Intestine (n/fold)	20.98±10.49^{a}	33.27±11.21^{b}	31.29±6.50^{ab}
GC Skin (n/200µm)	14.56±2.85^{a}	18.22±2.48^{b}	14.44±2.66^{a}

Data are mean values ± SD. Values with different alphabetical superscripts are significantly different (P < 0.05).
MFL – Mucosal Fold Length; MT – Muscularis Thickness; LPW – Lamina Propria Width; GC – Goblet Cell counts

Ongoing analysis of expression of key immunoregulatory genes and a comprehensive 16S rRNA metabarcoding analysis of intestinal samples is being undertaken to identify the mechanisms which underpin improved mucosal data observed.

EFFECT OF DIETARY AUTOLYSED BREWER'S YEAST ON THE GROWTH PERFORMANCE, INTESTINAL HEALTH, AND IMMUNE RESPONSE OF NILE TILAPIA *Oreochromis niloticus* FRY

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The Nile tilapia (*Oreochromis niloticus*) is one of the most common aquacultural species globally, including in Nigeria and several African countries. Despite the remarkable growth of global aquaculture, the sector still faces serious challenges in Nigeria and other sub-Saharan African countries, which undermines sustainability in those countries. With this, there is a constant need to improve existing knowledge and to develop new diets using sustainable additives such as yeast derivates. 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. The present study was conducted to evaluate the potential of dietary supplementation of autolysed brewer's yeast (ABY) on Nile tilapia growth performance, feed utilisation and intestinal health.

A 5-week feeding trial was conducted in a RAS with Nile tilapia fry (0.45 g). Four diets were formulated to meet the known nutrient requirements 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 (40 fish/15L tank) were fed one of the four diets (n = 3 tanks per treatment) at 5% of biomass per day. At the end of the feeding trial, intestinal samples were taken for gene expression and histological analyses (data not shown).

Table 1: Experimental diets (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
Proximate Composition (%, dry matter)				
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

The growth data showed that the final body weight, specific growth rate, and FCR of the fish fed ABY1 was significantly better (p< 0.05) than fish fed the control diet (Table 2).

Table 2: Growth performance of Nile tilapia fed the experimental diets.

	Control	ABY1	ABY2	ABY4
Initial weight (g)	0.46±0.01	0.45±0.02	0.46±0.02	0.45±0.01
Final weight (g)	1.13±0.07^{a}	1.38±0.21^{b}	1.21±0.12^{ab}	1.12±0.02^{a}
Weight gain (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}

Data are mean values ± SD. Values with different alphabetical superscripts are significantly different (p< 0.05).

Histological analysis of the mucosal fold height, lamina propria, muscularis thickness, and goblet cell counts did not reveal any significant differences (p< 0.05) among the treatments.

Gene expression analysis demonstrated a significant upregulation (p<0.05) of interleukin 10 (*il10*), toll-like receptor 2 (*tlr2*), and occluding (*ocln*) gene expression in the ABY1 treatment group compared to the control. In contrast, fish fed the ABY1 diet exhibited a significant downregulation (p<0.05) in tumor necrosis factor alpha (*tnf*α) gene expression (data not shown).

These findings suggest that feeding ABY specifically at a lower incorporation rate level is effective in improving the growth performance and intestinal health of Nile tilapia.

ENHANCING THE SURVIVAL OF THE AFRICAN BONY TONGUE *Heterotis niloticus* FRY THROUGH TRANSPORT AND HUSBANDRY TECHNIQUES

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Aquaculture continues to be an important sector in the African economic development agenda. To further strengthen and diversity the industry, in response to the requests by farmers, there is an essential need to increase the number of cultured species in Africa. The African bony tongue, *Heterotis niloticus* has a huge potential for culture. However, the major challenge hindering its commercial production is its high larval and fry mortality, which have been linked to inadequate nutrition and handling stress. The aim of this work was to share the experiences acquired in handling fry and juvenile *H. niloticus* and to contribute to the knowledge base for managing its hatcheries. It summarizes the best practices for managing water quality, collecting larvae/fry, feeding preferences, transporting fry, and the beneficial effects of using the green water technique in *H. niloticus* larviculture. To understand the conditions under which the species thrives, the breeding behaviour and water quality in larval nests and surroundings were was monitored during the breeding season. Additionally, a feeding trial was conducted with *H. niloticus* fry from 6 to 27 DAH (days after hatch) using six feeds: *Artemia* nauplii; rotifers; 50% *Artemia* nauplii and 50% rotifers (w/w); egg custard, egg yolk and compared to fry that were not fed. Moreover, a simulated transportation trial was conducted for four different periods 24, 48, 72 and 96 hours with mortality and water quality (temperature, DO, pH and ammonia) monitored before and after transportation of fish inside the bags. Finally, fry were reared in two rearing media: “clear water” and “green water” with *H. niloticus* fry at 8DAH for 6 weeks. The results indicate that *Artemia nauplii* is best live feed organism for *H. niloticus* larviculture after yolk sac absorption. Additionally, there was deterioration in the water quality and a gradual increase in mortality with increase in transportation periods. Fish reared in “green water” systems, had a 20% higher survival rate and showed better growth. These studies on *H. niloticus* larviculture have given insights into some best practices in terms of husbandry techniques, transportation strategies and feed preferences at each growth stage. Additionally, the green-water rearing approach demonstrated high practical efficacy in the growth and survival of *Heterotis niloticus* fry; farmers can easily adopt this and we continue to work on ways to improve hatchery practices for the species.

TRADE IN FRESH SEAFOOD: ECONOMICS OF SCALE AND QUALITY

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Trade in perishable products is complex as evidenced by the large literature in operations research on managing perishable products inventories and distribution (Mirabell and Solina, 2022). Despite its complexity trade in perishables, including seafood, has grown over time (Asche, Straume and Vårdal, 2021).

This paper investigates trade in fresh seafood when there is economics of scale in order sizes. Suppliers often reduce the unit purchasing price for large orders due to cost savings. For perishable products larger order sizes lead to lower average product quality in the destination market. All else equal, a larger order is consumed over a longer period, which then reduces its average quality. This leads to inefficiency when there are economics of scale on order sizes. Trade in perishables benefit from larger buyers or access to larger markets, allowing utilization of economics of scale without sacrificing product quality.

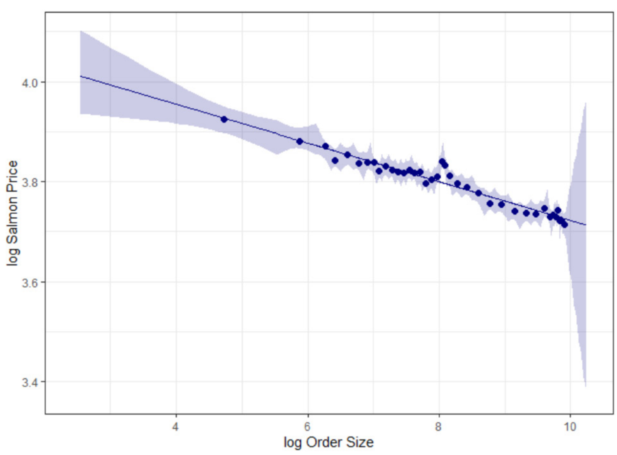
We leverage customs data on Norwegian seafood exports and document that

- A robust order size discount on price exists in trade of fresh seafood from Norway.
- Variation in order size is important to account for variation in firm-to-firm trade.
- Larger buyers trade in larger order sizes and pay lower prices than smaller buyers.
- The price discount to larger buyers can be explained by the order size discount.

We further explore the implications of perishability and economics of scale in trade by developing a trade model that includes decisions over both order size and order frequency. The model shows how perishability leads to higher prices, lower order sizes to maintain quality, lower sales and trade profit. At the aggregate level perishable products excludes small buyers. We use customs data to test model predictions and quantify the impact of perishability on Norwegian seafood exports.

Asche, F., Straume, H.M. and Vårdal, E., 2021. Perish or prosper: Trade patterns for highly perishable seafood products. *Agribusiness*, *37*(4), pp.876-890.

Mirabelli, G. and Solina, V., 2022. Optimization strategies for the integrated management of perishable supply chains: A literature review. *Journal of Industrial Engineering and Management (JIEM)*, *15*(1), pp.58-91.



EFFECTS OF LOW-LEVEL MIXTURES OF PHARMACEUTICALS ON TWO NON-TARGET MACRO-INVERTEBRATE ANIMALS

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Over the past twenty years or more, there has been increasing interest in the effects of pharmaceuticals in the aquatic environment, particularly in Europe and North America. Thousands of pharmaceuticals are in use worldwide, many are not fully degraded in wastewater treatment plants (WWTPs) and are continuously released in the aquatic environment resulting in concentrations in the low $\mu g\ l^{-1}$ range in the receiving waters. The effects of these drugs, especially at environmentally relevant concentrations, are still unknown. Moreover, few ecotoxicity test species are recommended by Organisation for Economic Co-operation and Development (OECD) and so a dearth of information exists for many organisms.

This study examined the biological effects of prolong low-level exposure of the mixtures of erythromycin, diclofenac, and ibuprofen on the growth, feeding and mortality of aquatic macro-invertebrates (*Gammarus pulex* and *Asellus aquaticus*). It was found that the mixtures of erythromycin, diclofenac and ibuprofen decreased growth rate, feed intake was reduced but mortality was not significant for both *G. pulex* and *A. aquaticus.* The effects of these pharmaceuticals on the growth, feeding and mortality of the test animals were a result of the actions of the drugs and not attributed to a more general stress response. Although pharmaceuticals are indispensable to human health their usage and discharge to the aquatic environment coupled with their ecotoxicity to aquatic life may lead to ecological problems in the near future. Furthermore, this research confirms the suitability of the test species (*G. pulex* and *A. aquaticus*) as ecotoxicological test species that is both amenable to laboratory culture and sufficiently sensitive to provide reliable quantification of environmental risk.

ESTIMATION OF GENETIC PARAMETERS AND GENOME WIDE ASSOCIATION ANALYSIS (GWAS) FOR HEAD SIZE IN MEAGRE *Argyrosomus regius*

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Meagre is a relatively new species in Mediterranean aquaculture industry, and it is considered a fast-growing sciaenid with an increasing commercial importance. Recently, the genome of the meagre became available, therefore methods which have been utilized in other Mediterranean species, such as GWAS, are becoming achievable and are expected to advance selective breeding in the species. The aim of the present study is to investigate the inheritance and genetic architecture of the head size as well as its relationship with the body weight in meagre. 762 fish at the age of 770 days-post-hatching (DPH) were weighed and a digital picture for each fish was taken. The images were used to analyze body shape, head centroid size (HCS, as a ratio to the overall centroid size) by means of geometric morphometrics. Apart from the phenotype, fin-clips were collected for each fish. Samples were genotyped using the double-digest random amplified DNA (ddRAD) methodology and after quality control and use of the available genome information, 4,573 SNPs remained. The genomic heritability of the HCS and the genomic correlation with the body weight were estimated using maximum likelihood methods with the ASREMLF90. To analyze the body weight at 770DPH, the body weight at 394 DPH was used as a covariate in the analyses.

Furthermore, a univariate GWAS for the HCS was performed to identify associated QTL using the GEMMA. An alternative Bonferroni correction was performed in which the 0.05 and 0.01 were divided with the independent SNPs only and the haplotype blocks instead of the total number of SNPs. A high genomic heritability of the head size [0.49 ± 0.06] and a high genomic correlation [0.86 ± 0.04] with the body weight were found. Focusing on the GWAS, a QTL on chromosome 8 was detected, which explains approximately 2.62% of the total phenotypic variance of the HCS (Fig. 1). The present findings suggest that the head size (HCS) is a heritable trait but given its high positive genetic correlation with the body weight, any potential selection towards the reduction of the head size is expected to negatively influence its body weight. Therefore, given the verification of the reported QTL a Marker Assisted Selection scheme might be very beneficial in a future breeding program for meagre.

Acknowledgements
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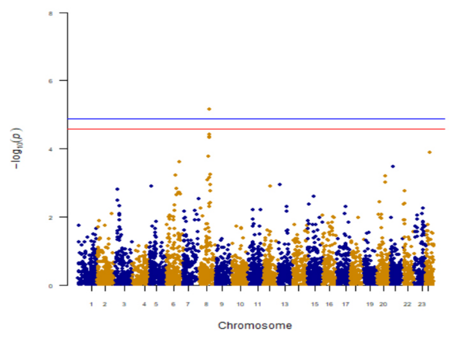

Fig 1. Manhattan plot for head size. The blue (initial value 0.05) and the red line (initial value 0.1) illustrate the threshold after the alternative Bonferroni correction (at the genomic level).

BIO-ECONOMIC ASSESMENT OF NON-CONVENTIONAL PROTEIN SOURCES IN NILE TILAPIA (*Oreochromis niloticus*), USING NOVEL FUTA AQUA-FEED FORMULATON SOFTWARE

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This study evaluated the bio-economic assessment, growth performance, and nutrient utilization of non-conventional protein sources as substitutes for traditional feed ingredients in Nile tilapia (*Oreochromis niloticus*) diets. Four test diets were formulateded with various combinations of non-conventional protein sources, including agricultural by-products, rendered animal meals, and insect components, detailed in Table 4. These diets were formulated using the novel FUTA aquafeed software and administered to tilapia over a period of 56 days. Biological parameters such as weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival rate were compared with the control diet containing the conventional fishmeal. Economic analysis, based on feed formulation costs and production yields, was also conducted. The results presented indicated that significantly higher ($P>0.05$) mean weight gain, FER, PER and better FCR was recorded in fish fed with a diet comprising 30% poultry viscera meal and 10% Mopane worm meal (Diet C), in comparison to the control and diets containing Palm kernel cake (Diet A) and blood meal ($P<0.05$). However, the control diet recorded the best profitability per unit biomass produced. These findings highlighted the potential for the widespread adoption of specific non-conventional feed ingredients, including blood meal, poultry intestine, mopane worms, water fern, palm kernel meal, and wheat meal, to decrease feed costs without compromising tilapia growth.

TABLE 01: Gross Composition of the Experimental Diet (g/100g) for Culturing *Oreochromis Niloticus*

FEED STUFFS	CONTROL	DIET A	DIET B	DIET C
Blood meal	0.00	32.50	0.00	0.00
Palm kernel cake	0.00	13.00	0.00	0.00
Water fern	0.00	9.00	0.00	0.00
Wheat meal	0.00	0.00	19.00	0.00
Mopane worm	0.00	0.00	20.00	22.0
Poultry Intestine	0.00	0.00	0.00	18.00
Ground nut cake	8.00	0.00	0.00	0.00
Fish meal	44.10	0.00	0.00	0.00
Soya bean meal	7.90	0.00	23.44	16.00
Maize	30.0	26.50	25.00	25.00
Soya bean oil	6.00	5.00	5.00	5.00
Alginate	2.00	2.00	2.00	2.00
Vitamin	2.00	2.00	2.00	2.00
Methionine	0.00	5.00	5.00	5.00
Lysin	0.00	5.00	5.00	5.00

TEACH ME HOW TO FISH: POLICY OPTIONS FOR PROFITABLE CATFISH AQUACULTURE

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The global contribution of aquaculture to total fish production and consumption has continued to increase both in absolute and relative terms, not least in many aquaculture nations. Similarly, the contribution of aquaculture to national fish production in Nigeria has increased over the years, mostly instrumented by the increase in catfish production. As the second largest producer in Africa, aquaculture is making a significant contribution to increasing income, dietary diversification, empowering women and youth, and general economic growth. All these gains in aquaculture development have been driven by increased interest in the sector, largely due to its profitability, which has led many entrepreneurs and business-oriented individuals to go into fish farming. To guard the gains of aquaculture in Nigeria and sustain its numerous benefits, policies that will promote the profitability of aquaculture must be put in place and encouraged.

We utilized economic, operational, and socioeconomic data obtained from catfish farms in Nigeria to identify potential policy options that would promote the profitability of the catfish farmers. These were grouped into learning, tactical and infrastructure-based policy-relevant factors. We used analysis of covariance (ANCOVA) to measure the effect size of the policy-relevant factors on profit. This was measured as the effect they have through the performance variables. Production scale was included as a covariate to account for variation due to scale of operation.

Product price, feed conversion ratio, feed price, and other costs exert the strongest effects on profit in the sector (Fig.1). Characteristics related to knowledge and learning, such as experience and access to knowledge sources, have the highest overall effect on profitability. They notably impact the key performance variables, which collectively contribute significantly to profit (Fig. 2). It is recommended that policies that would promote capacity enhancement of farmers should be promoted for increased profitability of the farmers. Other policy issues were also considered.

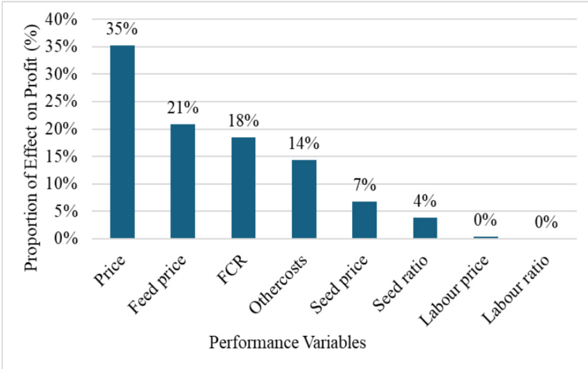
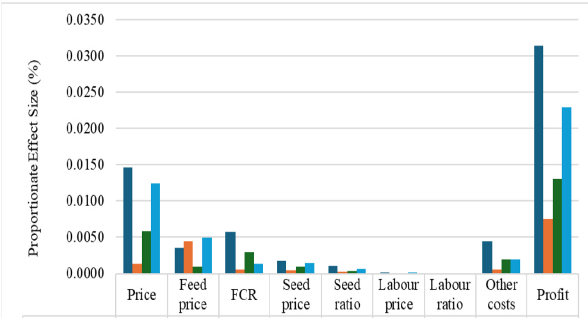


Fig. 1. Effect of performance variable on profit.



	Price	Feed price	FCR	Seed price	Seed ratio	Labour price	Labour ratio	Other costs	Profit
Learning	0.0146	0.0035	0.0058	0.0018	0.0011	0.0002	0.0000	0.0045	0.0314
Tactical	0.0014	0.0044	0.0005	0.0004	0.0002	0.0000	0.0000	0.0005	0.0075
Infrastructure	0.0059	0.0009	0.0030	0.0009	0.0004	0.0000	0.0000	0.0020	0.0131
Scale	0.0125	0.0049	0.0013	0.0014	0.0007	0.0002	0.0000	0.0020	0.0230

Fig. 2. Effect of policy-relevant factors on profit through performance variables.

MONITORING RECIRCULATING AQUACULTURE SYSTEM (RAS) MICROBIOMES THROUGH SHALLOW METAGENOMICS

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Microbiomes within recirculating aquaculture systems (RAS) provide functions critical to fish health and maintenance of optimal water quality throughout the fish rearing cycle. In natural marine environments microbial diversity is often vast, on a scale that renders monitoring through whole-sample sequencing information a prohibitively expensive and computationally taxing endeavour. As in many marine environmental contexts, to date the RAS industry largely relies on single-gene amplification studies to provide overviews of either taxonomic diversity or presence of particular functional genes in the system. However, the information from these data types is limited, proven as biased, and may not elucidate the breadth of genomic factors which could be used for monitoring RAS function. We propose that, as a closed system, RAS is inherently conducive to "shallow" metagenomic monitoring methods.

Although most environmental metagenomic studies rely on deeply sequenced samples comprising tens of millions of sequences each, the complex yet relatively stable nature of microbiomes in RAS allows for combination of data from samples sequenced at a fraction of the typical depth. Sequencing all sample DNA via metagenomics avoids introduction of confounding variables from DNA amplification steps and is a generally unbiased, comprehensive method. Metagenomics can 1) describe RAS microbial dynamics over time 2) identify functionally key community members in RAS and 3) characterise organisms that may signal non-optimal system function. Here, we demonstrate the feasibility of recovery of functional genes and metagenome-assembled genomes (MAGs) from RAS biocarrier samples across complete rearing cycles of 2 modules using an open-source computational pipeline applied to shallowly sequenced samples. Through combining sequence data of groups of samples determined as compositionally similar, we recovered 203 medium to high quality MAGs from the complex (yet limited) biocarrier community within RAS modules.

UNLOCKING THE POTENTIAL OF POET CFP: CORN-FERMENTED PROTEIN AS A SUSTAINABLE INGREDIENT IN EUROPEAN SEABASS DIETS

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Attention is rising to valorizing plant by-products as ingredients for aquafeeds, complying with the circular bioeconomy approach. In this context, two trials were performed to test the potential of POET CFP, a corn-fermented protein derived from dry-mill bioethanol production, to replace corn gluten meal (CGM; trial 1) or CGM plus soybean meal (SBM; trial 2) in practical diets for European seabass.

A control diet was formulated similar to practical diets (48% protein and 18% lipids), including 20% fish meal and 15% CGM and SBM. For the first trial, four other diets were formulated similar to the control but with POET CFP replacing CGM at 25%, 50%, 75%, and 100%. For the second trial, three other diets were formulated similar to the control but with POET CFP replacing CGM and SBM at 33%, 66, and 100%. The trials were performed simultaneously, with triplicate groups of European sea bass with an initial body weight of 26 g fed these diets for 85 days.

In the first trial, dietary replacement of corn gluten meal for POET CFP did not affect the fish's growth performance, feed intake, feed utilization, or whole body composition. In the second trial, no significant differences in final body weight, weight gain, feed intake, and feed efficiency were also observed. However, polynomial contrasts showed a significative linear trend for these parameters to increase with the dietary inclusion of POET CFP. Whole-body dry matter and lipid content linearly decreased with the dietary increase of POET CFP. In both trials, the histological morphology of the posterior intestine was unaffected by dietary POET CFP inclusion.

Overall, the results of this study indicate that POET CFP can completely replace corn gluten meal and soybean meal in diets for European sea bass without affecting the growth performance and feed utilization of European seabass juveniles.

NANOENCAPSULATED *Lippia sidoides* ESSENTIAL OIL AS AN ANTIMICROBIAL AGENT AGAINST FISH-PATHOGENIC *Aeromonas spp.*

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The advancement of sustainable aquaculture necessitates innovative approaches to disease management, mainly through utilizing natural antimicrobial agents. These agents possess bioactive properties that can enhance animal health and welfare. Currently, the exploration of essential oils in aquaculture has focused on their roles as antioxidants, antimicrobials, antifungals, anesthetics, immunostimulants, and stress reducers, highlighting their potential as an alternative approach to developing sustainable aquaculture practices. Given their high volatility, low water solubility, and poor environmental stability, encapsulating essential oils has emerged as a promising strategy. This study introduces a novel approach utilizing nanostructured lipid carriers (NLCs) to encapsulate essential oils from *Lippia origanoides* Kunth (syn. *Lippia sidoides*), a Brazilian aromatic plant known as pepper-rosemary. Its leaves contain an essential oil rich in substances of recognized antimicrobial properties, including thymol, carvacrol, and caryophyllene. The nanostructured lipid carriers, chosen for their biocompatibility and low toxicity, effectively might encapsulate *L. sidoides* essential oil. Our encapsulation technique employs a hot emulsification method using high-speed homogenization followed by ultrasonication, along with a selection of surfactants (poloxamer, quillaja saponins, and whey protein concentrate), aiming to enhance the oil's solubility, stability, and efficacy against aquacultural pathogens such as *Aeromonas veronii*, *Aeromonas caviae*, and *Aeromonas hydrophila* isolated from fish. The bactericidal activity of the lipid formulations was assessed by measuring the minimum inhibitory concentration (MIC) and bactericidal concentrations (MBC) using the microdilution broth method, as recommended by the Clinical and Laboratory Standards Institute. The main results are presented in Table 1.

The developed lipid formulations were water-dispersible, and their characteristics - mean particle size, polydispersity index, and zeta potential - were assessed, along with their pH and conductivity. The evaluation of the MIC and MBC of the nanostructured lipid carriers (NLCs) formulations showed that they significantly inhibited the growth of pathogens. The choice of surfactant was found to influence antimicrobial efficacy. Remarkably, formulations incorporating quillaja saponins demonstrated increased effectiveness in specific assays, indicating their potential as a significant component in the antimicrobial delivery system.

Table 1. Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the NLCs loaded with *Lippia sidoides* essential oil against *Aeromonas spp.*

	Lipid formulation 1 (poloxamer)		**Lipid formulation 2** (quillaja saponins)		**Lipid formulation 3** (whey protein concentrate)	
	MIC ($\mu g\ mL^{-1}$)	**MBC**	**MIC** ($\mu g\ mL^{-1}$)	**MBC**	**MIC** ($\mu g\ mL^{-1}$)	**MBC**
A. veronii KX385275	120	480	58.8	117.5	118.4	118.4
A. caviae KU605565	120	>480	117.5	235	236.9	236.9
A. hydrophila MH591949	120	>480	117.5	117.5	118.4	118.4

EFFECTS OF FEEDING VARIABILITY ON SALMON FILLET QUALITY AND HEALTH IN NORTHERN NORWAY

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Introduction

In fish farming, optimizing feeding for optimal growth is crucial. Feeding strategies for Atlantic salmon are influenced by factors like day length and temperature. However, drawing definitive conclusions from feeding studies can be challenging. Understanding the best feeding practices for post-smolt Atlantic salmon in sea cages is limited. Our study aimed to determine the most effective feeding regimes for salmon health and performance. We hypothesized that varied feeding frequencies of overfeeding across seasons would impact fish growth, welfare, resource utilization, and feed waste. Our approach aimed to strike a balance between underfeeding, hindering growth, and excessive overfeeding, leading to waste, to establish best practices in Atlantic salmon feeding.

Material and Methods

The 41-week feeding trial at LetSea's R&D sea facility in Northern Norway involved large smolts (±700g). Fish were distributed across 12 sea cages (125 m3), each with 150 fish. Conducted in triplicate, all cages received conventional industrial feed. Four study groups followed different feeding strategies: FS1 (2 meals/day + 0% overfeeding), FS2 (1 meal/day + 0% overfeeding), FS3 (1 meal/day + 10% overfeeding), and a control group (CF) with 2 meals/day and 10% overfeeding. Post-trial, fish were weighed, and welfare scoring conducted. Parameters including fat content, color, melanin, gaping, and texture were compared among groups. Muscle histology and product quality samples were collected, with fat and color analysis using Near-infrared spectroscopy and digital Salmofan.

Results

One-way ANOVA showed significant differences (p-value = 0.007) in the weights mid-experiment (mid-weights); however, we observed no significant differences in the end weights. Significant differences were also observed in the SGR values among the experimental groups and control. We did not observe any corresponding trend concerning temperature and oxygen changes in the feeding responses. Results of the effects on welfare and fillet quality will be presented at the Conference. In summary, this study suggests that feeding regimes can be used to obtain a healthy Atlantic salmon of good quality and desirable growth during a typical commercial production cycle. The study shows that a chosen feeding strategy can impact several growth and quality parameters in Atlantic salmon.

FUTURE DEVELOPMENT AND PRODUCTION GROWTH IN NORWEGIAN SALMON AQUACULTURE: THE ROLE OF REGULATION

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The Norwegian aquaculture industry have experienced remarkable success in terms of production growth, and both the industry and the government have ambitions for further growth and development of a sustainable aquaculture industry. However, in recent years, the growth has levelled off, and increasing sustainability concerns have sparked debates about potential solutions and calls for governmental initiatives for improvement. Transitioning to more sustainable practices in salmon aquaculture is imperative, but achieving this goal requires effective governance at all levels to mitigate negative impacts and ensure alignment to societal objectives, while still fostering increased production.

Regulation of salmon aquaculture production in Norway has been under governmental oversight since the industry's beginning in the 1970s. Regulatory frameworks have evolved over time in response to emerging challenges and shifting political priorities. Government intervention plays a crucial role not only in environmental protection, but also for addressing social considerations and societal expectations, such as job creation and economic development in coastal regions, and ensuring equitable distribution of economic benefits. Numerous regulations have exerted influence on the industry's trajectory, alongside other contributing factors. Notably, the licensing system hold significant importance, governing the expansion of production, as well as access to suitable production area.

Since 2017, with the introduction of a traffic light system, growth has primarily been governed by the environmental performance (with the use of an indicator) of industry actors within designated geographic areas. While this marks the implementation of a novel regulatory framework aimed at managing both growth and environmental concerns within the industry, it has not been without criticism and unintended consequences. With current production technology and regulatory framework, growth potential is limited. Thus, the industry's further progression depends on political prioritization and the selection and implementation of regulatory measures.

We investigate the potential impacts of regulatory alterations on the industry's future trajectory, encompassing aspects and effects of measures such as the introduction of new licensing systems, stricter operational standards, alterations in area allocation, among others. Several factors have the potential to drive production growth, including enhanced production efficiency, adaption of barrier technologies, and access to new geographical areas. Nevertheless, implementation of stringent regulations, such as those concerning fish health and environmental protection, production flexibility constraints, and scarcity of suitable production area, may curtail growth prospects. Consequently, a spectrum of regulatory interventions may either impede or expedite potential industry developments and various potential pathways for the industry in the forthcoming years. Additionally, the design and implementation of regulatory measures must consider governance capacity, the existing policy-mix, and the inevitable tensions and conflicts arising from divergent political and industry agendas, while addressing all dimensions of sustainability.

A NOVEL IN-SITU OXYGENATION SYSTEM FOR RAS AND TANK AQUACULTURE

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Oxygenation in tank culture systems (e.g. RAS and race-ways) is one of the key factors affecting fish growth and welfare, and one of the major important production costs. Recent developments in RAS tank design faced the problem about how to make dissolved oxygen available for the animals in the tank (in-situ), especially in shallow (1.5m) and large surface area, or deep (4-6m) and circular tanks.

The analysis of available oxygenation solutions and potential new oxygen transfer technologies considered relevant aspects such as oxygen input efficiency and dissolved gas distribution over time and space in the tank, as well as energy demand, individuality, and independency.

After a thoroughly analysis, a novel in-situ oxygenation system was developed. It consists of an independent low power consumption unit with a ceramic membrane that operates at both low gas and water pressure. Table I shows the amount of oxygen input level and energy demand of the oxygenation system (named Canox).

As a self-sufficient and plug-and-play device, the unit can be quickly installed and retrofitted into existing tanks. The Canox oxygenation device is installed directly in/at the tank and uses the internal tank circulation and water flow for a very even oxygen distribution, ensuring a good oxygen supply. Due to the low gas and water pressure, there are no generation of harmful pressure release bubbles. Moreover, the normal behaviour of the fish and shrimp were not affected. The unit works completely independent of the main water circulation system and transfers oxygen into the tank even if the main water flow is stopped.

Existing technologies (low head oxygenators, U-tubes, oxygen cones, among others) were found to be inappropriate for an in-situ oxygenation. Other technologies were characterised by an extremely high cost at similar gas input efficiency levels.

Table I. Oxygen input level (L/h, kg/h of oxygen), energy demand (kg of oxygen/kW), and efficiency (%) for two different Canox models.

Model name	Oxygen input		Energy demand	Efficiency
	L/h	kg/h	kg/kW	%
Canox 20[1]	300-600	0.4-0.8	8	70 – 100[2]
Canox 40	4.500	6.0	3.4	70 – 100[2]

[1] with motor controller, adjustable between 5-100% of duty

[2] values will depend on tank size, load, and unit configuration

EXPLORING DIVERSITY AND ANTIBIOTIC RESISTANCE IN THE ANGUILLARUM CLADE (GENUS *VIBRIO*) WITHIN EASTERN ADRIATIC BIVALVE AQUACULTURE

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Vibrio is ubiquitous genus found in marine and brackish environments. Despite being commonly found in seawater, sediment and marine organisms, numerous *Vibrio* species are considered pathogenic for marine organisms and humans. Bivalves are highly susceptible to bacterial colonization due to their capacity to accumulate bacteria in their tissues through filtration. Focus of this study is Anguillarum clade, known for bivalve and fish pathogens. 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.

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 bimonthly over the course of one year. For bacteria isolation, *Vibrio* selective medium was used, while we identified *Vibrio* clades with MALDI-TOF mass spectrometry. As final step, we performed Multilocus Sequence Analysis (MLSA) with selected phylogenetic marker genes for species identification. *Vibrio* isolates belonging to Anguillarum clade, were tested for antibiotic resistance *via* disk diffusion on Mueller Hinton agar for 12 selected antibiotics which are frequently used in aquaculture and medicine.

MALDI-TOF MS results showed that only 23 isolates belonged to Anguillarum clade. All isolates were found during sampling during colder months (T ≥ 13°C). As for antibiotic resistance, all isolates were resistant to ampicillin. Also, we recorded high resistance to antibiotic imipenem (83%), commonly used in human medicine. To some extent, isolates were resistant to erythromycin (48%) while 52% of isolates were intermediately resistant. More than half of isolates (56%) were multi-resistant, meaning that they were resistant to two or more antibiotics.

This study shows first report on the diversity and antibiotic resistance of Anguillarum 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.

IN-FEED PLANT EXTRACTS AS NOVEL ANTIVIRAL THERAPEUTICS AGAINST VIRAL NERVOUS NECROSIS INFECTION IN SEA BASS

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The search for novel antiviral compounds to prevent or mitigate the losses caused by viral infectious diseases is of great importance since they cause high economic losses in the aquaculture sector. In this work, we evaluated *in vivo* the antiviral activity of five plant extracts encapsulated in chitosan and administered in the fish feed: *Vitis vinifera, Camelia sinensis, Rosmarinus officinalis, Glycyrrhiza glabra* and *Punica Granatum*. These plant extracts have previously shown antiviral activity *in vitro* against nervous necrosis virus (NNV). This virus affects a large number of wild and farmed fish species and is distributed globally. The disease caused by this virus, the viral nervous necrosis (VNN), is considered a worldwide and disastrous disease that could be classified as one of the most important diseases in marine fish, specially for sea bass (*Dicentrarchus labrax*) fish farming. In this study, the therapeutic and prophylactic activity of five plants extracts (encapsulated in chitosan and formulated in the feed) was evaluated in vivo. Sea bass of 5-6 gr were fed with the plant extracts supplemented feeds for up to 10 days and intestine, head kidney, red blood cells (RBCs) and spleen were collected at 3 and 10 days of feeding. We evaluated the impact of in-feed plant extracts on fish health by means of blood biochemistry and hematological analyses. We evaluated the plasma-based biochemical parameters, including plasma proteins (total protein, albumin, globulin, creatine kinase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, amylase, and lactate dehydrogenase), plasma metabolites (creatinine, urea, glucose, cholesterol, triglycerides, bicarbonate, bilirubin, and lipopolisacaride), and plasma ions (Calcium and phosphorus). As well, we evaluated the hematological parameters, including leukocyte parameters (white blood cells, lymphocytes, neutrophils, monocytes, eosinophils, basophils, and platelets), and erythrocyte parameters (RBCs, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin). The immune response induced by the plant extracts supplemented feeds was evaluated by means of RT-qPCR and RNA-sequencing. Finally, we evaluated the protection conferred by the plant extracts supplemented feeds against red-spotted grouper nervous necrosis virus (RGNNV) challenge.

Acknowledgements
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ANTIVIRAL ACTIVITY OF PLANT EXTRACTS AGAINST VIRAL HEMORRHAGIC SEPTICEMIA VIRUS

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In the last decades aquaculture seems to be the only real alternative for global fish food supply. However, diseases affecting aquaculture produce high economic losses, so chemical agents are usually used, but they can affect the ecosystem. Plant extracts are an alternative because of their pharmacological properties. In this work, we evaluated the antiviral activity of four plant extracts: *Vitis vinifera, Rosmarinus officinalis, Glycyrrhiza glabra* and *Punica Granatum*, which have shown their activity as immunostimulants or antivirals, to search for new antiviral compounds against the viral hemorrhagic septicemia virus (VHSV).

The antiviral activity of the plant extracts was evaluated in EPC (epithelioma papulosum cyprini cell line from *Pimephales promelas*) cell line, which is susceptible to VHSV infection. Working concentrations of the plant extracts were selected based on bibliography and firstly assessed by a cytotoxicity assay. Then, the antiviral activity of the plant extracts, before or during the course of infection, were assessed: i) by pretreating the cell monolayer with the extracts 24 hours prior to infection; and ii) by treating the cells during the course of infection, 2 hours after infection. Viral loads were evaluated by means of a focus forming units assay.

The results showed that all plant extracts had antiviral activity, reducing the viral load *in vitro* more than a 50% in all of the cases. Further research will be focused on their application in fish feed and administration *in vivo*. In conclusion, the use of these plant extracts as antivirals is presented as an alternative to mitigate viral diseases in aquaculture.

BOOSTING TECHNOLOGICAL DEVELOPMENT IN THE SALMON INDUSTRY IN NORWAY

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To boost technological development in the salmon aquaculture industry, the Norwegian government initiated a competition for development licences in 2015. 104 applications were received over the next couple of years, and several years later a total of 24 applications were approved to receive development licenses to accomplish and pursue 24 different technological projects. The development licenses constitute an ambitious political project to promote breakthrough innovations in the aquaculture industry. Through major technological innovations, the environmental and area problems in aquaculture were to be solved. The results so far point towards a technologically diverse future consisting of many new and different solutions, and great variety in production systems.

Based on interviews, and in-depth document studies we report on how the development license scheme impacted technological development, knowledge sharing, and industry structure in the Norwegian salmon industry. In particular, we discuss how license terms to share knowledge during the course of technological development was realized, and how requirements for open knowledge sharing materialized as a surge of patent application. Patent applications quadrupled in the years the competition was open and has since steadily declined. 7 years after the first license was allocated, only 5 out of 24 projects has converted their licenses from development licenses to ordinary commercial licenses. Which also means that only 5 projects have so far completed their projects, and have submitted a final report from the project. These, and other aspects of this ambitious political project for technological development, is discussed.

ASTRAL ATLANTIC AQUACULTURE NETWORK: FOSTERING INTERNATIONAL COOPERATION IN THE ATLANTIC AREA

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ASTRAL (*All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture*) is a Horizon 2020 project focused on integrated multi-trophic aquaculture (IMTA), aiming to define, support, and promote this type of 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.

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. The project is also committed to reinforcing existing international efforts to advance acceptance and awareness about the sustainable exploitation of marine resources.

The Atlantic Aquaculture Network is the effort to gather stakeholders and exert networking influence towards "promoting institutional changes in aquaculture management", aiming at social acceptance, reputational capital, and social license.

The backbone of the network is the Aquaculture Helix, a virtual community and marketplace around ASTRAL to support its long-term establishment. Through the Helix, the Atlantic Aquaculture Network provide a community for understanding and applying a sustainable harnessing of Atlantic Ocean resources. It brings together and systematically connect industrial partners, SMEs, scientists, policymakers, social representatives, and other stakeholders, promoting data exchange, knowledge sharing, business opportunities and capacity building.

ASTRAL is an EU H2020 project (GA 863034) funded under the Blue Growth programme.

Further information: www.astral-project.eu/

DIETARY INCLUSION OF NORTH ATLANTIC FISH OILS AFFECTS THE UTILIZATION OF OMEGA-3 FATTY ACIDS IN FARMED ATLANTIC SALMON IN THE SEAWATER PHASE

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North Atlantic fish oils, such as mackerel oil and herring oil, contain lower levels of EPA and DHA compared to South American fish oils like anchovy and sardine oils. However, they are very rich in marine long-chain monounsaturated fatty acids, especially cetoleic acid (22:1 n-11). Cetoleic acid can make up around 17-25% of the total fatty acids in a North Atlantic fish oil, compared to around 1% in a typical South American fish oil.

In previous studies, we have shown that cetoleic acid can stimulate the synthesis of EPA and DHA in liver cells from both humans and salmon. Furthermore, we found that salmon smolts fed a herring oil-based diet rich in cetoleic acid had higher apparent retentions of EPA and DHA in the whole body, compared to salmon fed a sardine oil-based diet with low cetoleic acid content.

A feeding trial with Atlantic salmon in sea cages, 100 fish per cage, was conducted to investigate how cetoleic acid rich diets influence the growth, feed utilization, whole body retentions of omega-3 fatty acids and visceral adipose tissue depots. Atlantic salmon of approximately 1 kg were fed three experimental diets with increasing levels of herring oil in triplicate cages until the fish reached 4 kg.

The inclusion level of cetoleic acid in the diets did not affect the growth rate or feed utilization, but improved the retention of omega-3 fatty acids in whole body, affected the gene expression of several inflammatory markers and lipogenic enzymes, reduced condition factor, and improved visceral fat score and slaughter yield.

USING A LENGTH-WEIGHT RELATIONSHIP BASED ON WILD LUMPFISH (*Cyclopterus lumpus* L.) FOR ESTIMATION OF BODY CONDITION OF LUMPFISH IN AQUACULTURE

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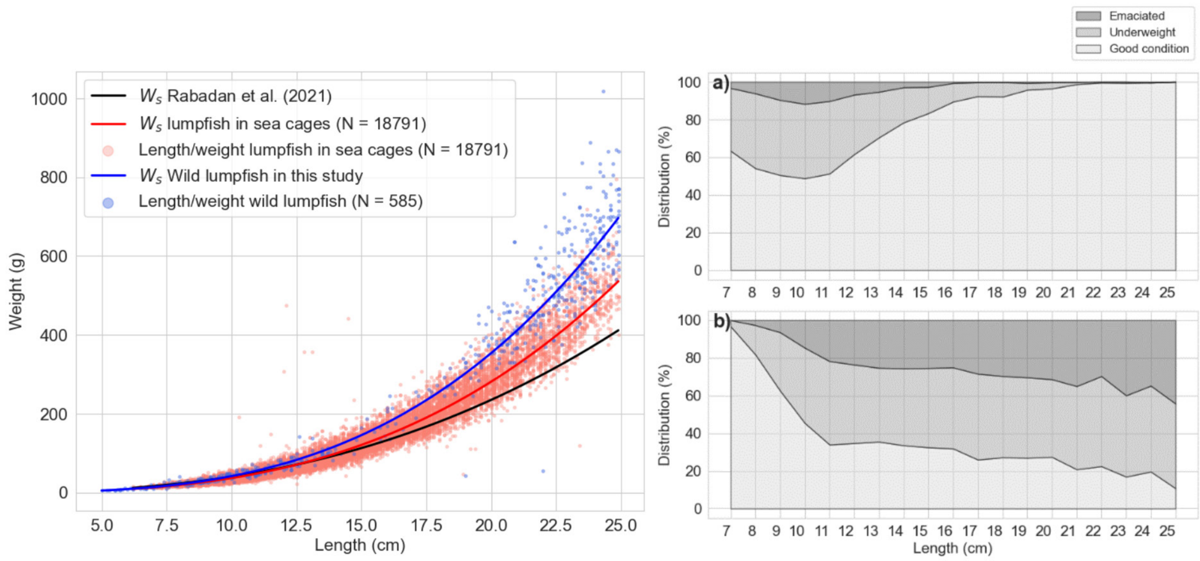
The use of lumpfish (*Cyclopterus lumpus* L.) as cleaner fish in Atlantic salmon aquaculture has emerged as a promising solution to combat the issue of sea lice infestation, as they have proven effective under the right conditions. The aquaculture industry, however, is facing challenges in ensuring the welfare and survival of lumpfish in salmon cages. Part of welfare monitoring of lumpfish in salmon cages is estimation of body condition. Previous research on standard length-weight relationships for lumpfish has primarily relied on growth patterns of lumpfish in salmon cages, which might not align with the natural growth patterns of the fish. Therefore, a correct species-adapted method is required to accurately estimate the body condition of lumpfish in salmon cages. In this study, we argue for a different approach: calculating the standard weight for lumpfish based on length-weight relationships observed in wild lumpfish. We assert that this approach aligns more closely with the natural state of the fish and may offer a more accurate representation of their condition. Our observations revealed a considerable difference in the length-weight growth patterns of wild lumpfish and lumpfish in salmon cages. Wild lumpfish displayed a positive allometric growth pattern, while the lumpfish in salmon cages displayed a slightly negative allometric growth pattern. The difference in growth patterns between wild lumpfish and lumpfish in salmon cages has important implications for the assessment of lumpfish body condition in aquaculture settings.

In conclusion, this study highlights the necessity of using appropriate length-weight relationships for lumpfish in salmon cages to accurately assess their body condition.



SALMON LOUSE *Lepeophteirus salmonis* VACCINE DEVELOPMENT – DIFFICULTIES AND PROMISING SOLUTIONS

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Today's frequent use of non-medical treatment methods against salmon louse (*Lepeophtheirus salmonis*) introduces severe welfare challenges for Norwegian salmon farming. Finding new salmon louse treatments not involving extensive handling such as a salmon louse vaccine, can provide a significant improvement. Parasites are, however, far more complex than bacteria and viruses, having several mechanisms to evade the host's immune system. Therefore, vaccines against ectoparasites are extremely challenging to make. Nevertheless, much effort has been done to develop a salmon louse vaccine, where the most promising vaccine candidates tested are expressed in the louse intestine. However, vaccines with intestinal antigens require an enhanced antigen concentration to be effective demanding stronger adjuvants, and the side effects perhaps becomes too severe for commercialization. By analysing the lice's gut content, we have found that the reason for this may lie in the louse's intestinal function, where digestive enzymes are secreted into the intestinal lumen degrading blood components and probably also vaccine induced antibodies. Antigens acting outside the louse at the skin interface may therefore have a greater potential as vaccine candidates.

One of the most important features allowing a parasite to settle onto a host is the secretion of immunomodulatory factors. The salmon louse secretes such factors into their mouth tube, and these are deposited onto the host skin during lice feeding where they dampen inflammatory responses and kills immune cells. Such host modulative proteins can thus have great potential as vaccine candidates since antibodies against these proteins do not need to encounter the louse's digestive system to have an inhibitory effect. Such a vaccine must induce the secretion of antibodies into the salmon's mucus, which can here bind to and inhibit the action of secreted lice components and might enable the salmon to resist salmon louse infestations fully or partially. However, as immune suppressive proteins have the potential to generally dampen immune responses producing an immune suppressed salmon, we have mutated the antigens in such a way that they are left non-functional but still folded correctly so that the induced antibodies can recognize native proteins. By implementing these antigens into DNA vaccines, mutants have been produced and tested on a larger scale, where we have modified the antigens and included flagellin as a molecular adjuvant for enhanced antibody production. Also, many of the proteins are relatively small proteins, and these are not always the best at inducing antibody responses. Though, by increasing the size of one antigen by injecting a construct encoding four proteins connected by a linker, the serum antibody response was enhanced, and specific antibodies could also be detected in mucus.

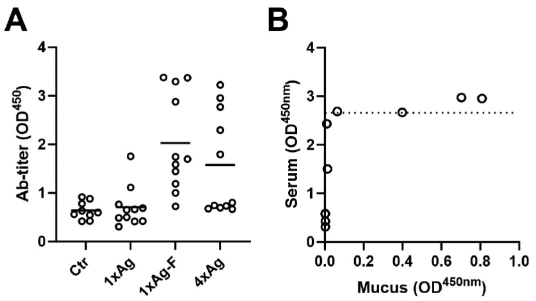

Figure 1: Level of specific antibodies (Ab) in vaccinated Atlantic salmon. A) Serum Ab in fish injected with no antigen (Ctr), constructs encoding one (1xAg) or four antigens connected with a linker (4xAg). All groups were also injected with flagellin as adjuvant, except for one group where the Ag was fused to flagellin with a linker (1xAg-F). B) Correlation of Ab in serum and mucus in fish injected with the 4xAg construct.

STUDY OF BACTERIAL MICROBIOTA OVER THE COURSE OF TILAPIA GROWTH CYCLE IN AN AQUAPONICS SYSTEM

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Aquaponics combines hydroponic crop production with recirculating aquaculture. These systems comprise various compartments (in this case: fish tank, biofilter, sump, hydroponic floating raft, settler, and aerobic digester provided by Green in Blue). Efficient developing of aquaponics in terms of water quality, fish welfare, plant health and food safety requires a thorough understanding of the microbial composition for each compartment of the system. In this study 1 m^3 dechlorinated tap water aquaponic system stocked with 50 Nile tilapia (*Oreochromis niloticus L.*, stocked at 5.7 kg per system) and 72 lettuce plants (*Lactuca sativa* Batavian Red) for 22 lettuce cycles of 5 weeks. The experiment was conducted in a small scale, closed-loop, simple recirculating aquaculture system (RAS) running for six months from 18 January ended on 21 July 2023. Several physico-chemical water parameters were monitored throughout the cycle to follow the general evolution of the system and correlate potential microbiota variations with water parameters and establish connection to the nitrogen cycle.

DNA sequencing results in Figure 1 clearly displays that aquaponics microbiome is complex and each compartment possesses its own bacterial community ($p<0.05$). An important genus in the biofilter is *Nitrospira*. This highlights its crucial role in nitrification as no *Nitrobacter* or *Nitrosomonas* is detected in the biofilter. Nocardia. A substantial proportion of denitrifying *Flavobacterium* genus common in soil, aquatic systems and plant-associated habitats were detected in sump. Also, *Cetobacterium* genus related to fish gut.

As conclusion, metagenomics allows optimization of the operational parameters of the aquaponics system to prevent pathogen proliferation and pathogen cross-transfer among compartments, as well as to enhance efficient functioning of aquaponics biofilters.

Acknowledgement: Aquaponics from wastewater reclamation (AWARE). GA N. 101084245

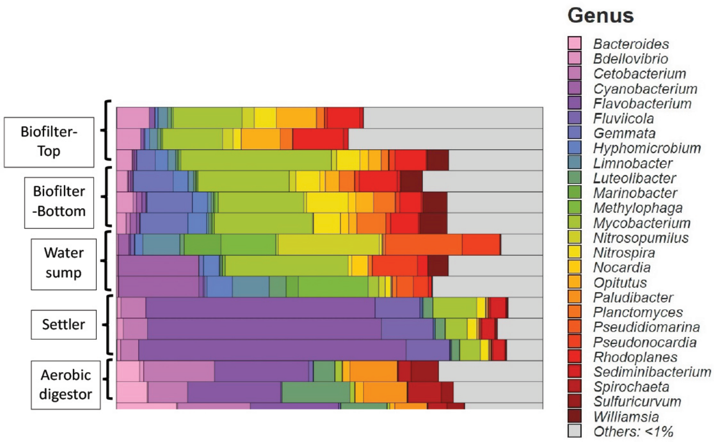

Figure 1. Microbial profiles at different compartments of the aquaponic system present at the end of the experiment at genus level.

STREAM – A SATELLITE-BASED ANALYSIS TOOL FOR RAPID EVALUATION OF AQUATIC ENVIRONMENTS

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Coastal resources are prone to intertwined effects of climate variability and anthropogenic stressors. With their massive societal and economic benefits through fisheries, aquaculture, and recreation, it is imperative for decision-making entities to integrate the highest-quality data and observations into decision support systems, thereby enhancing coastal management and monitoring. To further enrich existing observational capabilities, we have developed an expedited data processing system that ingests, processes, and displays water quality (WQ) maps (i.e., chlorophyll-a, Secchi, total suspended solids) from high-resolution imagery (10 – 30 m) of Landsat and Sentinel-2 missions. This web-based platform, STREAM (a satellite-based analysis tool for rapid evaluation of aquatic environments), offers globally validated WQ products developed using a processing engine that relies on a machine-learning model. For its interface, we harness various tools and capabilities that have already been developed as part of NASA's near-real-time data processing systems (e.g., Fire Information for Resource Management System). It allows end-users to visualize WQ maps, identify pixel values, and view time-series plots for a given pixel or a region. STREAM will enable low-latency (< 6 hours) detection of anomalous WQ conditions for robust and timely decision-making. The system is currently live and supports processing at select regions.

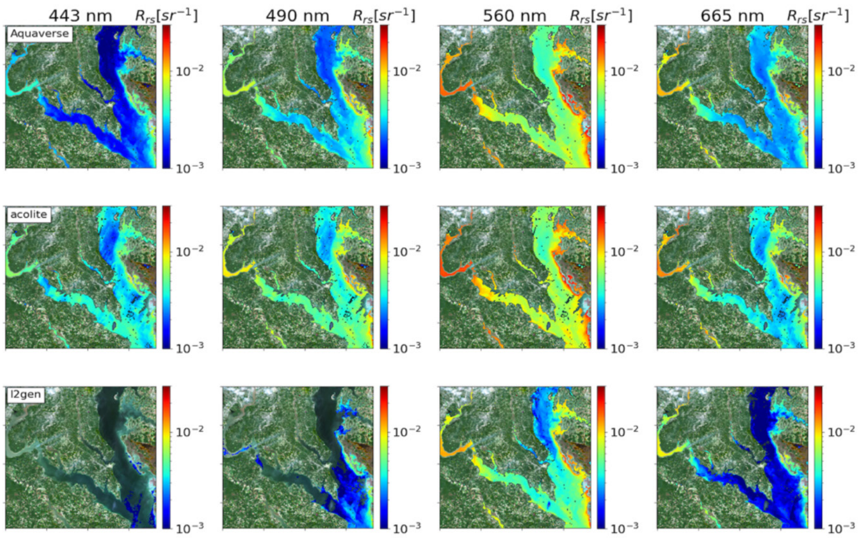

Figure 1. Chesapeake Bay on Sep 29th, 2021. Our (20-meter) Sentinel-2 reflectance products (top row, generated from our processing engine termed Aquaverse) are compared with equivalent products from other processors. Reflectance products are supplied to our machine-learning model to generate chlorophyll-a, Secchi, and TSS maps.

DO LOW-QUALITY EGGS YIELD LOW-QUALITY LARVAE? THE CASE OF EURASIAN PERCH

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Introduction

Since the 1990s, the Eurasian perch (*Perca fluviatilis* L.) has been identified as a significant fish species for enhancing aquaculture diversity in Europe (Fontaine and Teletchea, 2019). Although commercial production of this species is already established, ongoing research is still advancing the understanding of its biology, reproduction technologies and larval rearing in controlled environments (Żarska et al., 2017; Palińska-Żarska et al., 2020). It is believed that high quality eggs in fish significantly contribute to better survival and growth rates in fish larvae, positively impacting overall productivity in aquaculture (Bobe 2015). However, it is still not clear whether the "normal" larvae (the ones not showing any symptoms of developmental abnormalities) coming from various egg quality, are performing differently while cultured. In present study we compared rearing efficiency of Eurasian perch larvae obtained from eggs of high-quality and low-quality either grown group-wise or being mixed.

Material and methods

Ribbons with high and low quality eggs (n=5 from each category) were selected based on differences in hatching rate (Fig. 1). Ribbon from each female was incubated separately. Next, larvae obtained from high quality eggs (group HQ) and low quality eggs (group LQ) were pooled within the groups and reared separately. Additionally, a mixture (in equal proportions from HQ and LQ) of larvae were also created constituting mixed-quality group (MQ). Each group was reared in triplicates in the same recirculating aquaculture system (RAS). During standardized larval rearing basic zootechnical parameters (i.e. deformity rate, total length - TL, wet body weight – WBW, mortality, percentage of eating larvae, swim blader inflation effectiveness – SBIE), were compared. The obtained data were analysed using two-way ANOVA, and Tukey's *post-hoc test* ($p<0.05$). All statistical data were performed using Statistica software by StatSoft.

Results

Significant differences ($p<0.05$) in hatching were observed between HQ and LQ eggs, with hatching rates of 48.4±5.7% and 19.29±3.9%, respectively. Significant differences were also found between FERT-R and HATCH within HQ group. In LQ group, significant differences were observed between FERT-R, EYE-S, and HATCH (Fig. 1). However, no significant differences ($p>0.05$) were found during the rearing trial in terms of: TL, WBW, the percentage of eating larvae, SBIE (Fig. 2), and mortality rate (Fig. 3) between groups.

Discussion

The most widely accepted definition of egg quality is, its potential to produce a viable embryo (Kjorsvik et al. 1990, Bobe 2015). In our study we have observed considerable differences in terms of egg quality which resulted in significantly lower hatching rate. However, surprisingly no significant differences were observed in terms of performance of larvae obtained from either HQ or LQ groups. Also, there were no differences in MQ group, what indicates that larvae obtained from various egg quality were not exhibiting different within-stock interactions. These results indicate that European perch larvae which managed to hatch, had the same chance for survival and proper development, regardless of whether they came from eggs of high or low quality. While, in fact incubating low quality eggs will bring the breeder a smaller number of larvae (due to higher mortality during embryonic development, which will lead to a lower hatching rate, and a bigger number of deformed larvae that are more likely to die), the remaining individuals can be considered as high quality ones. Therefore, the widely observed within-group variability (typical for Eurasian perch; Palińska-Żarska et al. 2020) is stemming from other factors, which still need more attention and should be addressed by a more specific experimental operation.

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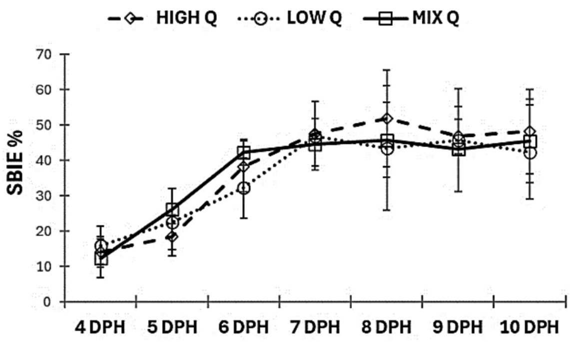

Fig.1. Survival of embryos from high and low-quality eggs at different embryonic stages: FERT-R – fertilization rate, POST-MBT – post mid blastula transition, EYE-S – eyed-eye stage, HATCH – hatching, HIGH Q – high quality eggs, LOW Q – low quality eggs. Asterisk – indicate significant differences between the groups. Small letters indicate differences within HIGH Q group and capital letters within LOW Q group ($p<0.05$).

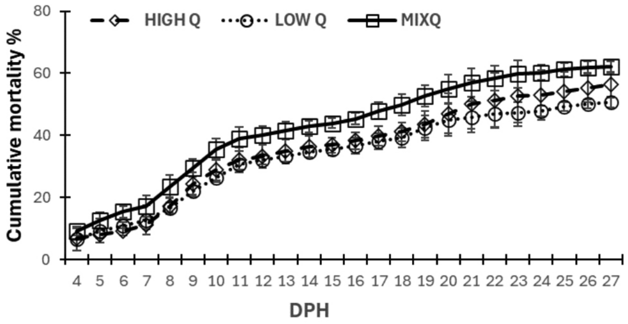

Fig. 2. Swim blader inflation effectiveness (SBIE, %) of Eurasian perch larvae. DPH – days post hatch. No statistical differences found ($p>0.05$).

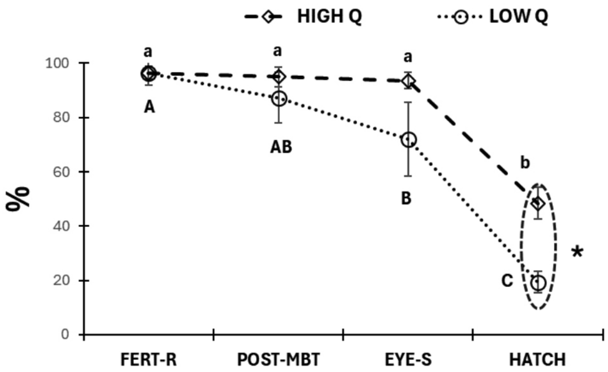

Fig. 3. Cumulative mortality (%) of Eurasian perch larvae during rearing trial. No significant differences found ($p>0.05$).

Acknowledgment

Research founded by National Science Centre of Poland (SONATA BIS project, number UMO-2020/38/E/NZ9/00394).

References

Bobe, J. (2015). Egg quality in fish: Present and future challenges, Animal Frontiers, Volume 5, Issue 1, January 2015, Pages 66–72.

Fontaine, P., Teletchea, F. (2019). Domestication of the Eurasian perch (*Perca fluviatilis*). In: Teletchea, F. (Ed.), Animal Domestication. IntechOpen, London, UK, pp. 137–159.

Kjorsvik, E., Mangor-Jensen, A., Holmefjord, I. (1990). Egg quality in fishes. Advances in Marine Biology 26: 71-113.

Palińska-Żarska K., Woźny M., Kamaszewski M., Szudrowicz H., Brzuzan P., Żarski D. (2020). Domestication process modifies digestion ability in larvae of Eurasian perch (*Perca fluviatilis*), a freshwater Teleostei. Scientific Reports, 10(1): 2211.

Żarski D., Palińska-Żarska K., Łuczyńska J., Krejszeff S. (2017) The type of spawning agent affects the egg composition during out-of-season spawning but not during in-season spawning in Eurasian perch, *Perca fluviatilis*. General and Comparative Endocrinology 245: 19–29.

COST ACTION EEL SUPPORT: SOLVING BOTTLENECKS IN EEL REPRODUCTION TO SUPPORT SUSTAINABLE AQUACULTURE

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World-wide, eel populations have decreased strongly in numbers since the 1970s. The eel farms still depend on catches of wild juvenile eels, or 'glass eels', which are then raised to market size. Only a restricted number of glass eels is available for aquaculture and societal concern exists about the unsustainable level of their harvesting. Successful propagation in captivity could supply aquaculture with glass eels and close the production cycle. Eel aquaculture can become sustainable then and, by releasing the natural population from fishing pressure, also contribute to sustainable management of the natural population.

With our international consortium of partners that has tremendous experience in eel research, we aim to share our knowledge and collaborate to force breakthroughs in the propagation of eel in captivity. This is an absolute necessity as the partners currently depend on national funding and lack an international networking umbrella. The COST Action EEL SUPPORT will use the available networking tools to jointly share the state-of-the-art, to identify knowledge gaps, to develop collaborative strategies to fill these gaps, and to synthesize and review this knowledge in order to: i) design optimal protocols for broodstock conditioning from glass eel to an eel in early puberty, or 'silver eel'; ii) design optimal protocols to artificially mature and propagate the eel to produce larvae, and iii) design hatchery technology for rearing larvae to the glass eel stage. This way, EEL SUPPORT will contribute to closing the production cycle and supporting sustainable aquaculture and management of natural populations.

The COST Action EEL SUPPORT was kicked of in Brussels on September 27 2023 and will run for four years. Currently, the Action has 70 participants representing 25 countries, including New Zealand and Japan. The first annual meeting took place early June 2024 in Demre (Turkey) and the Action was promoted in July at the 2nd International Eel Science Symposium in Liverpool (UK) and now in August during this eel session at AQUA 2024 in Copenhagen (Denmark).

For more information on this Action, please check the EEL SUPPORT website at www.wur.eu/eelsupport. If you are interested to contribute to the Action, please check the COST Action CA22163 website at www.cost.eu/actions/CA22163.

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COST Action CA22163
EEL SUPPORT

TOWARDS EXPLORING SPERM QUALITY INDICATORS RELATED TO FERTILIZING ABILITY OF CRYOPRESERVED SPERM IN EURASIAN PERCH, *Perca fluviatilis*

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The cryopreservation of fish sperm offers a versatile technique for enhancing reproduction, breeding, and conservation efforts. Although sperm cryopreservation technology for Eurasian perch (*Perca fluviatilis*) can be successfully used at a commercial-scale in aquaculture, there is still a need for finding suitable markers of cryopreservation effectiveness and fertilizing capacity of frozen-thawed sperm (FTS). Such markers may be used as selection criteria in cultured fish and for optimization of reproductive protocols.

In this study, we have performed extensive analysis of sperm collected using highly standardized protocols from 20 males and subjected them to thorough evaluation for various quality parameters. Alongside classic sperm motility parameters (using computer-assisted sperm assessment [CASA] system), we also checked for various other parameters characterizing crucial cellular components, such as, ROS+ production, mitochondrial membrane potential, ATP levels, integrity and fluidity of cell membranes in Eurasian perch sperm. Later, these analyses were also performed on the same sperm subjected to standardized cryopreservation protocol. Next, we tested fertilizing capacity of the post-thawed sperm samples during controlled *in vitro* fertilization assay. Briefly, for this purpose we have used sperm-to-egg ratio of 10000:1 and fertilizing ~1g of eggs (~500 eggs) from 3 females for each male (n=20; in triplicates). In total 180 egg samples were fertilized and later monitored for fertilization and embryonic developmental rates.

We observed positive correlations between fertilizing ability of FTS and average path velocity (VAP, $\mu m\ s^{-1}$) and straight-line velocity (VSL $\mu m\ s^{-1}$). This aligns with the observations made in previous studies where spermatozoa's kinematic parameters were key factors in the fertilization success. Interestingly, we observed negative correlations between cellular membrane integrity, both in fresh and FTS with fertilization rates. This contradicts the well proven fact that sperm membrane intactness is one of the most important quality indicators. However, since cryopreservation has a detrimental effect on spermatozoa and resulted in decrease of sperm motility of about 20%, we can speculate that there is a possibility for leakage of intracellular components from these non-alive spermatozoa which could influence on those that survive/ fertilize. One of the possibilities can be leakage of ATP from spermatozoa, since we found its level to be positively correlated with fertilization in our study. It should be emphasized that extracellular ATP has already been reported to improve fertilization rates in other taxa, but not studied so far in any fish species. In conclusion, along with this study we bring first set of validated markers which can be used as predictors of FTS fertilizing capacity in Eurasian perch and may serve as inspiration for other aquaculture species.

This work was funded by National Science Center of Poland (SONATA BIS project, number UMO-2020/38/E/NZ9/00394).

EVALUATING THE USE OF BIOFLOC PRODUCED IN A RAS AS A FEED INGREDIENT USING *ARTEMIA* AS A MODEL

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Biofloc is a microbial biomass produced in aquaculture through the assimilation of fish excretions, including ammonia and solid waste. Yogev and Gross (2019) had demonstrated the integration of a microaerophilic bioreactor with a recirculating aquaculture system (RAS) for water treatment, through the assimilation of fish excretions into bacterial biomass, producing biofloc. This biofloc contained about 40% protein and its potential as a fish feed ingredient had been tested in barramundi (*Lates calcarifer*), showing improved health and disease resistant (Nayak et al. 2023).

Feeding trials in fish aiming to reveal effects on growth and health are lengthy and complex and require animal ethic permits. Brine shrimp (*Artemia*) are known for their non-selective filter-feeding behaviour, they are easily accessible (as commercial cysts), have a short generation time and are therefore ideal for small-scale and short trials. *Artemia* had been previously used as a model for crustacean in studying host-pathogen interactions (Baruah et al. 2014). **Our aim** in this study was to explore the use of artemia as a model for fish and/or shrimp feeding trials, to evaluate the effect of feed formulations on health and growth.

Methods: *Artemia* were fed with biofloc in combination with commercial fish feed (Raanan Fish Feed BA74, 45% protein, 14% lipid) at inclusion levels of: 10, 15, 25, 50 and 100%. Growth was analysed daily for 7 days and samples were collected for immune gene expression analyses (on day 7). *Artemia* was challenged with *Shewanella jiangmenensis* (on day 4).

Results: Feeding with 25% biofloc improved *Artemia* growth and their survival following bacterial challenge, compared to control and to diets containing higher (50%) and lower (15%) biofloc inclusion levels (Fig 1). Feeding *Artemia* with 100% biofloc resulted in slower growth compared to control feed (not shown). Nayak et al. (2023) demonstrated that while the growth rate of barramundi supplemented with biofloc (20%) was lower compared to the control group, they exhibited significantly higher resistance to bacterial challenges and appeared to be immunostimulated, as indicated by elevated expression of immune-related genes.

In conclusion, biofloc as a feed ingredient exhibited a similar immunostimulatory effect in barramundi and in *Artemia*. Unlike the results in barramundi, *Artemia* growth was elevated when biofloc was added at optimal concentrations.

Reference:
Nayak S, Yogev U, Kpordzaxor Y, et al (2023), Aquaculture, https://doi.org/10.1016/j.aquaculture.2022.738780
Norouzitallab P, Baruah K, Vandegehuchte M, et al (2014), FASEB J, https://doi.org/10.1096/fj.14-252049
Yogev U, Gross A (2019), J Clean Prod, https://doi.org/10.1016/j.jclepro.2019.04.003

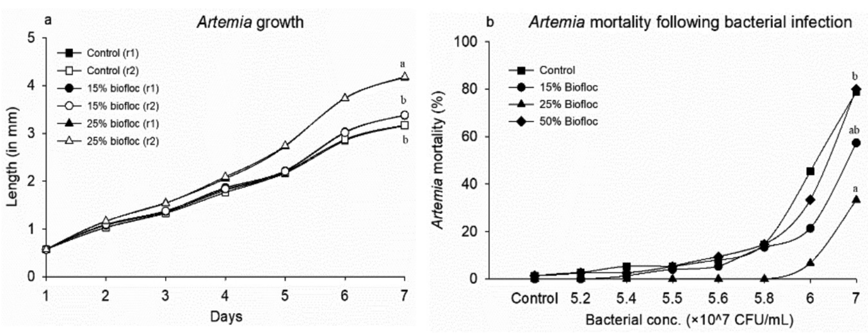

Fig1: *Artemia* fed with diets supplemented with different amounts of biofloc (15, 25 and 50%). Growth **(a)** and mortality following infection with *S. jiangmenensis* **(b)** are presented. r1 and r2 in graph **(a)** represent replicates 1 and 2; Different lowercase letters denote significant differences among different diets ($p < 0.05$).

A FULL-SCALE SEQUESTRATION OF SEDINEMTS FROM COMMON CARP (*Cyprinus carpio* L.) FARM IMPROVES BIODIVERSITY OF THE AQUATIC ENVIRONMENT AND SETS FARMERS ON THE CIRCULARITY PATH

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Traditional common carp (*Cyprinus carpio*) farming, mainly realised in earthen ponds, functions highly embedded in the landscape and is regarded as a semi-natural ecosystem. Semi-intensive farming systems typically involve a three-year cycle for common carp production, in which fish rely on natural pond food and are additionally supplemented with locally produced cereals. To mitigate the environmental impact of carp farming, producers adhere to official regulations that limit carp production to 1500 kg increased biomass per hectare, considering the nutrient release effects. One of the objectives of SAFE project is to minimise the impact of pond farm on environment and improve the viability of the freshwater aquaculture by development of sound circular economy approaches. Therefore, to reduce the impact of the farms on the river systems and to increase profitability of the farms the SAFE team built a full-scale, low-cost, and low-tech system to capture sediments discharged from the farm. Moreover, we have assessed efficiency of the system to capture sediments, quality of the captured sediments and condition of the environment before and after application of the system.

System for sediment sequestration consisted of 69 barriers made of 3-set straw bricks arranged in a herringbone layout (136 m) and nailed to the bottom of the main discharge channel. The system has been in operation for the full period of pond emptying, i.e. from October of 2023 to January of 2024. Every week water parameters before and after the system, as well as at eight evenly distributed sampling sites were assessed on-site using multiparametric probe (e.g. pH, temperature) and water samples were collected and secured to perform additional hydrochemical analyses (e.g. DO, NO_3, NH_3, P, % of TSS). After the trial sediments sequestrated by the system were sampled and analysed to assess chemical and toxicological parameters as well as grain size. Next, sequestration efficiency of the systems has been assessed concerning weight sediment deposited between and inside the brick barriers. To assess whether the system have a positive impact on the biodiversity the communities of benthic macroinvertebrates were collected and assessed, as well as condition of three aquatic bioindicators (noble crayfish, *Astacus astacus*; thick shelled river mussel, *Unio crassus*; river water-crowfoot, *Ranunculus fluitans*) installed downstream the system.

The system reduced speed of the discharged water (meandering) and 85 tonnes of sediments settled between barriers. Moreover, each of the 207 straw bricks was infiltrated on average with 10 kg of discharged sediments. Laboratory analyses showed that sequestrated sediment did not contain pesticides, antibiotics and chemical composition and grain size distribution showed its potential as a substrate or co-substrate in agriculture or horticulture. The use of sediments has been already proved in a production of beta-glucan-rich oyster mushroom (*Pleurotus ostreatus*). Aquatic bioindicators installed downstream the sequestrating system survived the discharging period in good condition, showing that installation of the system brings ecological benefits.

Funding
This work was supported by the funding from the European Union's Horizon Europe programme under grant agreement no. 101084549.

BEHAVIORAL LATERALITY AS A PREDICTOR OF PERSONALITY IN THE THREESPINED STICKLEBACK

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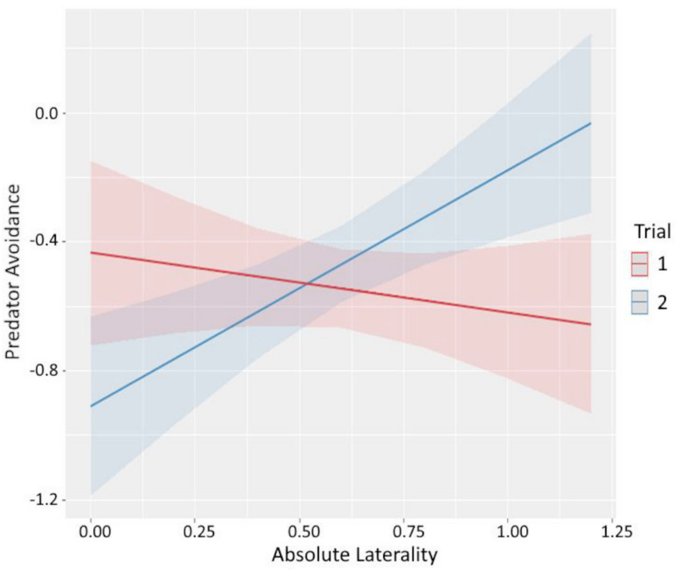
Animal personality is defined as differences in behavior between individuals within a certain group or population that are consistent across time and context. Recently, it has been proposed that animal personality can be used as a framework to develop innovative techniques in animal welfare. Nonetheless, identifying animal personality in practice can be time- and resource-consuming, making such implementation less feasible in a commercial setting. Behavioral laterality is the preference for an animal to use one side of the body respect to another to interact or respond to stimuli in the environment. In many species, it has been found that emotions are processed differently by the two hemispheres, and that individuals with different personality process emotional behavior differently. This suggests that individual differences in laterality and personality should covary. To test the robustness of such correlation, we manipulated the environment during development to examine the effects on both laterality and personality, as well as their covariation. More precisely, it is known that perceived predation and conspecifics in the early environment can influence the development of both laterality and personality. If emotional reactions are lateralized in sticklebacks, we expect personality scores to covary with laterality, regardless of the treatment experienced by the individual.

Newly hatched Threespined Sticklebacks were split in 4 treatment groups in a 2v2 design, manipulating predator exposure and social environment during the first 3 months of development. Consequently, they have been tested twice for laterality and boldness. Compared to control individuals, those who experienced predation threat during development showed a stronger lateralized behavior, while those who experienced a richer social environment showed a bolder behavior. Interestingly, boldness and laterality covaried regardless of the treatment, even though such relationship was evident only in the second trial. Such result shows how laterality, a relatively easy to test behavioral measure, has potential to be used as a proxy of personality, more specifically boldness; more research is needed to understand the effect of context on such relationship, which is possibly related to the exposure of the individuals to the same testing environment, eliciting different parts of the brain in reaction to the difference in familiarity.

Paolo Panizzon, Jakob Gismann, Bernd Riedstra, Marion Nicolaus, Culum Brown, Ton Groothuis. Effects of early predation and social cues on the relationship between laterality and personality. *Behavioral Ecology*, 35, 3. https://doi.org/10.1093/beheco/arae012



Predicted fitted line of the covariation between Absolute Laterality and Predator Avoidance, split by Trial. There was a strong, positive covariation only in the second trial.

REPRODUCTIVE PERFORMANCE OF GILTHEAD SEABREAM *Sparus aurata* IN A RECIRCULATING AQUACULTURE SYSTEM

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Recirculating aquaculture systems (RAS) offer significant biosecurity and water parameter control for broodstock management, but also allow for the accumulation of metabolites released through the fish gills, urine, and feces in the tank water, due to low water renewal. In spawning tanks, accumulating cortisol and sex steroids during the reproductive season can lead to endocrine, behavioral or welfare disorders of fish. In the present study, the spawning kinetics and reproductive performance of gilthead seabream *Sparus aurata*, kept in a recirculating aquaculture system (RAS) with natural cycling temperature vs. a flow-through system with constant temperature are compared. At the same time, the concentrations of cortisol and sex steroids, in both fish plasma and tank water, in the two systems are also compared.

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Two duplicated gilthead seabream broodstocks were formed: one was exposed to a flow-through system with constant well water temperatures (18-20°C, Flow-through), and the other one was under a RAS system with cycling thermal regimes (15-23°C, RAS group), both under simulated natural photoperiod. Their spawning kinetics (relative fecundity and fertilization percentage), egg quality parameters (embryo survival, hatching and 5-day larval survival) and plasma and water cortisol and sex steroid levels were followed.

The reproductive season of both groups began in December, when mean daily relative fecundity of both groups exhibited its lowest values, compared to the following months. Mean daily relative fecundity ranged between 7,500 and 46,000 eggs Kg^{-1} female body weight (Figure 1A) and was similar between groups. Mean fertilization percentage was also similar between groups ranging between 80 and 94% (Figure 1B). Plasma and water cortisol and sex steroid levels were analyzed.

The full results of the study will determine whether stress and reproductive hormones accumulate in the RAS systems, and examine their effect on reproductive function and egg production and quality.

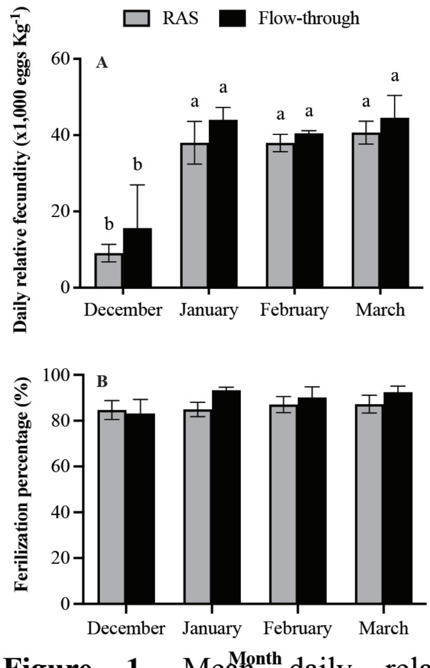

Figure 1. Mean daily relative fecundity (A), and mean fertilization percentage (B) of two duplicated gilthead seabream broodstocks kept under a RAS or a flow-through system.

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DIFFERENTIAL EXPRESSION OF MICRO RNAS (miRNAs) IN GONADS OF GILTHEAD SEABREAM *Sparus aurata*

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MicroRNAs (miRNAs) play a crucial role in regulating reproduction-related genes in various organisms, including teleosts. They regulate processes such as germ cell differentiation, gametogenesis, steroidogenesis, and apoptosis. The gilthead seabream *Sparus aurata* is of particular interest as it is a protandrous hermaphrodite that functions as a male for the first two years of life. Afterward, a proportion of fish reverse sex to female. As a result, some individuals exhibit bisexual gonads during the reproductive season.

The present study aims to explore the role of miRNAs in the bisexual testes and mature ovaries of the gilthead seabream. To achieve this aim, six fish (three females and three males) aged six years old were sacrificed. The two different parts of the bisexual testis (mature male, M and immature female, fM), as well as ovaries of mature females (F) were excised and preserved for histology and RNA extraction. Subsequently, sncRNA libraries were generated, sequenced, and differential expression analysis was conducted.

After quality and adaptor trimming, the average number of reads per sample was 13 million reads. The read length distribution displayed two main peaks: one at 21-24 nt, corresponding to miRNAs, and one at 25-30 nt, corresponding to piwi-interacting RNAs (piRNAs). As expected, expressed miRNAs formed three distinct groups, with mature females being closer to immature females. The number of differentially expressed miRNAs among groups are shown in a Venn diagram (Figure 1). Putative miRNA targets are explored and discussed.

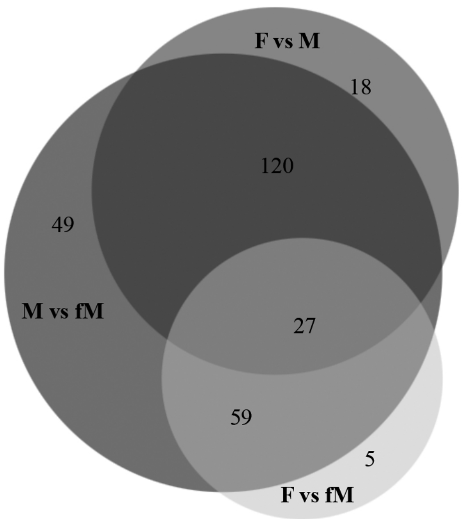

Figure 1. Venn diagram showing the comparisons in the number of differentially expressed miRNAs between three different gonadal types of gilthead seabream: the mature male (M) and the immature female (fM) of the bisexual testis and the mature ovary (F).

BEST/WORST FEEDING WINDOW ANTICIPATION THANKS TO AI AND PHYSICO-CHEMICAL PREDICTION

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Introduction

In the current context of climate change, aquaculture is facing new challenges. Extreme events are increasing, and variations in temperature and dissolved oxygen, the main drivers in feeding decisions, become more difficult to predict. This impacts the processes and routines of fish farms, forcing farmers to react to environmental changes rather than to anticipate them. Based on AI and machine learning coupled with biology, Bioceanor has developed a new bio-guided approach to adapt the feeding time window of fish within the next 24 hours, based on environmental condition predictions

Results

Based on temperature and oxygen prediction, we can identify the ideal and less favorable periods for feeding for the next working day. Figure 1A is illustrating a pre-deployment analysis where 57.9% of feed is given outside the optimal periods, resulting in inefficient productivity and economic loss. Figure 1B is illustrating the recommendations given to the farmer for the next days. By adjusting DFI according to oxygen levels, it is possible to optimize feed conversion and reduce the percentage of feed given outside of the optimal period. Proactive and adaptive feed management based on oxygen level forecasts not only ensures that feed is provided at the right time, but also improves farm performance and minimizes environmental impact by reducing waste.

Discussion and conclusion

Our value proposition is unique to anticipate future oxygen concentration to assist farmers not to feed fish when oxygen is below a given threshold, and rather use optimal oxygen windows.

- Not feeding during Worst Feeding Window avoids having feed not consumed by the fish and be lost in the sea, reduces FCR and increases eutrophication.
- Feeding during BFW, when the fish has the greatest appetite during the day, ensures a maximal DFI and an increased SGR.

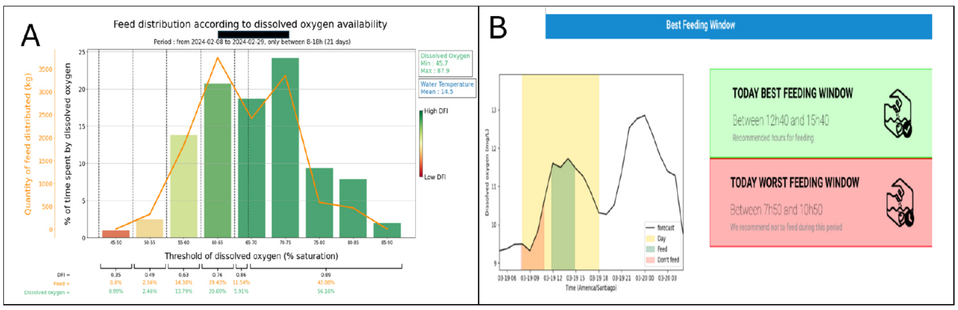

Figure 1: A. Example of feed distribution (orange line) according to dissolved oxygen (histogram). B. Example of best feeding window recommendation thanks to Oxygen prediction 24h ahead.

ENERGY USE AND GREENHOUSE GAS EMISSIONS IN AQUACULTURE: THE ROLE OF CERTIFICATION

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Food systems contribute unequivocally to climate change through direct emissions of greenhouse gas (GHG) emissions for energy, deforestation, loss of soil carbon, biogenic methane and other gases, production of fertilisers and other inputs, and downstream supply chain activities such as transport and packaging. Collectively, food systems account for up to a third of anthropogenic emissions. Government, non-governmental, and industry-led efforts have recently focused on reduction targets, substantiating claims, and identifying meaningful actions to support transitions to cleaner energy and lower emissions.

Aquaculture production accounts for four per cent of global GHG emissions. Emissions vary markedly across species and systems, including production of feed and deforestation in feed supply chains, destruction of climate-critical habitats such as mangroves, and high energy needs for land-based production (e.g., ponds, recirculating aquaculture systems). In addition to the direct and indirect reduction opportunities, the largest contribution of the industry to combatting climate change may be via expanding production of low-emission products to replace high-emission land-based animal protein sources such as beef and lamb.

High-quality, transparent, consistent data are needed to inform reductions, track progress towards targets, and support initiatives by processors, retailers, and governments. To date, understanding of GHG emissions and energy intensity of aquaculture has relied on published studied with mixed methods and uneven coverage. Access to up-to-date, regularly collected and reported farm data is necessary. Farm certification can play a critical role in connecting producers, retailers, and other actors along the supply chain to improve data availability while also helping to ensure transparency, consistency, and verification.

Here we present an overview of the energy use and GHG requirements in the Aquaculture Stewardship Council's new Farm Standard, the basis for their development, approach to implementation, and expected contributions to industry and academia. These requirements include farm input record-keeping, emissions accounting, and reporting across all farmed species and all production methods. The requirements extend to include target-setting relative to established benchmarks and implementation of management plans to address recognized drivers of emissions and work towards those targets. Together these requirements will support efforts by producers, processors, retailers, and other stakeholders in working towards climate solutions and transitioning towards climate-responsible food systems.

CHEMOSENSITIVITY OF INVASIVE BLUE CRAB *Callinectes sapidus* ON COMMERCIALLY IMPORTANT LAGOON BIVALVES

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The blue crab *Callinectes sapidus* Rathbun 1896 has become one of the most common invasive alien species (IAS) in the Mediterranean Sea. Thanks to its large environmental tolerance, high swimming capacity, and high fecundity, it is nowadays almost spread along the entire Mediterranean coastal area. In 2023, the occurrence of *C. sapidus* in the Mediterranean Sea reached the dimension of a massive invasion with direct impacts on the economy of artisanal small-scale fisheries. This species is a predator whose exponential expansion is raising concerns about the possible ecological consequences and the threats posed to native species. Indeed, invasive predators can have significant impacts on ecosystems by altering food webs and causing declines in native species populations. Here, we explored the chemosensitivity of *C. sapidus* in the detection and selection of prey in the field of predator-prey interactions.

In our experiments, we tested the food preferences of the blue crab by exposing it to different prey species: *Mytilus galloprovincialis*, *Ruditapes decussatus* and *Cerastoderma glaucum*. Male and female crabs were tested separately to identify possible feeding differences related to sex. The prey was presented to *C. sapidus* in four different manners: 1) alive, whole prey and 2) alive prey, but following removal of one valve, thus allowing the predator to use different sensory systems (smell, sight, touch) for food detection/selection, (3) agar-dissolved prey, so that the predator might only rely on the sense of smell and 4) prey shells filled with a different agar-dissolved prey (i.e. shells with smell of an alternative prey) to uncouple visual vs. olfactory inputs. Experiments lasted two hours and were videotaped for later analysis of the predator's behaviour.

Results showed that, after tactile exploration, all prey was chosen by both males and females. Female crabs appeared more consistent than males in their choice, while males seemed more likely to make random prey selection, in agreement with the fact that their higher claw strength may allow them less selectivity in the choice. Therefore, the prey choice might be related to the size of the crab and especially to the strength of the claws. The same behavior of prey choice was observed when the prey supply was carried out in prey shell filled with agar-dissolved prey.

On the whole, our results suggest that the blue crab relies on both olfactory and visual inputs when making the choice.

This study provides valuable insights into the prey selection strategies of the blue crab *C. sapidus*, and how predators respond to different chemical cues emitted by various prey species. The study of the chemosensitivity of *C. sapidus* provides valuable insights into the mechanisms driving predator-prey interactions and ecosystem dynamics, with implications for conservation and management efforts. This approach is also useful for evaluating the effectiveness of baits to effectively attract blue crabs into traps and thereby counteract their expansion.

EFFECTS OF FEEDING PROTOCOLS ON OXIDATIVE STRESS RESPONSES OF ATLANTIC COD (*Gadus morhua*) LARVAE

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Atlantic cod aquaculture still faces 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 to ensure the quality of juveniles. Although rotifers and Artemia are the most common start feed for cod larvae cultured by intensive methods, barnacle nauplii are also a potential live feed for marine fish larvae. Although much progress has been made concerning feeding protocols for the intensive culture of cod larvae, further research is needed to optimize inert diets and a better combination of co-feeding protocols with live feed. This work aimed to evaluate the effects of two experimental microdiets and one live feed protocol on oxidative stress of Atlantic cod larvae.

Cod larvae from Ode AS (Stadsbygd, Norway) were randomly distributed in triplicate 400 L tanks at 2 days post-hatch (dph) and kept until 70 dph (trial end). The light regime was 24h and temperature ranged from 9 to 11 °C. All tanks were fed with a similar live feed protocol: a sequence of enriched rotifers, plankton eggs (Cryo-µ), small barnacle nauplii (Cryo-S), and large barnacle nauplii (Cryo-L), or a sequence of enriched rotifers and Cryo-L. The 3 microdiets were introduced at 27 dph in co-feeding with live feed, and alone from 45 dph onwards. Diet CTRL was a commercially available diet, and diets D1 and D2 were experimental prototypes. At the end of the trial (70 dph), pools of cod larvae were collected for the analyses of lipids peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activities in homogenized whole larvae.

Figure 1. Average CAT and SOD activities (left) and LPO (right) in Atlantic cod larvae at 70 DPH fed two experimental (D1 and D2) and one control (CTRL) dietary treatments. Different lowercase letters indicate significant differences between treatments ($p<0.05$).

CAT and SOD activities did not present significant differences between the groups at 70 dph. However, LPO was significantly lower in the D1 group when compared to D2 and CTRL groups. LPO is considered a valuable biomarker of oxidative stress, and these results combined with a significantly lower incidence of skeletal anomalies in group D1 (Henriques J. 2024, submitted to AQUA2024) highlight the potential for optimizing feeding protocols for Atlantic cod larvae.

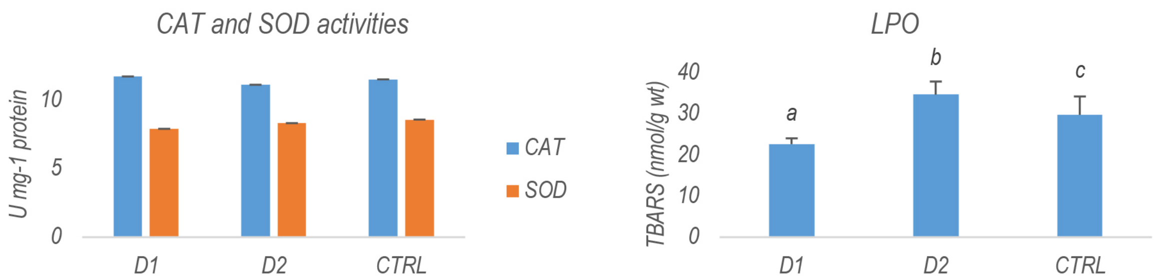

Figure 1. Average CAT and SOD activities (left) and LPO (right) in Atlantic cod larvae at 70 DPH fed two experimental (D1 and D2) and one control (CTRL) dietary treatments. Different lowercase letters indicate significant differences between treatments (p<0.05).

NUTRIENT STOCKS AND FLUXES OF CARP POND SLUDGE

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Introduction

In the Waldviertel region, carp pond farming has a long tradition. Besides the production of fish, ponds play an important role as landscape elements, wetland ecosystems and water retention and storage basins in agricultural landscapes. The question of whether ponds act as nutrient and sediment sinks or as sources is a topic of ongoing debate in the community (e.g. Potužák et al. 2012, 2016). Presumably, this is largely dependent on the catchment area and the management of the pond. During the harvesting of the pond, the pond sludge is disturbed and – depending on the structural design of the outlet – potentially transported out of the pond. For pond farmers in the EU, the outflow of high nutrient loads during harvesting can present a significant challenge. On the one hand, contamination of receiving waters during fish harvest might result in a conflict with the EU Water Framework Directive. On the other hand, excessive loss of sludge and, consequently, a reduction in nutrient availability, has the effect of reducing pond productivity and therefore affect the economic quality of the pond (less nutrients = less fish production = less economic income). However, until now, research has not focused on carp pond sludge quantity or quality.

Material and Methods

The volume and mass of sludge in a carp pond in the Waldviertel region (Lower Austria) were quantified through a combination of methods (directs measurements of sludge depth, airborne-supported mapping of spatial sludge extent, spatial interpolations, sludge sampling, laboratory analysis and difference models). Two flight campaigns were conducted in the autumn of 2021 and 2022, during which the pond was drained. The investigation of the sludge based on measurements with bamboo rods, to determine depth at spatially distributed locations, and sludge collection using an aquatic sediment corer. This methodology allowed the sampling of sludge cores to a maximum depth of 50 cm. In the laboratory, Total Organic Carbon (TOC), Total Nitrogen (TN), Total Phosphorus (TP) of the sludge (in sections with a depth of 10 cm), the pond water and the three main inlets were determined. Furthermore, the sludge discharge and the associated nutrient loss during harvesting the pond we recorded.

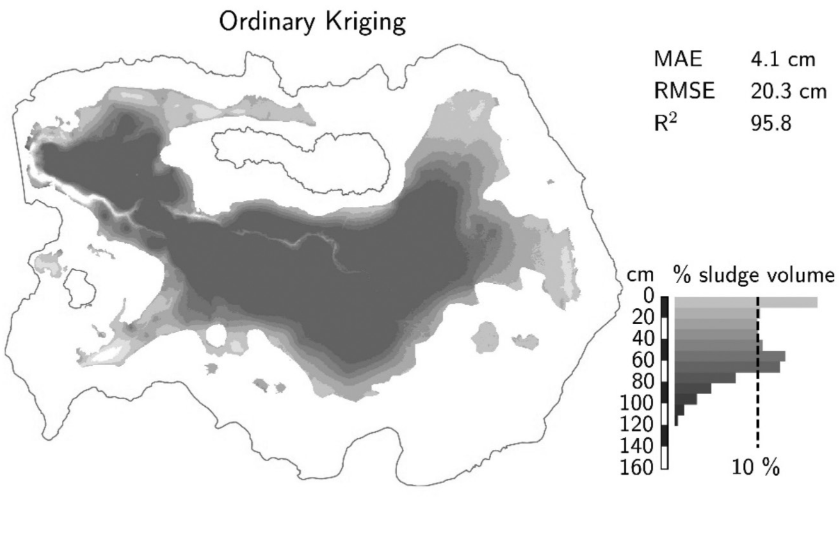

Fig.1: Shows mass, depth and distribution of sludge in the pond. Ordinary Kriging emerged within our study as the most accurate interpolation method.

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Results and Discussion

Although the examined carp pond is of considerable depth, the sludge layer was relatively shallow, with the thickest sludge accumulating towards the center. The analysis of sludge cores revealed an uneven depth distribution of sludge parameters, with a notable increase in TOC content up to a depth of 30-40 cm, followed by a sharp decline. The mass of the entire sludge layer was estimated to be 9,396 Mg (Fig. 1), with 2,130 Mg of TOC, 105 Mg of TN, and 5 Mg of TP contained within. The evaluation of TOC, TN, and TP storage indicates that the pond functions primarily as a sink for these nutrients. The accumulation of TOC and other nutrients in the sludge suggests significant retention, preventing an immediate release back into the water column or downstream ecosystems.

However, local erosion and displacement can cause temporary nutrient resuspension and redistribution. A comparison of aerial images from 2021 and 2022 revealed unevenly distributed areas of sludge loss or accumulation, indicating displacement processes during pond drainage. Despite fluctuations in sludge thickness, the overall sediment mass or volume remained relatively constant between the two years. It seems reasonable to assume that the upper layers of sludge are subject to mixing because of the movement and foraging behavior of carp. While the question of nutrient balancing is beyond the scope of this study, we were able to demonstrate that a lot of sediment and nutrients are stored in the pond sludge and significant amount of both are removed from the pond during harvesting. Such occurrences can have a detrimental impact on the quality of the receiving water.

DIURNAL VARIATIONS AND DEVELOPMENT OF DISSOLVED OXYGEN CONTENT IN AUSTRIAN CARP PONDS

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Introduction

The amount of dissolved oxygen (DO) being available in a waterbody can determine its suitability for Aquaculture. Contrary to Salmonids, carp (*Cyprinus carpio*) are known to survive rather low DO contents. They can survive with DO concentrations of 3 mg L^{-1} (Steffens 2014, Schäperclaus and Lukowicz 2018) and can withstand levels as low as 0.4 mg L^{-1} for some minutes (Dunham *et al.* 2002). Due to climate change, traditional Austrian carp farming ponds are now experiencing a change in the course of the year in terms of temperature and DO patterns (Böhm and Bauer 2015). Increasingly often, high water temperatures and high nutrient concentrations lead to oxygen deficiencies. That is why fish losses in summer become more frequent. In order to adapt the pond management to the altered circumstances it is crucial to know as much as possible about the development and variations of DO in carp ponds. Therefore, we have started a continuous monitoring of DO and water temperature in Austrian carp ponds.

Material and Methods

The study site is located at the district Waidhofen an der Thaya in the Waldviertel Region (Lower Austria). This Region is known throughout Austria for its carp production. The high nutrient concentrations found in carp ponds result in high biological activity. A continuous measurement of DO is therefore difficult, because a biofilm forms on the measuring device in continuous use and leads to excessive maintenance work. Since Mai 2024, we use a fully autonomous oximeter (SmonOX; Galvanic probe; Clark sensor). This device cleans itself after each measurement and the probe is stored above the water surface, which significantly reduces biofouling. Measurements with this device are conducted 26 times per day at a depth of 0.5 m at a carp farming pond with 41 ha in size.

Results and Discussion

Between 1.6. and 25.7.2024 DO under 3 mg L^{-1} was measured five times. On the 14th and 18th of July the lowest DO concentrations occurred during the early morning hours. As the amount of sunlight increased, so did the photosynthetic activity of algae and aquatic plants and thus the DO concentration rose (Fig.1). On July 15, we carried out an additional measurement at the bottom of the pond (depth of approx. 2 m) and found a DO of 0.2 mg L^{-1}. The DO at the surface was in a good range that day. Therefore, the pond was aerated to remove the stratification. On 16th and 17th we could not find signs of stratification. On 19th July we measured DO under 0.2 mg L^{-1} at the bottom. The data of our continuous measuring device indicates that, aeration of the pond successfully removes stratification, but lowers the DO of the surface. The lowest concentrations are typically found in the morning hours. However, due to aeration, DO levels can also drop around lunchtime or in the afternoon (Fig.1).

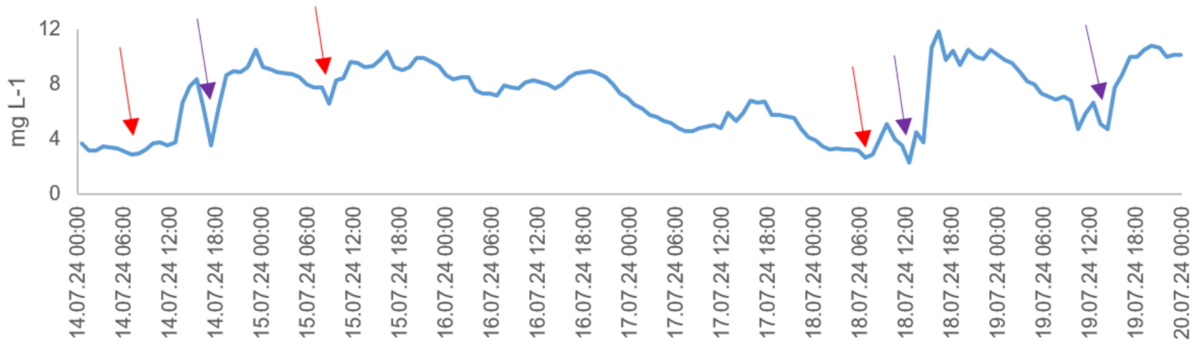

Fig. 1: DO between 14.7. – 19.7.2024. Red arrows mark the lowest concentrations in the early morning hours, which is common due to photosynthetic activity of the algae. Purple arrows mark the effect of the aeration (mixing the layers lowers the DO at the surface).

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Literature

Böhm, M., & Bauer, C. (2015). Changes in water parameters in carp ponds of northern Austrian (Waldviertel) over the last 30 years. Journal of Applied Ichthyology, 31, 15-17.

Dunham, R. A., Chatakondi, N., Nichols, A., Chen, T. T., Powers, D. A., & Kucuktas, H. (2002). Survival of F2 transgenic common carp (Cyprinus carpio) containing pRSVrtGH1 complementary DNA when subjected to low dissolved oxygen. Marine Biotechnology, 4, 323-327. https://doi.org/10.1007/s10126-002-0025-x

Schäperclaus, W., & Lukowicz, M. (2018). Lehrbuch der Teichwirtschaft, 5., aktualisierte Auflage. Eugen Ulmer KG, S. 680

Steffens, W. (1958). Der Karpfen. A. Ziemsen.

OUTCOMES OF A SHORT-TERM DIETARY TRYPTOPHAN INTERVENTION UPON VACCINATION IN A TELEOST FISH MODEL

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Tryptophan has been shown to play several roles in the modulation of fish stress and immune responses, and might serve as a complementary strategy alongside vaccination in aquaculture. The present study evaluated the synergistic effects of dietary tryptophan supplementation and vaccination in European seabass (*Dicentrarchus labrax*) juveniles' immunity and resistance to infection with *Tenacibaculum maritimum*.

European seabass juveniles (26.23 ± 7.22 g) were randomly distributed into three recirculating seawater systems. In a complete randomized design, fish were fed a control diet (CTRL) and two CTRL-based diets supplemented with tryptophan (TRP1 0.05% DM and TRP2 0.5% DM) in triplicate tanks of each system for 3 days. Fish from system 1 were then sampled for blood, liver, and head-kidney (0h). The remaining fish from system 1 were bath vaccinated and similarly sampled after 1 and 6 hours and 21 days. Fish were fed the same diets for 3 days more and after that, all fish were fed the CTRL diet until the end of the trial (21 days). Fish from system 2 were left undisturbed until the end of the trial, but were subjected to the same feeding strategy. Fish from system 3 were subjected to a bath challenge with *T. maritimum* (5 x 106 CFU mL^{-1}) and cumulative mortality was monitored daily for 10 days.

Tryptophan surplus for 3 days did not significantly affect disease resistance. However, results suggest that tryptophan surplus may induce variable physiological effects. Non-vaccinated fish fed TRP diets presented higher hepatic superoxide dismutase (SOD), elevated plasma cortisol and down-regulation of immune-related genes, in a dose-related manner. These results suggest that TRP diets per se, might have triggered regulatory mechanisms by increasing cortisol production.

At 6 h post vaccination, TRP1 dietary treatment inhibited SOD activity and plasma cortisol levels. In addition, the expression of head-kidney tryptophan hydroxylase was downregulated, while plasma total IgM levels and humoral immune parameters were enhanced. These data could indicate a beneficial role of the low tryptophan supplementation level (TRP1) upon vaccination, enhancing humoral responses while counteracting stress-associated cortisol increase.

Regarding the long-term synergistic effects of a 3 days-tryptophan supplementation with vaccination (sampling at 21 days), fish fed TRP2 showed lower expression of indoleamine 2,3 dioxygenase and plasma alternative complement pathway activity compared to TRP1. However, when vaccinated fish were provided with a lower level of tryptophan supplementation (TRP1) their molecular profile was more similar to that of CTRL-fed, non-vaccinated fish. Despite no signals of increased vaccination efficiency were verified (plasma IgM levels), results suggest that tryptophan might at least have a homeostatic effect on the immune system of fish subjected to an acute stress such as a bath vaccination procedure.

Overall, the findings suggest a complex interplay between dietary tryptophan supplementation, immune responses, and immunization mechanisms in fish.

This work was supported by IMMUNAA project (PTDC/CVT-CVT/7741/2020), financed through Fundação para a Ciência e a Tecnologia (FCT). DP, IC, MM, BC, and RA were supported by FCT, Portugal (UI/BD/150900/2021, 2021.04867.BD, 2022.03304.CEECIND, 2020.00290.CEECIND and 2022.03248.CEECIND, respectively).

EVALUATING NON-INVASIVE BIOMARKERS FOR ASSESSING RESPONSES TO ACUTE TRANSPORT STRESS IN ATLANTIC SALMON *Salmo salar* SMOLTS

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In the context of consumer concerns over animal welfare accompanying a growing demand for premium, sustainable animal protein, current research is concentrating on the evaluation and development of novel non-invasive tools to assess animal health.

For this, Atlantic salmon (*Salmo salar*) smolts, reared under standard conditions and weighing an average of 79 g, were subjected to a 6-hour transport at a density of 23.5 kg.m^{-3}. At several time-points 18 fish were sampled, before transport (control), immediately after (6-hours), and after a 24-hour recovery period. Proteomics and transcriptomics (miRNA) analyses will be performed on both skin mucus and water samples. Standard samples were also collected: liver samples to examine oxidative stress responses and plasma for metabolites and humoral immune parameters. Skin tissue samples were also collected for RNAseq. Results from skin mucus and water samples will allow to infer correlations between minimally invasive and non-invasive biomarkers.

Oxidative stress biomarkers in the liver showed significantly decrease in lipids peroxidation 24 h post-stress, as well as higher redox equilibrium. In plasma metabolites, lactate levels also dropped significantly 24 h post-stress, indicative of healthy liver function and normal physiological activity. Immune parameters revealed decreased plasma antiproteases activity 6 h post-stress. The results also demonstrated elevated lysozyme and nitric oxide levels post-transport, indicative of an active innate immune response following acute stress. Regarding the oxidative and immune balance, the decrease in peroxidase levels reflects decreased need for oxidative damage control, highlighting a transition from an acute stress response to a recovery phase. Skin transcriptomic analysis revealed higher significance at recovery with more variables showing substantial changes in metabolic and immune activity at 24 h post-stress.

Together, the data highlights the resilience and adaptability of the fish to acute transport stress, providing insights into their physiological and immune mechanisms. Mucus and water analyses will be scrutinized for correlation with these findings.

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EFFECT OF BICULTURAL RAERING OF PIKEPERCH (*Sander lucioperca*) AND RUSSIAN STURGEON (*Acipenser gueldenstaedtii*) ON GROWTH AND FISH CONDITION PARAMETERS IN RAS

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Intensive aquaculture employs bi- or polycultural methods inspired by pond aquaculture, where mixed fish stocks increase productivity and profitability (Turnšek et al., 2019). Polyculture, a technique according to Dalsgaard et al. (2013)building, and operating intensive land-based RAS for different species. This study compiles and assesses published literature along with un-published hands-on experiences with rearing different species in RAS in the Nordic countries, including Atlantic salmon (Salmo salar, stands as a beacon in aquaculture, ingeniously harnessing the potential of multiple fish species to optimize productivity, resource utilization, and ecological sustainability.

Juvenile pikeperch [n=1224; total length (TL) = 131.5 ± 6.9 mm and body weight (BW) = 18.2 ± 2.7 g] and sturgeons [n=576; total length (TL) = 168.1 ± 12.5 mm and body weight (BW) = 18.2 ± 3.6 g] were stocked in five groups (with triplicates): pikeperch (P100) and Russian sturgeon (P0) monocultures, and three types of bicultures with different stocking ratios of pikeperch and sturgeon (P90, P80 and P70). The distribution of fish species varied across different groups. In group P100, all 120 fish were pikeperch. Group P90 consisted of 108 pikeperch and 12 sturgeons. Group P80 had a mix of 96 pikeperch and 24 sturgeons. In group P70, there were 84 pikeperch and 36 sturgeons. Lastly, group P0 was entirely stocked with 120 sturgeons. The Daily feed ratio 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), thermal growth coefficient (TGC), 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 (NH_3), and triglyceride (TAG).

Following the 84-day trial, there were no significant differences in fish biometric parameters (TL, SL, BW) among all tested groups, regardless of species (pikeperch: $F(3,360) = 1.0$; sturgeon: $F(3,360) = 1.0$; $p > 0.05$; SL: pikeperch: $F(3,360) = 3.7$; sturgeon: $F(3,360) = 0.2$; $p > 0.05$; BW: pikeperch: $F(3,360) = 1.5$; sturgeon: $F(3,360) = 0.2$; $p > 0.05$). Pikeperch was the species most affected by biculture compared to sturgeon at the end of the experiment. The final TL, SL, and BW differed depending on the treatments (ANOVA: $F(3,596) = 28.1$, $p < 0.01$ for TL; $F(3,596) = 29.5$, $p < 0.01$ for SL; $F(3,596) = 36.8$, $p < 0.01$ for BW). For all these parameters, the values for fish in P100 were lower than those in biculture Groups P90, P80, and P70 (Figure 1).

Pikeperch's SGR was lowest in Groups P100 and P90 and highest in Groups P80 and P70. No significant differences were observed in the SGR of Russian sturgeon among the groups. Pikeperch in Groups P80 and P70 had the highest TGC, while the lowest was in Groups P100 and P90. The highest TGC for Russian sturgeon was in the monoculture Group P0. Pikeperch survival rate ranged from 96.0% to 98.6% across all groups, with no mortality in sturgeon groups. Feed conversion ratio (FCR) was highest for pikeperch in Group P100 and for sturgeon in Group P90. Significant differences in hepatosomatic index (HSI) and Fulton's condition index (FSI) were found only within pikeperch groups. No significant differences were found in somatic indices for both species. Caudal fin erosion was most pronounced in pikeperch from the P100 group.

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Acknowledgements
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References

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., Pedersen, P.B., 2013. Farming different species in RAS in Nordic countries: Current status and future perspectives. Aquacultural Engineering 53, 2–13. https://doi.org/10.1016/j.aquaeng.2012.11.008

Turnšek, M., Morgenstern, R., Schröter, I., Mergenthaler, M., Hüttel, S., Leyer, M., 2019. Commercial Aquaponics: A Long Road Ahead, in: Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M. (Eds.), Aquaponics Food Production Systems. Springer, Cham, pp. 453–485. https://doi.org/10.1007/978-3-030-15943-6_18

REDOX EFFECT ON NUTRIENT SOLUBILISATION IN MARINE AQUACULTURE SLUDGE

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In aquaculture, recirculated aquaculture systems (RAS) show potential circularity due to their capacity to reutilize water and concentrate nutrient-rich effluents While successful implementation of circular production has been demonstrated in freshwater RAS with aquaponic systems, 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. Their efficient uptake and assimilation of key nutrients, including nitrogen, phosphorus, and carbon, align closely with the nutrient composition of RAS effluents. Despite nitrogen's proven bioavailability for algae cultivation from RAS overflow water, the bioavailability of phosphorus, carbon, and micronutrients in RAS sludge remains a significant hurdle. While carbon recovery from waste has been explored, the recovery of dissolved phosphorus has not been fully elucidated.

This study investigates the redox effect 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 to support *Ulva sp.* growth. Key parameters, including ORP, dissolved oxygen, various nitrogen and phosphorus species, iron, manganese, acetate, and chemical oxygen demands, were monitored to understand the effects of redox on their solubilisation. The nutrients were chosen as the nutrients affecting the most *Ulva* growth based on the literature.

The outcomes of the study, currently undergoing analysis, will be showcased in the forthcoming presentation.

SMART-IMTA, A SYSTEM FOR MONITORING AND ADAPTION IN REAL TIME FOR INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA), COMBINING PRODUCTION OF SOLE AND MACROALGAE

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In a scenario where global food systems are being challenged due to the expected population growth, together with resource impoverishment and other environmental constraints, seafood has been identified as a vital source of food and a key component of a healthy diet. Nonetheless, decades of unsustainable overfishing practices are depleting aquatic ecosystems at a time when nearly one-fifth of all animal protein consumed by humans comes from seafood, reason why aquaculture has gained traction over wild fisheries. However, intensified aquaculture raises environmental and resource-related questions, mainly due to waste-streams, dependence on wild fisheries for aquafeed, disease outbreaks and the potential introduction of invasive species resulting from escapes (for instance in case of near-shore systems) in ecosystems where they do not belong.

INNOAQUA's main objective is to pave the path towards the upcoming sustainable and diversified EU land-based aquaculture industry by leaning on the demonstration and mainstreaming of innovative algae-based food and solutions, using ecology, circularity and digitalization approaches.

This presentation deals with two specific goals of the project, a) to implement an ecosystem approach for sustainable management of aquaculture production and b) to demonstrate tools to limit the waste in aquaculture cultivation and processing. In practical terms, these objectives are achieved through the implementation of an IMTA system at SEA EIGHT's commercial hatchery in northern Portugal. An area with over 200m^2 of macroalgae productive area (mainly for *Ulva* and *Gracilaria*) is being installed by A4F, to handle approximately 40m^3 of effluent from SEA EIGHT's Recirculated Aquaculture System (RAS). The SMART-IMTA includes the development and implementation of two main digital innovations by INESC TEC: (i) a novel Multisensing Module allowing for real time water quality monitoring and; (ii) a Digital Twin Backbone (DTB) that will facilitate the ingestion, transformation and storage of the data flows and make them accessible to other processes. Ultimately this technology will allow the management of the macroalgae production component without the permanent action of a seaweed expert, contributing significantly towards the implementation of the IMTA concept in other commercial RAS.

GRINNAQUA: GREEN INNOVATION STRATEGIES FOR ANIMAL HEALTH MANAGEMENT: TOWARDS SUSTAINABLE AQUACULTURE

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GRINNAQUA aligns strategically with the Farm-to-Fork Strategy to enhance CIIMAR's research capabilities, its innovation, and secure its leadership in the aquaculture industry. The collaborating with prominent European aquaculture research institutions, each with specialized expertise, will elevate CIIMAR's competitiveness. INIA-CSIC, in Spain, excels in vaccination techniques and fish acquired immunity. The University of Bergen, in Norway, is renowned for its expertise in animal health, while the Roslin Institute at the University of Edinburgh, in the UK, brings exceptional knowledge in genetics and animal breeding.

This collaborative initiative leverages the combined scientific and technical resources of all involved institutions, focusing on urgent challenges in the aquaculture industry. The project aims to explore the preventive potential of functional feeds in protecting rainbow trout against haemorrhagic septicaemia virus and safeguarding Atlantic salmon from sea lice infestations. To elevate CIIMAR's excellence, the partnership will also implement specialized training sessions and immersive summer schools.

In summary, the GRINNAQUA project is pivotal in enhancing CIIMAR's innovative capacity and establishing it as a leader in the blue revolution of the aquaculture industry. This enduring partnership is expected to create synergies that significantly strengthen a more resilient and sustainable aquaculture landscape across Europe.

Keywords: Genetics, Vaccination strategies, Animal welfare, Collaborative project, functional feeds

THE FIRST GREEN SHIPPING CORRIDOR FOR THE AQUACULTURE INDUSTRY IN SOUTH AMERICA – A CASE STUDY OF THE SALMON INDUSTRY IN CHILE

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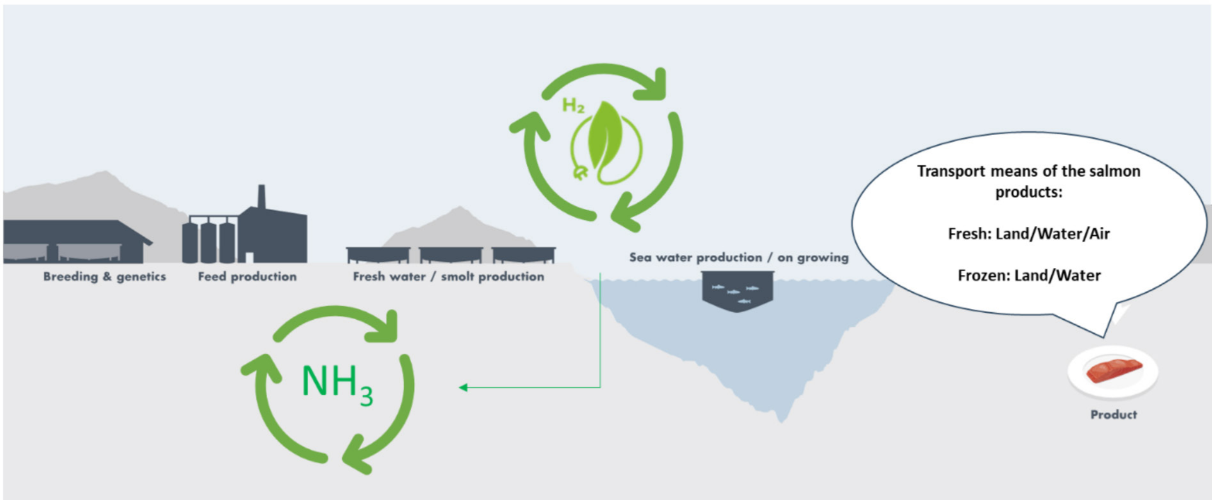
The green shipping corridors have been mostly considered for interoceanic transport of substantial amounts of goods, related to the use of large cargo capacity vessels, which is the most efficient transport mean to achieve it. Although efficient, sometimes without any regard to the necessary logistic chain to fill the ports with goods for these large vessels to be completely loaded, which is the most efficient condition considered. The logistic considering small feeder vessels, even land transport with enormous quantities of trucks.

The aquaculture industry in Chile represents the second larger productive industry overall yet the quantity of goods is not completely transported by sea, most of it is transported by land and air yet part of the production chain is completely involved in the water transport. Specifically, the transport of salmon, at different growth ages, from and to distinct parts such as piscicultures and fish farm centers. This process happening within local areas instead of open sea, which make it even more important because the impact of shipping is possible to see it locally in nearby coastal population areas, where the effects of greenhouse gases and contaminants (particle emissions) is quite significant especially in the health of people. Therefore, needs to be mitigated. This case study presents the commitment, the gaps found, and the results for the salmon industry in Chile that aims to decarbonize and to mitigate the effects of GHG and particle emissions, through the implementation of the first green shipping corridor for the aquaculture industry and the development of the first barge to transport green hydrogen first and the development of a barge to transport green ammonia. The shipping industry has been stated as one of the most difficult industries to be decarbonized, and this study case presents how necessary and important is to achieve it from the inland waterways to the open sea.



POSITIVE EFFECT OF MARINE PROBIOTIC SUPPLEMENTATION ON WHITE SHRIMP LARVAE SURVIVAL RATE AND ITS POSITIVE IMPACT ON MICROBIOTA AFTER AN AHPND STRESS

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The aquaculture industry is continuously interested in innovative practices with the aim of improving farm practices while ensuring sustainable development. This is reflected in a global demand for quality shrimp and a better environment. One of the key strategic priorities is to improve water quality by reducing organic matter in ponds. Organic matter is the source of ammonia, nitrite, nitrate and hydrogen sulfide for pathogen development. The *Bacillus* strains are known for their high levels of enzymes that are able to degrade organic matter, colonize the water column and therefore to reduce the bacterial load and consequently improve shrimp health. In this study, we evaluate the potential of marine probiotics as bioremediators and in feed supplementation but above all their resistance capacity against a *Vibrio parahaemolyticus* infection by reducing shrimp mortality and their impact on larvae and water microbiota.

A 7-week supplementation with a bacteria consortium added in water was performed in 800-liter ponds (n=1000, water temperature = 28°C) in triplicate on *Litopenaeus vannamei* PL-12. The physico-chemical parameters, mortality and weight were monitored during the supplementation phase. Larvae were then transferred in smaller ponds of 40L (n=60, 3 replicates) prepared during 2 days by inoculating water probiotics and challenged for 15 days with a *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND). The capacity of the bacteria consortium composed of three *Bacillus* strains on lithotamne support on shrimp survival rate was evaluated and demonstrated a survival rate improvement of +50% at the end of the challenge test compared to the control who did not receive probiotic supplementation. A metagenomic analysis was done by metabarcoding on water during the supplementation phase to see the impact of the probiotic on the composition of sea water. Aquatic probiotics can also have an impact on the microbiota's gut through the ingestion of water and organic matter by shrimp larvae. The variation in the microbiota gut composition was also analyzed. The results show the ability of the probiotic to colonize the water column and influence the surrounding microbiota. Metagenomic analyses were also done on dead and alive challenged larvae and a smaller quantity of the *Vibrio* family was found with a colonization of probiotic bacteria. This study demonstrated the ability of marine probiotics added to water ponds to help shrimp larvae fight against stress caused by *V. parahaemolyticus*.

A NOVEL ADDITIVE SPERMIDINE ENHANCES BREAKDOWN OF VISCERAL FAT IN *Salmo Salar*

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It is a major challenge to maintain healthy fish and high welfare standards, while also increasing the environmental sustainability of salmon feeds. Every year over 10 million Atlantic salmon deaths are reported from the marine phase, resulting in over £1.6 billion losses globally. Mass mortality events at this stage are increasing in all farming nations year on year associated with a range of infectious diseases and spectrum of different pathogens.

While farmed salmon were traditionally fed diets rich in fish oil, currently most of it is replaced with vegetable oil. Vegetable oils have more short chain n-6 Polyunsaturated Fatty Acids than fish oils, which promotes tissue adipogenesis leading to deposition of fat depots in the salmon viscera. The polyamine spermidine enhances life span in various species via upregulation of autophagy, a cellular mechanism of protein and lipid breakdown. It is cardioprotective, improves ß- oxidation and boosts the antiviral response in aging mice. In juvenile salmon, dietary supplementation of arginine, which leads to spermidine biosynthesis, improves metabolic status by enhancing ß- oxidation of long chain fatty acids. We hypothesized that adding spermidine to a vegetable oil enriched salmon diet will enhance the breakdown of lipids through autophagy, leading to a range of health benefits.

We fed salmon with a soy oil rich diet (80:20, soy oil: fish oil) supplemented with 5mg/Kg of spermidine. After 5 weeks, we measured free fatty acids (FFAs) in the visceral fat. FFAs were measured using HPLC coupled with a Triplequad mass spectrometer. Analysis was performed with Multiple Reaction Monitoring in negative mode, with at least two mass transitions for each compound. Quantification of FFAs was obtained using Supelco 37-component FAME-Mix in relation to deuterated internal standards.

Our results show an increase in the release of long chain FFAs and n-3 FFAs in adipose tissue from visceral fat (Figure 1, A&B). Furthermore, we observed upregulation of Carnitine Palmitoyltransferase 1A (CPT1A) in the white muscle (Figure 1, C&D). CPT1A is a mitochondrial inner membrane enzyme required for the transport of FFAs to the mitochondria for ß- oxidation.

We propose that addition of spermidine in salmon diets can be immunomodulatory by providing ATP for active immune cells and release of desirable omega-3's which are anti-inflammatory.

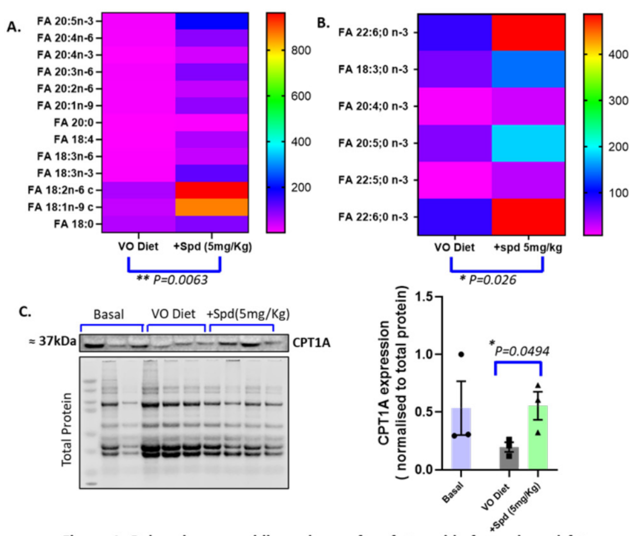

Figure 1. Polyamine spermidine releases free fatty acids from visceral fat (adipose tissue) (A) Long chain free fatty acids, (B) n-3 free fatty acids, (C & D) Enhanced expression of Carnitine Palmitoyltransferase 1A (CPT1A) in muscle. (N=3). ***VO= Vegetable oil, +Spd= VO diet supplemented with spermidine.***

ACCOUNTING FOR A MIXTURE OF DIPLOID AND TRIPLOID FISH IN GENETIC EVALUATION

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Induced triploidy is used in various fish and shellfish species to sterilize and improve growth and/or flesh quality in commercial productions, as well as to limit the risk of genetic introgression of farmed genotypes into wild populations. In particular, rainbow trout production of large fillets is based on triploid fish in fresh water. Selective breeding programs on sib performances are exclusively performed on diploid information. Maximizing genetic gain requires the evaluation of breeding values of the diploid candidates based on triploid sibs' performances. In mixed-family designs, this implies genotyping triploids and recovering their pedigree. Until recently, no reliable and open source tools were available to do so. We thus developed methods and R packages for genotype calling (https://cran.r-project.org/web/packages/GenoTriplo/index.html) and parentage assignment of triploids (https://cran.r-project.org/web/packages/APIS/index.html) to properly answer these issues. The relationships between progeny and parents are affected by the triploid nature of offspring as triploid fish inherit two copies of DNA from the mother and one from the father. Therefore, the genetic evaluation gathering information from both diploids and triploids must also account for the various allelic dosages in the relationship matrix used to run a BLUP animal evaluation. This issue was solved in the current study in order to estimate genetic parameters.

Two sib batches (one diploid, one triploid) of ~1,500 fish each were genotyped and phenotyped for production traits at 18 months. They came from the same parental cohort (190 dams × 98 sires) produced by Milin Nevez (Bretagne Truite, France). The BLUPF90 software was used for the genetic evaluation based on a bivariate animal model integrating the inverse relationship matrix provided by the R package 'polyAinv' (Hamilton & Kerr, 2019) that allows various ploidy levels. Heritability of diploid and triploid performances as well as their correlations were calculated using an AIREML algorithm and either a full diploid genetic model (wrong model) or a correct model accounting for the right ploidy level of any offspring. While the heritability values derived for diploid performances were similar for the two models (see Table below), the heritability estimates were largely overestimated for triploids under the wrong model. Using the correct genetic model, heritability estimates were very close between diploid and triploid performances. Genetic correlations were less impacted by the genetic model used, and were high in any case ($r_g > 0.70$).

	Under wrong model for triploids			**Under correct model**		
Trait	**h^2 diploid**	**h^2 triploid**	**r_g**	**h^2 diploid**	**h^2 triploid**	**r_g**
Weight	0.25±0.05	0.42±0.06	0.69±0.12	0.25±0.05	0.25±0.04	0.72±0.13
Head weight	0.22±0.05	0.41±0.06	0.70±0.13	0.22±0.05	0.24±0.04	0.73±0.13
Viscera weight	0.46±0.06	0.70±0.07	0.80±0.07	0.45±0.06	0.42±0.05	0.80±0.08
Headless gutted carcass weight	0.25±0.05	0.42±0.06	0.70±0.12	0.25±0.05	0.25±0.04	0.74±0.13
Total fat - Fatmeter	0.66±0.07	0.86±0.07	0.98±0.03	0.62±0.06	0.57±0.05	1±0.00
Fillet fat content	0.66±0.07	0.87±0.07	0.97±0.03	0.63±0.07	0.55±0.05	0.98±0.03
Subcutaneous fat surf.	0.58±0.07	0.60±0.07	0.93±0.05	0.55±0.07	0.39±0.05	0.95±0.05

Given these high correlations estimated between diploid and triploid performances, selection based on diploid information is efficient to improve a commercial production with triploid fish.

FOURTEEN CANDIDATE GENES GOVERNING SPONTANEOUS SEX-REVERSAL IN XX-RAINBOW TROUT

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Introduction

Revealing the molecular mechanisms of sex determination and differentiation may have important implications for research on reproductive development, sex-ratio control, and selective breeding in fish. Although phenotypic sex in rainbow trout is primarily controlled by a XX/XY male heterogametic sex determination system, spontaneous masculinization of XX fish is a phenomenon repeatedly observed at low frequencies. This spontaneous sex-reversal is a highly heritable trait and several QTL were detected (Fraslin et al., 2020), highlighting the existence of minor sex-determining genes that are independent of the major determinant carried by the sex chromosome (Omy29). In particular, two QTLs were located on Omy1 with a strong evidence while two other QTL were identified on Omy12 and Omy20. Variants from these four QTL regions were kept for further investigations in the present study using the new reference genome USDA_OmykA_1.1.

Materiel and methods. Sequenced samples

The initial data set was produced by Fraslin et al. (2020), and the 4 QTL detected in the previous study were further investigated in the current work, using realignment against the new reference genome USDA_OmykA_1.1. Here we only considered the sequence data of 23 dams with at least 10 progenies evaluated for phenotypical sex and extreme proportions of sex-reversed offspring, either ≥25% or ≤6% of neomales among their XX daughters. **Genotyped Samples.** A set of 192 SNP located within the 4 QTL were selected to design two 96 SNP genotyping arrays using microfluidic real-time PCR Fluidigm Kasp chemistry. These arrays were used to genotype 315 fish from 6 diverse French populations of rainbow trout with at least 30 XX fish and a minimum of 8 neomales per line. **Statistical analysis.** An exact Fisher test was run for the discovery population as well as for each of the 6 validation populations using the R 'fisher.test' function to test the independence of genotypes at each SNP and phenotypic sex. SNPs were considered as significant when p_value < 0.005. To deal both with "large p, small n" issue and correlations among SNPs, we applied a machine learning method on the discovery data set using haplotypes of 80 subsequent SNPs using the R package 'RandomForest'.

Results and discussion

We confirmed on several commercial lines, the importance of several genomic regions as containing minor sex-determining genes involved in spontaneous sex-reversal in rainbow trout. The candidate genes based on positional and functional information are SYNDIG1, TLX1, HELLS and GBF1 on Omy1, HCN1 on Omy12, and ARFGEF3, AKT3, KHDRSB2, DST, CILK1, CSMD1, RPS6KA2, LRRC59 and CASKIN2 on Omy20. These results are consistent with a model of sex determination in which spontaneous female-to-male sex-reversal in rainbow trout is associated with genetic factors able to reduce germ cell proliferation and arrest oogenesis, as suggested by works in zebrafish and medaka (Siegfried et al., 2008; Nishimura et al., 2018). This study paves the way for identifying the main causal mutations responsible for sex-reversal of XX rainbow trout.

Acknowledgment

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References

Fraslin C., Phocas F., Bestin A., et al. (2020). Scientific Reports 10:17693.
Nishimura T., Yamada K., Fujimori C., et al. (2018). PLoS Genet. 14, e1007259.
Siegfried K. R. and Nusslein-Volhard C. (2008). Dev. Biol 324, 277–287.

EFFECT OF PRIOR FAMILIARISATION WITH THE TEST ARENA ON FEEDING BEHAVIOUR ASSESSMENT IN THE PACIFIC WHITE SHRIMP (*Penaeus vannamei)*

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Penaeus vannamei is one of the most bred crustacean species in the world. In farms, feed is, by far, the main expenditure. Feed and premix manufacturers aim to find affordable but attractive formula. However, the methods for measuring the attractivity of a feed remain poorly reproducible. One solution is to design a test arena and a standardised procedure allowing to automatically track shrimp during a feeding event and assess pellet attractivity. In this test arena, lowering the level of stress is paramount not to bias their feeding behaviour. In a previous study, we showed that the use of orange light induced less stress behaviours in shrimps than other colours (Pichon et al., in prep).

The objective of this study is to test the effect of prior familiarisation with the test arena on stress and feeding behaviour during the testing phase.

Thirty PL20 *P. vannamei* shrimp were divided into two groups of fifteen shrimp: the test group and the control group. The test group was familiarised with the test arena 1h per day over three consecutive days and was tested the following week. The control group was tested without prior familiarisation to the test arena. Each group consisted of five batches of three shrimp. The batches were randomly tested (once per day) with: no food, blank diet, blank diet + 0.5% KS50 or blank diet +1% KS50. KS50 is a functional amino acids mix obtained from poultry feather by the company BCF Life Sciences.

At the beginning of a test, the batch of shrimp was first acclimatised 15 min in the starting zone, they were then given access to the whole test arena and filmed for 20 min. The food was gently placed in a petri dish at the opposite of the starting zone, called the feeding zone (FZ), 1min before the end of the acclimation period. We measured shrimp's stress (loops and tail flips) and feeding (latency to reach the FZ, time spent in the FZ and number of transitions to the FZ) behaviours.

Careful evaluation of the effects of the different features of the test arena (e.g., light) and procedure (e.g., familiarization, acclimation, …) on stress behaviours will provide important insights to design a standardised procedure. This will allow an accurate assessment of feed attractivity in shrimp.

USE OF A SINGLE-CELL PROTEIN IN A MARINE FISH LARVAL DIET: EFFECTS ON GROWTH PERFORMANCE OF GILTHEAD SEABREAM (*Sparus aurata*) LARVAE

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In recent years, there has been a growing interest in finding innovative and eco-friendly protein sources for the aquaculture industry. Single-cell protein (SCP) has emerged as a promising candidate, particularly for juvenile fish diets. Formulating diets for larval stages, however, is inherently complex, making the incorporation of alternative protein sources not always a top priority. Therefore, research on its efficacy in larval fish diets is limited. Nonetheless, early inclusion of such ingredients could potentially enhance productivity during the juvenile phase and contribute to environmental stewardship. This study aimed to verify if SCP could be implemented as an effective protein source in larval diets without impacting the larval development of one of the most important cultured marine fish species in Europe, the gilthead sea bream (*Sparus aurata*).

Two different experimental co-feeding diets (D1 and D2, respectively) were formulated to have similar proximate composition. A SCP meal (FeedKind, Calysta, USA) was included as a novel ingredient in D1 at a level of 8%, while in D2, most of the protein sources originated from marine derivates as in diet D1, with an inclusion of a 5% vegetable protein source. Following pulverization and mixing of the dietary ingredients, agglomeration was performed using a laboratory scale spheronizer by atomization of a binder solution. The particles were dried at 60°C for 15 min followed by sieving to obtain 100-200 µm and 200-400 µm fractions of each diet.

Sea bream larvae were stocked at a density of 100 larvae.L^{-1} in 1000 L tanks, provided with water in a semi-closed recirculation system. The treatments consisted of providing the fish with the two different diets in triplicate tanks under a standard feeding regime, and being maintained under optimal conditions for this species.

As can be seen in Table 1, the performance of the fish in terms of survival, standard length, individual wet weight, and overall tank biomass was similar between the two diets. However, interestingly, the D1 group resulted in a higher percentage of bigger fish in its population than D2. To the best of our knowledge, this is the first study that shows the potential of incorporating this innovative ingredient into diets designed for marine fish larvae. This demonstrates the potential of integrating SCP into the larval diets of economically significant marine fish species.

Table 1: Performance of gilthead seabream larvae fed with the experimental diets D1 and D2

Treatment	Average survival (%)	Average Ind. Standard length (mm)	Average Ind. Wet Weight (g)	Average of tank biomass (g)
Diet D1	39 ± 5.9%	15.07 ± 2.28	0.082 ± 0.009	2857 ± 624
Diet D2	43 ± 2.0 %	15.31 ± 1.5	0.070 ± 0.004	2830 ± 133

DISTRESS FOR COMMON CARP (CYPRINUS CARPIO L.)

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Introduction

Stress can have diverse effects on fish, but the signs of stress in fish differ depending on the fish species, the type of stressor, the duration and the intensity of the stress. In addition, it is important to investigate the effects of stress on gene expression patterns at the brain level separately for different brain parts to respect their differences in functionality. The present study reveals that negative stressors have different effects on the gene expression patterns in four brain parts of common carp. As stressors chasing, confinement and air exposure were used for 1 min and the fish have been sampled 30 to 90 min after that to investigate the time course of stress responses. In addition, neurotransmitter levels in the different brain parts have been determined as well.

Material and methods

For the experiment investigating responses to acute stress, the fish were treated with air exposure, chasing or confinement for 1 min. Control groups were reared in parallel and left untreated during the entire experiment. Neurotransmitter analyses and qPCR analyses were subsequently used to analyze stress responses at the brain level. For the gene expression analyses, immediate early genes (indicating brain activity) and genes already known to belong to the stress axis were selected. The neurotransmitter analyses included serotonin and dopamine and their main metabolite, which were determined using commercial ELISA kits. The gene expressions investigated in the different brain parts have been used to build mixed models with a fully Bayesian approach using the *brms* package in R studio. Additionally, the gene expression data sets together with the neurotransmitter levels were subjected to principal component analyses (PCA) to show parameter clusters that correspond to the respective treatments of the fish.

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 the neurotransmitters. These results are compared to the insight that have been gained for the chasing and confinement treatment. A number of genes allows discrimination of between the stressors and will be presented. In summary, this study indicates that distress is perceived differently in carp at the brain level. The obtained results may help developing indicators for stress in the future.

Acknowledgement

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GROWTH PERFORMANCE OF THREE HALOPHYTE SPECIES IN AN AEROPONIC SYSTEM WITH DIFFERENT SALINITIES

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To apply an aeroponic system to a marine culture, it is necessary to use plants that have value as a cash crop and can develop in saline effluents. However, halophyte plants differ in their degree of salt tolerance. This study aimed to assess the growth of three halophyte species when irrigated with water in different salinities, and thus evaluate their potential for integration with aquaculture.

The aeroponic system was constructed in three 3-floor shelves, where 27 plastic boxes with lids were placed (30 x 40 x 22 cm, 0.12 m² of planting area, three boxes per floor). Inside of the boxes, six nozzles were installed on a ring of 16 mm pipes that were connected to the water reservoir. A commercial fertilizer was used as a nutrient source. Every hour, a pump was activated with a timer and the nutrient solution was sprayed on the roots of the plants for 5 minutes. Three salinities were tested (freshwater (0 ppt), 10 ppt and 20 ppt) for the growth of three species (*Salicornia europaea*, *Plantago coronopus* and *Crithmum maritimum*), with three replicates each. Six plants per box were placed in hydroponic baskets attached to the lids. The light intensity was 180 µmols photons.m^{-2}.s^{-1}, and the photoperiod was adjusted to 12h during the 6-week trial.

The increase in salinity impaired the growth of most plants, although *S. europaea* showed a favorable response to the salt in the nutrient solution (table 1). Nonetheless, its low productivity is possibly due to the quality of the light used, featuring a higher spectrum of red. This can promote the flowering of plants, which was observed in this study, thus reducing their vegetative growth. *P. coronopus* proved to be the most salt-sensitive among the species tested, presenting a substantial reduction in biomass under the 20 ppt treatment. Based on these findings, as far as salinity is concerned, *P. coronopus* and *C. maritimum* are viable species for integrated aquaculture up to 10 ppt, and *S. europaea* up to 20 ppt when a suitable light source is used.

Table 1. Growth indexes after 6 weeks of three halophyte species (*Salicornia europaea*, *Plantago coronopus* and *Crithmum maritimum*) cultivated in an aeroponic system with three salinities.

Parameter	Treatment		
	Freshwater	**10 ppt**	**20 ppt**
Salicornia europaea			
Fresh weight shoots (g)	1.2 ± 0.8^{a}	3.6 ± 2.4^{b}	3.7 ± 2.6^{b}
Shoot length (cm)	7.1 ± 0.8^{a}	8.2 ± 1.9^{b}	7.9 ± 1.7^{ab}
Root length (cm)	3.4 ± 1.0	4.7 ± 2.8	3.8 ± 1.9
Productivity (g.m^{-2})	59.0 ± 41.8^{a}	180.9 ± 117.7^{b}	184.7 ± 131.2^{b}
Plantago coronopus			
Fresh weight shoots (g)	36.8 ± 28.9^{b}	16.9 ± 14.4^{ab}	4.4 ± 4.8^{a}
Shoot length (cm)	25.3 ± 6.5^{c}	17.2 ± 4.6^{b}	12.4 ± 1.5^{a}
Root length (cm)	26.2 ± 18.7	25.4 ± 13.5	10.7 ± 7.4
Productivity (g.m^{-2})	1838.9 ± 1443.2^{b}	844.3 ± 720.9^{ab}	217.5 ± 239.6^{a}
Crithmum maritimum			
Fresh weight shoots (g)	4.8 ± 2.6^{b}	5.0 ± 1.8^{b}	2.0 ± 0.6^{a}
Shoot length (cm)	19.6 ± 4.9^{c}	13.5 ± 2.8^{b}	8.5 ± 1.6^{a}
Root length (cm)	29.0 ± 6.7^{c}	23.6 ± 4.8^{b}	11.6 ± 2.7^{a}
Productivity (g.m^{-2})	239.7 ± 130.7^{b}	247.6 ± 91.3^{b}	101.9 ± 31.7^{a}

Data are a mean ± standard deviation. Non-parametric ANOVA (Kruskal-Wallis). Different superscript letters in the rows represent a significant difference by the Dwass-Steel-Critchlow-Fligner test for pair-wise comparisons.

UNDERSTANDING THE EFFECT OF TEMPERATURE IN DIFFERENT STOCKING DENSITIES ON *Litopenaeus vannamei* REARING IN BIOFLOC TECHNOLOGY SYSTEM

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The present study evaluated the zootechnical performance of *L. vannamei* exposed to two different stocking densities at four different temperatures with a potential recovery period afterward.

L. vannamei juveniles were stocked with an initial weight of 1.16 ± 0.11 g, and the experiment was divided into two phases. In the first phase (42 days), the selected temperatures were 22, 25, 28, and 31°C, with two stocking densities used for each temperature: 200 and 600 shrimp/m³. In the second phase, all experimental units were adjusted to 31°C for 28 days to assess potential growth recovery in treatments previously exposed to sub-optimal temperatures.

At the end of the first phase, temperature was the determining factor for growth performance and it did not affect survival, while stocking density did not significantly influence any parameter. By the end of the experiment, shrimp kept in sub-optimal temperatures during the first phase could recover and grow at higher rates, although they did not reach the body mass of shrimp kept in optimal temperatures during the first phase. High stocking density negatively affected survival and feed conversion rate when shrimp reached around 8 grams.

In conclusion, *L. vannamei* can be cultivated at low and intermediate temperatures in high stocking density for a limited period without compromising the survival. Despite the low growth rates, the shrimp was able to regain weight when optimal temperature was reestablished. However, high stocking density can negatively affect the productivity when a high biomass is reached. Therefore, it is possible to use different stocking densities for each phase of grow-out, enhancing the productivity and not compromising the survival rates.

Table 1: Zootechnical parameters (mean ± standard deviation) of *L. vannamei* juveniles reared different temperatures and stocking densities (after phase 1 and 2. Same letters indicate absence of statistical differences ($p > 0.05$) among treatments within the same day.

Treatment	T22-D200	T22-D600	T25-D200	T25-D600	T28-D200	T28-D600	T31-D200	T31-D600
				1ª phase (day 42)				
Survival %	98.7 ±1.2^{a}	99.0±1.0^{a}	96.9±1.4^{ab}	99.6 ±0.8^{a}	100.0 ±0.0^{a}	97.1 ±3.0^{ab}	100.0 ±0.0^{a}	93.3±3.5^{b}
Weight (g)	3.6±0.7^{e}	3.7±0.4^{e}	4.6±0.5^{d}	4.9±0.3^{d}	7.1±0.3^{c}	7.1±0.8^{c}	9.0±0.1^{a}	7.8±0.2^{b}
SGR %	2.7±0.6^{d}	2.7±0.1^{d}	3.2±0.3^{c}	3.4±0.1^{c}	4.3±0.1^{b}	4.3±0.6^{b}	5.0±0.2^{a}	4.6±0.1^{ab}
AFCR	1.7±0.05^{a}	1.7±0.06^{a}	1.6±0.04^{b}	1.5±0.03^{b}	1.3±0.06^{c}	1.4±0.03^{c}	1.1±0.03^{e}	1.2±0.04^{d}
				2ª phase (day 70)				
Survival %	98.7±1.2^{a}	93.6±2.4^{ab}	98.0±2.0^{a}	88.4±0.8^{b}	100.0±0.0^{a}	90.9±2.3^{b}	100.0±0.0^{a}	87.6±4.4^{b}
Weight (g)	10.8±0.6^{c}	10.5±0.1^{c}	10.8±0.2^{c}	11.0±0.2^{c}	13.6±1.2^{b}	13.4±1.6^{b}	16.6±0.2^{a}	14.2±0.4^{b}
SGR %	4.0±0.5^{a}	3.8±0.3^{a}	3.0±0.5^{b}	2.9±0.3^{b}	2.3±0.3^{c}	2.3±0.2^{c}	2.2±0.1^{c}	2.1±0.1^{c}
AFCR	1.1±0.03^{cd}	1.3±0.06^{b}	1.2±0.03^{c}	1.2±0.05^{bc}	1.1±0.03^{d}	1.2±0.03^{bc}	1.1±0.05^{d}	1.4±0.04^{a}

COMPARING GROWTH AND WELFARE OF ATLANTIC SALMON POST-SMOLTS IN A NOVEL LAND-BASED COMMERCIAL HYBRID FLOW-THROUGH SYSTEM (HFS) VERSUS IN OPEN SEA PENS

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The Norwegian salmon sector often uses Recirculation Aquaculture Systems (RAS) to produce 500-700 g post-smolts, which are transferred to sea. This strategy shortens the seawater grow out phase in open net pens, thus helping to reduce the severity of problems like sea lice infestations, escapees or organic discharge in the environment. However, RAS are expensive and present a high biological complexity. In addition, RAS post-smolts can face difficulties adapting to sea water pens, potentially impacting their growth performance and mortality. As a result, some industry players are considering the use of Hybrid Flow-Through Systems (HFS) to produce post-smolts or market-size salmon. Using HFS aims to improve challenges resulting from the intense RAS conditions, yet still maintaining high control of the rearing environment. However, HFS are new systems, thus salmon growth and welfare in them must be assessed.

Our study aimed to document growth and welfare in a commercial batch reared at Bue AS HFS facility (Vestland, Norway), from smolt (90 g) in June 2023 to slaughter size (5 kg) in June 2024. In addition, this performance was compared with groups of salmon transferred from the HFS to two traditional sea water sites, at 1 kg on 16 October 2023 (to SW pens A) or at 2 kg on 10 December 2023 (to SW pens B). Five samplings were carried out, 3 at Bue HFS only, and 2 more (only one by abstract submission) in the three sites (n=15-20 fish per tank/sampling).

On 12 March 2024 (4th sampling), body weight was over 50% higher at the HFS than at the two sea water sites (Fig. 1), and SGR was almost double (Table I). Weight and SGR in sites A and B were similar. Welfare-wise, salmon in site B displayed more winter ulcers, fin damage and mortality than the in HFS and site A, which had similar values. Feed utilization (FCR) was also worse in site B. Mean temperature was similar in the three sites, while stocking density was highest in the HFS, medium in site B and lowest in site A (Table I).

The better fish growth in HFS might be explained by the absence of handling and disturbance, plus the controlled environment in a land-based system. Both groups A and B experienced transfer events that can induce stress, reduced growth, susceptibility to disease and mortality. In the case of site B, the transfer caused scale loss, more skin ulcers and mortality. In the case of site A, fish had the extra disturbance of one de-lousing treatment. In conclusion, lack of handling and a high-quality controlled environment (like in HFS) can boost salmon growth.

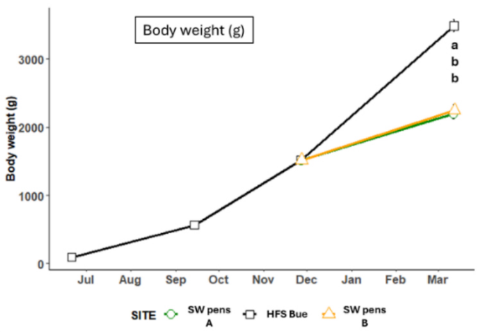

Figure 1. Mean body weight in the three sites over time. The graph displays means ± SEM. Letters “a” and “b” indicate significant differences between sites.

	Mean T (ºC)	Site transfers	De-lousings	St. density (kg/m3)	SGR (%)	FCR	Mortality (%)	% Relevant ulcers	% Dorsal damage	% Caudal damage
SW pens A	6.0	1	1	2	0.35	1.27	2.7	10	13	0
SW pens B	6.2	1	0	10	0.39	1.53	10.6	33	23	18
HFS	6.4	0	0	35	0.73	1.21	0.7	7	13	0

Table I. Production, performance and welfare parameters in the three sites between 16 Oct 23 – 12 Mar 24. Ulcers and fin damage (%) observed on 12 March 24 (sampling 4).

A COMPARATIVE MULTIDISCIPLINARY STUDY OF SENEGALESE SOLE (*Solea senegalensis*) PRODUCTION IN RAS OR IN IMTA-RAS WITH SEA LETTUCE (*Ulva ohnoi*) INCLUDING MICROBIAL MANIPULATION

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Integrated Multitrophic Aquaculture in Recirculating Systems (IMTA-RAS) mitigates the environmental impacts of fish production by coculturing macroalgae. *Ulva* spp. efficiently absorb nutrients, such as nitrate and phosphate, and transform them into biomass for food, feed, fuel, and bioactive molecules. Moreover, *Ulva*'s laminar blade offers support for beneficial bacteria, such as *Phaeobacter* spp., antagonists of fish pathogens. A strategy based on these bacteria has been developed at a laboratory scale (Pintado et al. 2023), maintaining *Phaeobacter* longterm on *Ulva* blade under low light or dark conditions.

The novelty of the present work is the analysis from a holistic perspective of a commercial-scale pilot scale IMTA-RAS co-culture of *Solea senegalensis* with *U. ohnoi* as a biofiltration system, and to test at that scale a fish health and welfare strategy based on the introduction of a *Phaeobacter* strain isolated from wild *Ulva*. Three systems, RAS, IMTA-RAS, and IMTA-RAS with *Phaeobacter*, were compared using a multidisciplinary approach. This included the analysis of: i) fish growth, welfare markers and quality, ii) *Ulva* growth and biochemical composition, and iii) water physicochemistry. Moreover, 16 rRNA metagenomic studies of the microbiota of fish (skin, gills, intestine), feed, *Ulva*, and the culture system (tank walls, biofilter, recirculating water) will be conducted.

In each system, Senegalese sole of around 85 g were allocated in three 1 m^2 parallel tanks (23 kg per tank) and fed a commercial diet 3 times/night at a 0.85% feeding rate. In IMTA-RAS, each fish tanks was connected to two *Ulva* tanks, allocated at a density of 3 g/L, one with light ($800 \pm 20\ \mu mol.m^{-2}s^{-1}$) in a 12:12 photoperiod and another in darkness. *Ulva* density and productivity was maintained by weekly harvesting, and the tank in darkness was switched to light and vice versa. All lots were sampled after an acclimation period (18 days) and at the end of the trial (8 weeks). Water parameters and nutrients were measured weekly.

The integrated analysis of the results will allow an understanding of the behaviour of the three systems, demonstrate the advantages of IMTA-RAS and assess the impact of introducing *Phaeobacter* on fish health and welfare. By operating IMTA-RAS systems, the aquaculture sector could diversify, decrease its environmental footprint, producing in a healthier way.

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BEHAVIOURAL PERFORMANCE OF TURBOT (*Scophthalmus maximus*) JUVENILES IN NOVEL ENVIRONMENT PARADIGMS

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Animal welfare concerns have gained increasing attention within the aquaculture industry, mainly to improve living conditions of farmed fish species and reduce stress, which can impact meat quality. Consequently, behavioural indicators of stress have emerged as valuable tools for assessing fish welfare, as they are non-invasive, easily implementable and generally effective in detecting early stress symptoms. However, there is a knowledge gap in the behavioural biology of several aquaculture species, which impedes their practical application. Hence, this study aimed to describe the behavioural response of the turbot *Scophthalmus maximus* to different novel environment paradigms, previously validated to evaluate fish stress responses.

Juvenile turbots (average length: 2.86 ± 0.45 cm) were acclimatised for 1 month at 18 ± 1 ºC in 8 L community tanks at Sparos facilities (Olhão, Portugal). Then, fish were randomly assigned to a control (CTRL) or stress (ST) treatment. Fish from each experimental group were individually subjected to one of the following behavioural assays: 1) Open field (OF), 2) Novel tank (NT) or 3) Scototaxis (SC) test (n= 16 fish/group/test). While CTRL fish were directly exposed to the behavioural assay, ST fish were subjected to an acute stress event prior to the behavioural testing. For each trial, fish behaviour was recorded for 10 minutes and different anxiety-like behaviours were measured using Ethovision XT software®.

In the OF test, a significant reduction in freezing behaviour was found for stressed fish (*t*-test, $p<0.05$; Fig. 1A), whereas no differences were observed for thigmotaxis behaviour. In the NT, neither stressed nor control fish displayed the expected fish bottom-dwelling response (*t*-test, $p<0.05$; Fig. 1B). However, stressed fish spent significantly more time in the upper part of the NT arena than control fish (*t*-test, $p<0.05$). Lastly, the SC test revealed that both the ST and CTRL groups displayed a preference for the light zone (*t*-test, $p<0.05$). Additionally, stressed fish registered reduced entries to the dark zone compared to control fish (*t*-test, $p<0.05$; Fig. 1C). Collectively, our findings revealed diverged responses among stressed and non-stressed turbot for all conducted assays, highlighting potential anxiety-like indicators for *S. maximus*. Moreover, the observed anxiety-evoked responses of turbot to these assays were generally opposite to those observed in other fish model species, such as the zebrafish *Danio rerio*. Overall, this study provides novel insights into the behavioural research of turbot, a commercially important species, denoting species-specific responses to novel environment paradigms, which might be valuable for improving the welfare assessment of this species as well as enhancing its welfare conditions in aquaculture settings.

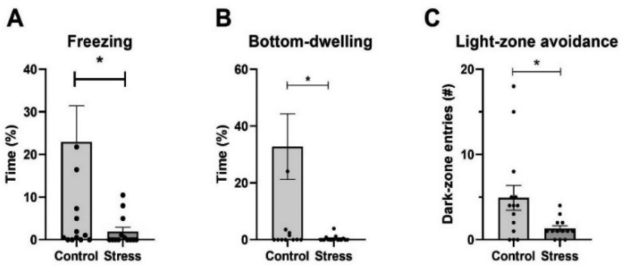

Figure 1. Anxiety-like behaviours of turbot *Scophthalmus maximus* in the **A.** Open field, **B.** Novel tank and **C.** Scototaxis test. Data are presented as mean ± SEM and asterisks indicate statistical differences between stressed (red) and control (grey) fish by means of *t*-tests.

CAN BILLIONS OF BLUE MUSSELS *Mytilus edulis* CONTRIBUTE TO ECOSYSTEM SERVICE AND SUSTAINABLE FOOD AT THE SAME TIME. BENEFITS, CHALLENGES AND MYTHS

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We are facing several global crises in the area of climate, biodiversity and food. Blue mussels have great potential to counteract these crises with little or no disadvantages. EU with Green Deal and Farm to fork consider the blue mussel production as an important tool in the green transition of the food system, although several stakeholders see mussel production as a huge polluter. This presentation will focus on the blue mussel production in Denmark, challenges related to legislations and permissions, and environmental impact and benefits. To assess environmental impacts, the results from a new study will show how biodiversity, and oxygen consumption is impacted by two mussel farms in poorly circulated water areas and by two mussel farms in good circulated water areas. All four farms in the nutrient-rich Limfjorden in Denmark. The results are used to define recommendations for policy makers based on ecosystem and biomass related considerations.

STERILITY AS A FOUNDATIONAL TRAIT FOR AQUACULTURE

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Aquaculture is growing to meet the needs of a growing world population, and aquaculture genetics must move quickly to help producers meet the expectations of stakeholders. To responsibly introduce animals that harness the power of genetic improvement into commercial production systems, the farmed fish should be sterile. The Atlantic salmon industry is an example where concerns over mixing of escapee farmed fish with wild relatives has disrupted industry growth.

The ability to produce sterile fish for commercial grow out provides a solution to concerns over genetic contamination from farmed fish that might escape and mate with wild relatives. This solution may allow expansion of farming concessions and the ability to focus on genetic improvement innovations in broodstock, mitigating regulatory concerns. Farming sterile fish would also provide benefits in improved growth and feed efficiency, and reduced aggression and stress. Finally, farming sterile fish may open up farming areas where concerns over establishment of feral populations prevents expansion.

Genome editing offers the opportunity to make targeted changes in the genome, introducing genetic variation that will result in rapid and substantial improvements in performance, health and sustainability. We describe here a genome editing approach to generating 100% sterile fish for commercial aquaculture.

INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) IN THE ATLANTIC

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Introduction

IMTA is an innovative option for enhancing the sustainability of aquaculture. By culturing a combination of low trophic and higher trophic species, the co-products of one crop are converted into fertilizer, food and energy for the lower trophic species. IMTA is shown to increase circularity and reduce waste from fed species and lower the nutrient load in the water (FAO, 2018). IMTA systems can reduce environmental impacts, diversify and increase production, lower investment risks, create jobs, increase consumers' trust, as well as support sustainable aquaculture and the circular bioeconomy (Chopin, 2015).

Methodology

RAS Biofloc System Brazil, during super-intensive shrimp production without water renewal, there is an accumulation of nutrients. The integration of IMTA species consuming biofloc (tilapia and oysters) and dissolved nutrients (seaweeds and halophytes) has been investigated to diversify products and maintain water quality. **Land-based Pump Ashore System South Africa** cultivates abalone in raceways with the green alga, *Ulva*, grown in adjacent interconnected paddle raceways using abalone effluent. *Ulva* serves as a biofilter allowing for increased water re-circulation and is used as a supplementary abalone feed. Sea urchins (*Tripneustes gratilla*) have also been trialled in this system. **Open-Water System Scotland** optimises cultivation techniques of macroalgae and shellfish, kelps and native oysters. The development of new cultivation systems explores options to minimise cultivation wastes through improved system design and reducing, reusing, and recycling polymer-based cultivation materials. **Open-Water System Ireland** explores the feasibility of the cultivation of Atlantic salmon, cleanerfish, European lobster, native oysters, scallops, seaweeds and spiny sea urchins in one IMTA system. Production technologies are assessed and optimised to enhance profitability and to mitigate environmental impact. **Prospective IMTA Argentina**, feasibility studies were carried out to assess local species (fish, crustacean, mollusc and echinoderm) and to identify appropriate sites within the Beagle Channel to facilitate IMTA.

Results

ASTRAL is examining the potential of these IMTA value chains throughout the growing seasons to establish criteria for optimal production conditions. Monitoring continues to establish baseline data for better yield and profitability, reduction of environmental impacts and the reduction of waste.

References

Chopin, T. 2015. Marine Aquaculture in Canada: Well-Established Monocultures of Finfish and Shellfish and an Emerging Integrated Multi-Trophic Aquaculture (IMTA) Approach Including Seaweeds, Other Invertebrates, and Microbial Communities. Fisheries 40, 28-31.

FAO. 2018. The State of World Fisheries and Aquaculture 2018. CC BY-NC-SA 3.0 IGO.

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PACIFIC WHITE SHRIMP, NILE TILAPIA, AND MACROALGAE INTEGRATED IN A BIOFLOC SYSTEM UNDER DIFFERENT FISH-FEEDING RATES

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The integrated multitrophic aquaculture can reduce the surpluses of solids and inorganic nutrients that tend to increase over time in a shrimp biofloc system, since the model integrates organisms from different trophic levels, all sharing the same production system and, consequently, the nutrients. Tilapia and macroalgae play an essential role in this system due to the ability to use flocs as a feed source and uptake inorganic nutrients, respectively. This study evaluated the effect of two feeding rates (1 and 3% of body weight day^{-1}) of tilapia *Oreochromis niloticus*, as an organic consumer, integrated with shrimp *Penaeus vannamei* as the main species and macroalgae *Ulva lactuca* as an inorganic consumer in a biofloc system regarding water quality, sludge produced, and performance of the organisms and system. The study was carried out for 52 days in a greenhouse at the Marine Aquaculture Station - FURG. The study consisted of an experiment with two treatments and three replicates each: 1) 1% body weight day^{-1} and 2) 3% body weight day^{-1}. Shrimp (4.28 ± 0.12), fish (16.98 ± 0.92), and macroalgae were stocked in 14, 4, and 4 m^3 tanks of useful volume, respectively, at a density of 230 shrimp m^{-2}, 30 fish m^{-3}, and 0.13 kg of macroalgae m^{-3} (considering the total volume of the system). Macroalgae were placed in a shallow float within 10 cm from the surface. The system remained in constant recirculation whereby water circulated from shrimp tank to fish tank through a submerged pump. Then, by gravity, water flowed to macroalgae tank and returned to shrimp tank. Dissolved oxygen levels were maintained above 5 mg L^{-1}, temperature remained around 29 °C, and salinity was maintained at 20 g L^{-1}. All water quality and shrimp performance parameters were unaffected ($p > 0.05$), but fish fed with 3% of body weight day^{-1} exhibited higher values for all growth performance parameters ($p < 0.05$), excluding survival ($p > 0.05$) (Table 1). Macroalgae growth was affected ($p < 0.05$), showing lower values in the treatment with the highest feeding rate. Total sludge produced in the highest feeding rate treatment was higher than that in the lowest ($p < 0.05$), but it did not result in higher sludge produced per organism biomass ($p > 0.05$). Increasing the fish feeding rate favored the final biomass and yield of the multitrophic system and did not affect the system conversion, generating an economic gain.

Table 1. Mean and standard deviation of growth performance for 52 days of study in a multitrophic system with biofloc stocked with 230 *Penaeus vannamei* m^2, 0.7 kg of *Ulva lactuca* m^{-3}, and 30 *Oreochromis niloticus* m^3 fed with 1 and 3% of body weight day^{-1} with three replicates.

	1% of body weight day^{-1}	3% of body weight day^{-1}
Shrimp		
Final weight (g)	11.38 ± 2.77	11.76 ± 3.14
Survival (%)	87.88 ± 2.83	85.22 ± 5.65
Weekly growth (g week^{-1})	0.96 ± 0.09	1.01 ± 0.08
Feed conversion ratio	2.00 ± 0.09	2.01 ± 0.09
Final biomass (kg)	45.93 ± 1.75	46.02 ± 1.59
Tilapia		
Final weight (g)	52.81 ± 16.90^b	99.79 ± 35.92^a
Survival (%)	93.33 ± 1.67	88.33 ± 3.00
Specific growth rate (% day^{-1})	2.18 ± 0.09^b	3.40 ± 0.10^a
Feed conversion ratio	0.47 ± 0.03^b	0.85 ± 0.05^a
Final biomass (kg)	5.92 ± 0.36^b	10.59 ± 0.91^a
Ulva lactuca		
Initial biomass (kg)	2.80 ± 0.0	2.80 ± 0.0
Final biomass (kg)	6.43 ± 0.41^a	5.69 ± 0.21^b
Specific growth rate (% day^{-1})	1.61 ± 0.12^a	1.37 ± 0.07^b
System		
Final biomass (kg)	58.28 ± 2.35^b	62.30 ± 0.80^a
Yield (kg m^{-3})	2.65 ± 0.11^b	2.83 ± 0.04^a
System conversion	1.61 ± 0.08	1.59 ± 0.03

Superscript letters indicate a statistical difference with $p < 0.05$.

Aspergillus niger β-GLUCAN ENHANCES ATLANTIC SALMON RESISTANCE TO ULCERATIVE DISEASE COMPARED TO COMMERCIAL YEAST PRODUCT

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Tenacibaculosis and winter ulcers, skin-associated diseases, have caused substantial economic losses in the global farming of salmon. The absence of effective vaccines and the problems associated with consistent antibiotic use necessitate the development of alternative treatments, such as oral feed additives. β-glucans have previously displayed immunomodulatory activity against skin-based disease in finfish and are, therefore, strong candidates. They are naturally derived from a variety of sources, and most bioactive β-glucans are produced by fungi. Evidence suggests that β-glucan structural diversity has a species-specific component, which may result in different immunomodulatory activity and potency. Despite this, there is a gap in animal health literature regarding diverse β-glucan sources because most research solely references *Saccharomyces cerevisiae* derived β-glucans. Thus, this study investigated the in-feed efficacy of novel *Aspergillus niger* β-glucan (Mycofence®) against ulcerative *Tenacibaculum maritimum* and *Moritella viscosa* infection compared to commercial *S. cerevisiae* β-glucan and a non-enriched control.

Atlantic salmon were fed one of five diets for 5-6 weeks and assessed for growth performance before *T. maritimum* or *M. viscosa* challenge. Feeding continued throughout challenge, wherein different diet groups were monitored for mortality and sampled for lesion severity and immune response through hematology and gene expression. Growth performance (weight gain and FCR), severity of lesion and hematology were unaffected by the diets. The transcript expression of immune markers in the gut (*sclra, cr3, igT* and *cxcl2*) and head kidney (*tlr5, cox-2* and *myd88)* and absolute quantification of *T. maritimum* and *M. viscosa* is under evaluation, and will be discussed. Mortality after *T. maritimum* challenge was comparably reduced by 0.2 and 0.3% Mycofence® and 0.1% yeast β-glucan, however, and was reduced significantly after *M. viscosa* challenge by 0.3% Mycofence® (see Fig. 1.). *A. niger*-derived β-glucan therefore provided comparable or improved protection against *T. maritimum* and *M. viscosa* infection to commercial yeast β-glucan. This finding highlights the relevance of investigating non-yeast sources of β-glucan as immunomodulators and reinforces the potential of *A. niger* β-glucan to the salmon industry as a sustainable and environmentally friendly approach to preventing disease outbreaks.

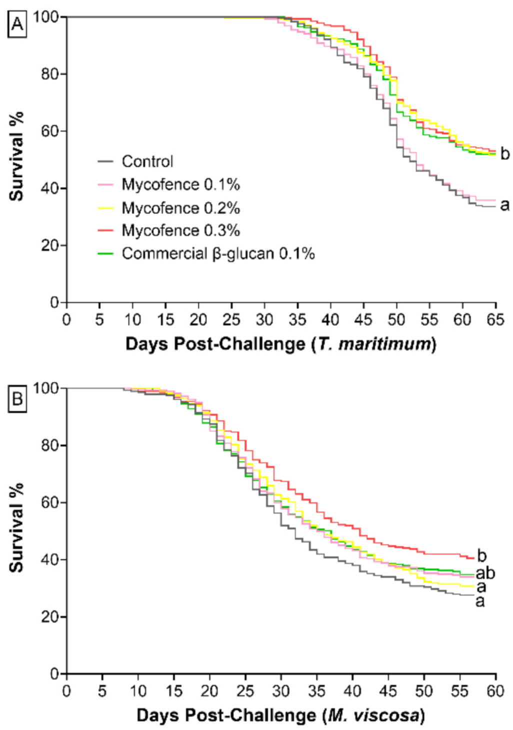

Fig. 1. Mortality in Atlantic salmon challenged with *T. maritimum* (A) or *M. viscosa* (B) after feeding trial for 5-6 weeks with Mycofence® (0; 0.1; 0.2 and 0.3%) or commercial yeast β-glucan. Different letters indicate a difference ($P < 0.05$) between treatments.

THE FACULTY OF FISHERIES AND PROTECTION OF WATERS AND CENAKVA RESEARCH INFRASTRUCTURE OF THE UNIVERSITY OF SOUTH BOHEMIA AS EUROPEAN EXCELLENCE RESEARCH AND EDUCATION CENTER

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The Faculty of Fisheries and Protection of Waters (FFPW) and the South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses (CENAKVA) at the University of South Bohemia (USB) in České Budějovice, Czech Republic, represent a prominent research and education institution specializing in high-quality basic and applied research. The faculty is dedicated to educating Bachelor's, Master's, and Doctoral degree students in the fields of fishery, water protection, and aquatic ecosystems. FFPW is renowned as one of the most active and well-equipped faculties in the Czech Republic, often recognized as the best in the country.

Currently, FFPW offers a Bachelor's degree programs in "Fishery" and "Protection of Waters" (available only in Czech), a Master's degree program in "Fishery and Protection of Waters" (taught in Czech and English), and Ph.D. degree programs in "Fishery" and "Protection of Aquatic Ecosystems" (offered in Czech and English). The programs are led by highly qualified, internationally recognized experts with extensive experience in teaching, publishing scientific articles, and engaging in project activities. For more details about the study programs, please visit: https://www.frov.jcu.cz/en/admissions/admission-procedures .

FFPW's laboratories cover a wide range of research areas, including fish reproduction, genetics, nutrition, germ cell biology, crayfish, aquatic invasive species, freshwater ecosystems, hydrobiology, aquaculture, toxicology, environmental chemistry, biochemistry, data management, and experiment design. These labs offer opportunities for short-term internships through the ERASMUS+ program. Additionally, FFPW organizes an International Summer School in Vodňany and Nové Hrady, providing students with practical research experience alongside field visits to fish farms and tourist attractions in the South Bohemian region. For more details, please visit: https://www.frov.jcu.cz/en/international-relations/summer-schools .

Apart from its educational initiatives, FFPW hosting CENAKVA is recognized nationally and internationally as a leading research center in freshwater aquaculture, fisheries, water quality, and aquatic ecosystems. Its research efforts are bolstered by funding from national (Ministries of Education/Agriculture/Environment) and international projects (such as HORIZONT 2020, HORIZONT EUROPE, Norway Grants, COST, INTERREG, JPI, and LIFE). The results are published in top-tier scientific journals and are applied in various businesses through patents, licenses, contracted research, technologies, and handbooks.

CENAKVA research infrastructure plays a crucial role in the coordinating research and international activities at FFPW. It adheres to the principle of open access infrastructure outlined in the Roadmap of the Czech Republic, aiming to firmly embed itself within European Research Infrastructure Consortiums (ERIC) in the fields of aquatic ecosystems and aquaculture.

BOTTLENECKS IN EUROPEAN EEL LARVAL & LEPTOCEPHALUS CULTURE

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The European eel (*Anguilla anguilla*) is a high-value species for commercial aquaculture in Europe, where wild-caught glass eels (transparent juveniles) are stocked in grow-out facilities to be cultured for human consumption or restocking purposes (FAO, 2023). However, the species has been categorized as "critically endangered" with the natural population at a historical low (Pike et al., 2020). Consequently, eel aquaculture requires closing the life cycle in captivity and efficient hatchery technology and techniques. Targeting this goal, developed broodstock feeds and assisted reproduction protocols, applying hormone therapy, have enabled a stable production of high-quality gametes and viable offspring (Tomkiewicz et al., 2019), while recent research has focused on the development of feeds and culture methodology for offspring (Politis et al., 2021; Benini et al., 2023; Bandara et al., 2024). This has led to larval growth and development into the leptocephalus stage (Fig. 1) incrementally extending longevity. Still, limited growth and high mortality in the early larval stages challenge the completion of this phase.

This presentation will overview progress in European eel larval culture and discuss bottlenecks needing attention to reach metamorphosis and glass eel stage in high numbers. Among other, we look at the still limited insights in larval demands to ambient culture conditions and stage specific nutritional requirements and preferences, but also the demands for an efficient hatchery operation and closed-cycle production of European eel.

References

FAO, 2023. https://www.fao.org/fishery/en/culturedspecies/anguilla_anguilla/en

Pike C, Crook V, Gollock M, 2020. *Anguilla anguilla*. The IUCN Red List of Threatened Species, 2020. https://doi.org/10.2305/IUCN.UK.2020-2.RLTS. T60344A152845178.en.

Tomkiewicz J, Politis S, Sørensen S, Butts I, Kottmann J, 2019. European eel – an integrated approach to establish eel hatchery technology in Denmark. In: Eels Biology, Monitoring, Management, Culture and Exploitation: Proceedings of the First International Eel Science Symposium, 2019. 5m Publishing, Sheffield, UK.

Politis SN, Syropoulou E, Benini E, Bertolini F, Sørensen SR, Miest JJ, Butts IAE, Tomkiewicz J, 2021. Performance thresholds of hatchery produced European eel larvae reared at different salinity regimes. Aquaculture 539, 736651.

Benini E, Bandara KA, Politis SN, Engrola S, Nielsen A, Conceiçao LEC, Santos A, Sørensen SR, Tomkiewicz J, 2023. Exploring first-feeding diets for European eel larval culture: insights at morphological, nutritional, and molecular levels. PLoS One 18, e0283680.

Bandara KA, Politis SN, Sørensen SR, Benini E, Tomkiewicz J, Vadstein O, 2024. Effect of Food Amounts on Larval Performance, Bacteriome and Molecular Immunologic Development during First-Feeding Culture of European Eel. Microorganisms, 12(2), 355.

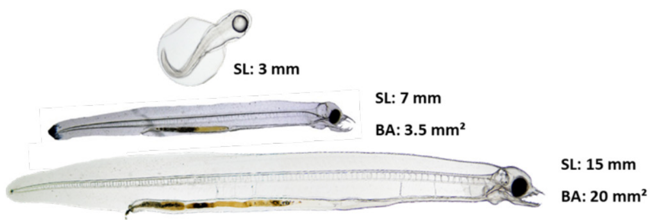

Figure 1: European eel larval stages and sizes.

INNOVATIVE SUSTAINABLE RECIRCULATING AQAUACULTRE SYSTEM AQUAFEEDS (InSuRAFeeds) FOR MIRROR CARP *Cyprinus carpio* AND RAINBOW TROUT *Oncorhynchus mykiss* UTILISING UK-SOURCED, NON-SOY, LOW-EMISSION INGREDIENTS, EXCLUSIVLEY

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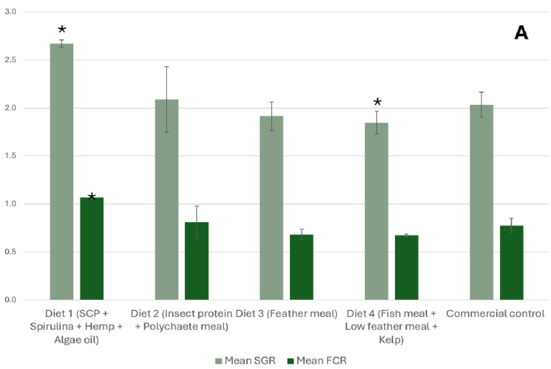
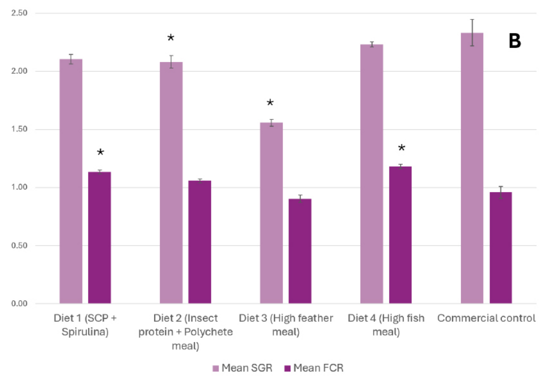
Within the industry, there is a growing demand for sustainable and resource-efficient aquaculture feed, particularly for Recirculating Aquaculture Systems (RAS), while minimizing environmental impacts and ensuring healthy and sustainable diets. This research aims to produce novel experimental RAS diet formulations, utilising non-soy, sustainably sourced, low-emission, UK-produced ingredients, exclusively, that are comparable to current commercially available aquafeeds, in terms of fish growth and nutrient digestibility.

Of the 30 ingredient suppliers who were approach to participate in the project, 21 fully engaged. Following comprehensive proximate composition analysis, 4 iso-lipidic and iso-nitrogenous experimental feeds were formulated to meet the known minimum nutritional requirements of mirror carp and rainbow trout, and manufactured in-house using a twin-screw extruder. Subsequent feeding trials (60-day carp: $BW_i = 13.7 \pm 0.4$g; 46-day trout: $BW_i = 86.7 \pm 2.2$g) were conducted in RAS at Pontus Research, with dietary regimes assigned randomly in triplicate. Standard health and growth metrics (WG, % survival, FCR, SGR, K-factor, VSI, HSI) were monitored, and faeces collected for digestibility analysis.

The proximate composition (CP, CL, Ca, P, Energy, Ash) and the quality of the 8 experimental diet pellets (bulk density, water stability, pellet size distribution, dust, and moisture) were comparable to those of the commercial controls (data not shown). Significant differences in FCR and SGR were observed between the experimental diets in comparison to the commercial control diets for both the mirror carp (Fig1A) and rainbow trout (Fig1B) and various other growth metrics (data not shown).

These results demonstrate the potential for both carp and trout, raised in RAS, to be administered aquafeeds comprised exclusively of UK-sourced ingredients, decreasing industry dependency on soy and the associated carbon footprint of aquafeeds.





FLOCponics: AN INTEGRATED BIOFLOC-BASED FOOD PRODUCTION SYSTEM FOR CIRCULAR AQUACULTURE

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FLOCponics is defined as the integration of biofloc-based (BFT) aquaculture with hydroponics. The technology is an alternative type of aquaponics system where recirculation aquaculture system (RAS) is replaced by a system based on BFT. The term “FLOCponics” was proposed to identify and unify the systems that have been previously called “BFT+hydroponics”, “BFT+aquaponics” or “BFT+plant production”. Aquaponics and biofloc-based aquaculture are considered environment-friendly approaches to food production, as both are intensive aquaculture systems with a strong focus on nutrient recycling and water saving. FLOCponics shares these characteristics. By adopting the principles of aquaponics and biofloc, FLOCponics can become an additional means to reduce the challenges of the global sustainable food supply. This new technology has been recently reviewed by our group of investigators which highlighted that FLOCponics is still in its initial research stage and inconsistent results were found regarding animal and plant yields in FLOCponics. Some investigations presented better or similar yield results in this system compared to traditional cultures, while others found the opposite. The further commercial application of FLOCponics requires research that provides a solid database, originating from experimental setups with characteristics similar to those of commercial production. One of the key challenges of using FLOCponics is the effective control of solids mainly in permanently coupled layouts, that may reach the hydroponic compartment and clough the plant’s roots affecting the nutrients absorption. Recently, our group of investigators refined the system’s design using an on-demand coupled layout and reported reduction of the critical issues related to FLOCponics systems and nutritional benefits of biofloc for tilapia production in FLOCponics. Nile tilapia juveniles fed with diets containing 24 and 28% of crude protein (CP) grew similarly to those in RAS-based aquaponics fed with a 32% CP diet, allowing an 8% reduction in the Nile tilapia dietary CP compared to on-demand coupled aquaponics using RAS. Lettuce growth was similar in FLOCponics, RAS-based aquaponics and hydroponics. A modelling study and simulation indicate that FLOCponics is 10% and 27% more efficient in using water and nitrogen, respectively, than the stand-alone biofloc system, and reduces 10% the amount of solids discharged, supporting the hypothesis that integrating a biofloc system with hydroponics makes biofloc-based fish culture more efficient in terms of resource use and wastes avoidance. An emergy synthesis study assessed the sustainability of tilapia juveniles and lettuce production in FLOCponics, biofloc and/or hydroponic systems, and found that most of the emergy indicators are similar for all systems. Based on the emergy performance, FLOCponics can be considered a promising sustainable food production approach, mainly considering that it is a system under development and there are still many opportunities for improvement. The integration of BFT with plant production fits with the circular economy concept and might contribute to social licenses and farm diversity. In terms of applicability, the FLOCponics system is likely to be applied in the short-term by farmers who already operate BFT in freshwater, adapting their structures to receive the hydroponics subsystem. For BFT production, FLOCponics seems to primarily increase the sustainable character of biofloc-based monocultures by recovering nutrients and expanding product diversity, rather than promoting higher animal growth performance.

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THE ROLE OF WOMEN IN AQUACULTURE AND SCIENCE IN BRAZIL

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Aquaculture activity in Brazil has grown significantly in recent decades, positioning the country as the 13th largest producer in the world, with a total production of 630,000 t in 2020 (FAO 2022). The activity is characterized by being practiced on small properties, mostly as a family activity. The country also registers a significant investment in science and technology, with more than 100 institutions dedicated to aquaculture in all regions of the country. However, as in most areas involving rural production, aquaculture presents strong gender inequality in the opportunities that the activity provides.

In 2024, of the seven Graduate Programs in Aquaculture that offer both Master's and Doctoral degrees, five make faculty information available on their websites. In them, the percentage proportions of men and women range from 55:45 to 93:7 (♂:♀), with an average of 73:27 (♂:♀). Among the 99 Research Productivity Fellows in the area of Aquaculture and Fisheries Resources of the National Council for Scientific and Technological Development - CNPq (a distinction intended for researchers who stand out among their peers when their scientific production is evaluated), the percentual proportion is 74:26 (♂:♀). However, when analyzing senior fellows (PQ-1A), who exercise greater leadership, the inequality stands out (100♂:♀).

Taking as an example the graduates of the PhD course in Aquaculture at the UNESP Aquaculture Center in 2016, 60% of the women were enrolled in universities, 22% in research institutes, 9% in extension, 2% in the private sector, 4% in postdoc and 9% gave up aquaculture to work in other areas, while men showed the proportions of 55% in universities, 20% in research institutes, 17% in extension, 6% in the private sector, 3% in postdoc and 3% in other areas. This scenario suggests greater interest by women in jobs with greater stability than in more competitive areas, such as the extension the productive sector and the private sector, where higher salaries are paid.

The participation of women in the productive sector is more difficult to assess due to lack of data and statistics. However, when analyzing the rural credit line "National Program for Strengthening Family Agriculture" (Pronaf) in 2021, and the specific financing line for the Brazilian female population (Pronaf-Mulher), it appears that credit to women in the area of aquaculture through Pronaf-Mulher is negligible, representing only 0.12% of the amount destined for this line of financing. This presentation will discuss the opportunities and challenges that women face to increase their participation in Brazilian aquaculture.

MACROALGAS DEL GENERO *Hypnea*, Y SU POTENCIAL PARA EL CULTIVO EN PLATAFORMA ARRECIFAL DE LAS ISLAS PROVIDENCIA Y SANTA CATALINA, RESERVA DE BIOSFERA SEAFLOWER

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Las macroalgas marinas son un componente fundamental en las comunidades de los ecosistemas marinos, por ser productoras primarias, fuente de alimento y hábitat muchos organismos. Además, se utilizan para el consumo humano y en productos cosméticos, nutracéuticos y fertilizantes, entre otros.

Hypnea spp., perteneciente al filo Rhodophyta (algas rojas), es de gran interés para diversas industrias, como la alimentaria, nutracéutica, cosmética, medicinal y de fertilizantes, debido a la presencia de carragenina en su pared celular. La carragenina posee propiedades emulsificantes, espesantes y gelificantes, lo que ha hecho que *Hypnea* spp. se convierta en la segunda fuente más importante de este recurso en los trópicos, después de *Eucheuma* / *Kappaphycus*, con Brasil como principal productor en la zona. Sin embargo, recientemente se han detectado errores en la clasificación de algunos morfotipos debido a la gran variabilidad fenotípica intraespecífica de la especie. Esto ha llevado al reporte de complejos de especies y, en el caso contrario, a la identificación errónea de especies crípticas, generando incertidumbre en los procesos productivos que deseen organizarse.

En el Archipiélago de San Andrés, Providencia y Santa Catalina, se están explorando otras especies marinas para el aprovechamiento de la comunidad, generando ingresos y diversificando el sector pesquero. Esto busca reducir la presión sobre especies tradicionales como la langosta espinosa y el caracol pala. La recolección de algas para consumo humano, principalmente de los géneros *Gracilaria* spp. y posiblemente *Euchema* sp., es una práctica tradicional. Debido al gran potencial de las especies de *Hypnea* spp., y ante su registro entre la flora nativa del Archipiélago, se ha propuesto la maricultura de algas como una opción atractiva que puede integrarse a la cadena de productores marinos y generar subproductos comercializables, por esto el presente estudio buscaba identificar la especie más viable para el desarrollo del cultivo por el estado de su población, la productividad del alga y el rendimiento de la carragenina.

Para ello, fue necesario evaluar el estado de la población de *Hypnea* spp. en la plataforma arrecifal de Providencia y Santa Catalina, determinando la distribución y abundancia de la población mediante el método del cuadrante. Con la colecta de los morfotipos encontrados, se realizó la identificación molecular de los genes COI y rbcL, complementada con identificación taxonómica, además de determinar el potencial de su cultivo y viabilidad. Se estableció que la distribución se encontraba principalmente en cercanías a la barrera arrecifal, en el costado de barlovento de la isla, en fondos de arena entre los 2 y 7 metros, con una cobertura compuesta por tres especies: *Hypnea stellulifera, Hypnea caraibica* e *Hypnea spinella*, siendo las dos primeras nuevos registros para Colombia. Sin embargo, la cobertura encontrada fue baja, menor al 10%. Respecto al potencial de cultivo y la viabilidad del aprovechamiento sostenible, se instaló un cultivo artesanal de la especie *Hypnea caraibica*, en el que se obtuvo una tasa de crecimiento relativo promedio de 11,82 ± 2,78 % día^{-1} y un rendimiento del 56%. Logrando valiosos aportes para la biodiversidad de macroalgas del país. La baja cobertura de estas algas resalta la necesidad de continuar los estudios sobre su biología para desarrollar estrategias sostenibles de aprovechamiento, ya que han mostrado un buen potencial de crecimiento y rendimiento.

EXPECTED SELECTION RESPONSES IN BREEDING PLANS AIMING TO LIMIT ENVIRONMENTAL IMPACTS OF TROUT FARMING

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With the growing societal concerns about the sustainability of food production systems, there is increasing interest in considering not only economic gains but also environmental impacts in the selective breeding of farmed species. In this study, we compared expected selection responses for alternative breeding programs aiming to limit the environmental impacts of the production of rainbow trout, one of the most important farmed fish species in Europe. The consequences of genetic improvement based on optimal selection indexes derived to minimize various environmental impacts were investigated in a simulated rainbow trout farm producing constant annual production volumes.

A cradle-to-farm-gate life-cycle assessment was performed to evaluate the environmental value of each trait that has been used in the breeding goals (H). The tested H included three different traits: the thermal growth coefficient TGC), the daily feed intake (DFI) and the survival rate (SR). Due to a lack of knowledge about the genetic links across these traits, we tested two correlation scenarios between the traits (A and B). We explored different impact categories as various environmental H and focused on freshwater eutrophication, terrestrial ecotoxicity and water dependence. Selection based on these H allowed an annual reduction of eutrophication and terrestrial ecotoxicity of 2 and 3%, respectively. For water dependence, the volume of water requires for the production was only reduced by < 0.1% annually. For the traits, annual genetics gains, expressed in % of genetic standard deviation (σg), ranged from -0.52 to +0.17 for TGC, -0.49 to +0.15 for DFI and -0.19 to +0.36 for SR.

We demonstrated interest in using environmental values (ENV) in H to minimize environmental impacts at the farm level, while maintaining high genetic improvements especially in feed efficiency-related traits. Nevertheless, we found high variability depending on the environmental impact category.

Another selection strategy should be considered to avoid negative consequences on SR when considering possible negative correlations between survival and production traits. Although our results are promising, their interpretations have to include the economic repercussions of such a selection strategy.

TABLE 1. Annual genetic gains (AGG) expressed (1) in physical units for the different breeding goals (H) with under brackets the gains expressed as % of the average environmental impact of the hypothetical farm and (2) expressed in % of genetic standard deviation (σ_g) for the three traits (TGC, DFI and SR) under selection according to two scenarios considering (B) or not (A) genetic correlations between SR and the other traits.

	Units	A	B
H freshwater eutrophication			
AGG_H	kg P eq	0.43 (3.2%)	0.45 (3.4%)
AGG_{TGC}	σ_g	-0.49	-0.52
AGG_{DFI}	σ_g	-0.46	-0.49
AGG_{SR}	σ_g	<0.01	-0.19
H terrestrial ecotoxicity			
AGG_H	kg 1,4-DCB	84.4 (1.9%)	88.7 (2.0%)
AGG_{TGC}	σ_g	-0.49	-0.52
AGG_{DFI}	σ_g	-0.46	-0.49
AGG_{SR}	σ_g	<0.01	-0.19
H water dependence			
AGG_H	m^3	77.4 (0.1%)	69.3 (0.1%)
AGG_{TGC}	σ_g	-0.10	+0.17
AGG_{DFI}	σ_g	-0.10	+0.15
AGG_{SR}	σ_g	+0.36	+0.36

SOLID WASTE MANAGEMENT IN SALMONIDS: INFLUENCE OF SPECIES, DIET COMPOSITION AND SALINITY OF REARING MEDIUM

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Introduction

Minimising the quantity of waste produced and its sustainable management are major concerns in aquaculture. The type of production system, species cultured, the salinity of the rearing medium, the composition of the diet and the waste removal technique used interact to determine the removal efficiency of waste produced and its subsequent accumulation in the production system. In the current study, we first investigated the impact of dietary starch, NSP and their interactions on solid waste production, its removal efficiency and the size and stability of faecal particles in three salmonid species: rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*). Following this, another trial was conducted with rainbow trout to understand the impact of the rearing medium's salinity and the diet's protein-to-energy ratio on the characteristics of faeces produced and its removal efficiency.

Materials and Methods

In the first trial, four diets were formulated using a 2 × 2 factorial design. The first factor, starch, was tested by including 0% gelatinised wheat flour (LS- low starch) or 20% gelatinised wheat flour (HS- high starch) in a plant ingredient basal diet. The second factor, NSP, was tested by adding 0% NSP source (low NSP) or 10% NSP source (high NSP). High NSP level was achieved by adding an equal mixture of soya hull (5%) and wheat bran (5%). Diets were tested in triplicates for each species, and feeding was done restrictively. The experimental duration was 42 days for rainbow trout and Atlantic salmon and 49 days for Arctic charr. Fish were fed restrictively twice a day.

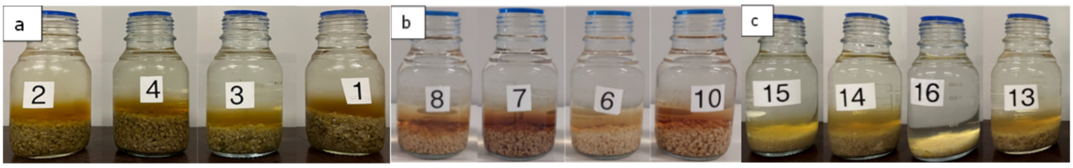

Image 1. Overnight collected faeces of rainbow trout (a), Atlantic salmon (b) and Arctic charr (c) fed restrictively during the experimental period. Bottles from left to right for each species represent faeces collected from fish fed with low starch low-NSP diet, low starch high-NSP diet, high starch low-NSP diet and high starch high-NSP diet respectively.

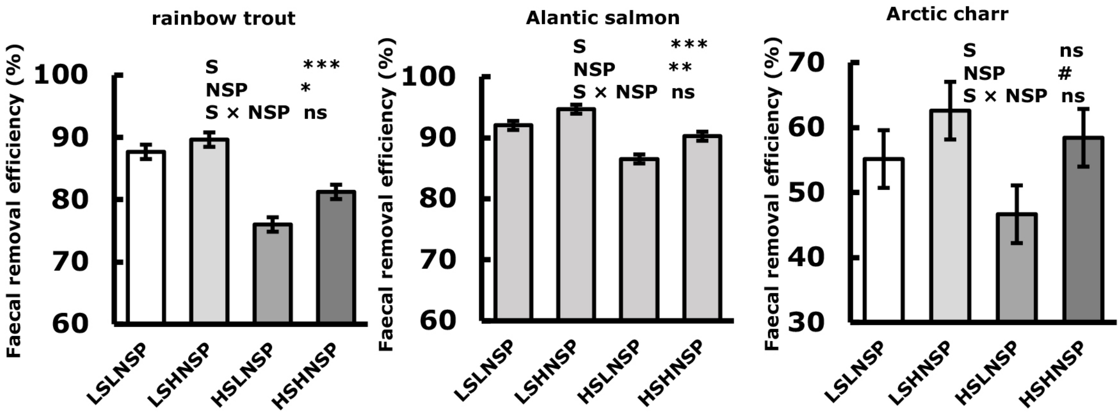

*Figure 1. Faecal removal efficiency by settling (%) in rainbow trout, Atlantic salmon and Arctic charr fed the experimental diets restricitvely during the experimatl perio; LS-LNSP – low starch low NSP; HS-LNSP – high starch low NSP; LS-HNSP – low starch high NSP; HS-HNSP – high starch high NSP; bars are means; error bars indicate standard error of means; ns- not significant, P>0.1; #, tendency, P<0.1; *, P<0.05; **, P<0.01; ***, P<0.001.*

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For the second trial, two diets varying in protein-to-energy ratio were formulated. Protein to energy ratio (P/E ratio) was varied so that the starch-to-fat ratio remained constant, indicating that higher starch levels also correspond to the higher fat content in the diet. High and low P/E ratio diets corresponded to P/E ratios of 25.5 and 15.8 mg/KJ, respectively. Each diet was randomly allocated to 6 replicate tanks and fed restrictively twice a day. The salinity of the rearing medium was kept at 1.5 ppt for an initial 4 weeks. In subsequent weeks, salinity was raised to 6, 12, 18, 24 and 30 ppt. Fish were maintained for three weeks at 30 ppt. Read-out parameters were measured at 1.5 ppt, 12 ppt, 24 ppt and 30 ppt.

In both trials, nutrient digestibility, faecal waste production and faecal removal efficiency were determined by sedimentation. Faecal particle size distribution (PSD) was measured pre and post-exposure to mechanical stress, giving insight into faecal stability.

Results

Visual observation of faeces collected in bottles indicated that most faeces consisted of fine faecal particles in Arctic charr in contrast to the dominance of faecal pellets or strings in rainbow trout and Atlantic salmon (Image 1). The amount of faecal waste produced was influenced by the interaction effect of starch and NSP ($P<0.05$). High dietary starch levels increased the proportion of smaller-sized particles, while high NSP content increased the ability of faecal particles to withstand mechanical stress in all three species. In rainbow trout and Atlantic salmon, high starch levels in the diet lowered faecal removal efficiency (Fig. 1a, $P<0.001$) but increased by high NSP content (Fig. 1a, $P<0.05$). The highest and lowest faecal removal efficiency averaged over all 4 diets was recorded for Atlantic salmon (91%) and Arctic charr (56%), respectively. The amount of non-removed faeces accumulating in the system was increased (Fig. 1b, $P<0.001$) by the high starch levels in rainbow trout and Atlantic salmon diet but remained unaffected (Fig. 1b, $P>0.05$) by the NSP content of the diet across the species. Among the three species investigated, Arctic charr had the highest (96 g) amount of non-removed faeces per kilogram of dry matter feed, followed by rainbow trout (17g) and Atlantic salmon (29 g) averaged over all four diets.

For the second trial, we are still awaiting the results from 30 ppt. Results presented below are based on our findings for 1.5 ppt, 12 ppt and 24 ppt. Preliminary results indicated that the removal efficiency of faeces was influenced by the interaction effect between the salinity of the rearing medium and the P/E ratio of diet ($P<0.05$). For a high P/E ratio diet, removal efficiency declined as the salinity was increased, while the effect of salinity was the opposite for a low P/E ratio diet. Due to significantly higher faecal waste production ($P<0.001$) and lower removal efficiency ($P<0.001$), the amount of non-removed faeces was higher at a low P/E ratio diet than at a high P/E ratio diet. The amount of faecal waste production and the non-removed faeces increased with the increase in salinity of the rearing medium.

Conclusion

Considerable differences exist between rainbow trout, Atlantic salmon, and Arctic charr regarding faecal waste production and faecal characteristics. High starch levels in the diet reduced faecal stability and removal efficiency in salmonids. Non-removed faeces by settling is determined by dietary starch content and not by NSP. Salinity of the rearing medium has potential to impact the quantity of faecal waste produced and its removal efficiency. To conclude, solid waste management measures for salmonids, should keep in consideration the differences in species, diet composition and rearing medium salinity.

TOWARD A NEW SUSTAINABLE MANAGEMENT FOR MUSSEL CULTURE OPTIMIZATION *Mytilus galloprovincialis*: A CASE STUDY IN THE IONIAN SEA (MEDITERRANEAN SEA, SOUTH ITALY)

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In recent years in many countries of European Union's (EU), mussel production has decreased, passing from 600,000 tonnes in the 1990s to around 430,000 tonnes in 2020. This lack of growth has been explained, by several environmental stressors, such as the lack of seed caused by predation by fishes, harmful algal blooms (HABs) with deterioration of marine environment; climate changes and their consequences as heat waves. Besides this, structural causes include conflict in spatial use, fewer areas available for mussel culture and establishment of several illegal farms, with consequent high density of farmed mussels, high competition for space and food, with detrimental effect on mussels' growth. This work aimed to develop new management and production systems for shellfish farms, to increase their competitiveness and improve the overall sustainability of this sector.

Experiments were performed from October 2022 to June 2023, in two commercial mussel farms located in the Second Inlet of the Mar Piccolo of Taranto (Italy). For each farm, 5 "chambers" were selected to house 4 experimental treatments plus a control. Mussel growth was compared among chambers with reduced number of collectors by 10%, 20% and 30% (18, 16 and 14, respectively) and chambers with 20 collectors but constituted by reducing the weight of mussels in the first grafting stage by 33%. At start mussels used in the experimental culture were randomly selected with a homogeneous distribution of size. The initial shell length (mean ± SD) was 37.4 ± 2.8 mm and the initial total dry weight of collectors was 686.53 ± 37.61 g. No significant differences in initial length and weight of collectors were observed between density treatments (ANOVA; $p > 0.05$). Absolute and Specific Growth rates (AGR and SGR) were calculated in mm day-1 and g day-1, respectively. Length–weight relationships (LWRs) was calculated using the equation $W=aL^b$. The increase in weight (%) of the collectors showed that the collector belonging to the r33 reduction exceeded the weight increase of the other treatments at the end of the experiment (Fig.1). This could be explained by a lower quantity of seed used in the first graft.

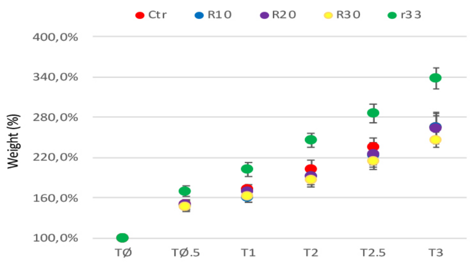

Fig.1. Weight (%) increase of collectors during experimental period.

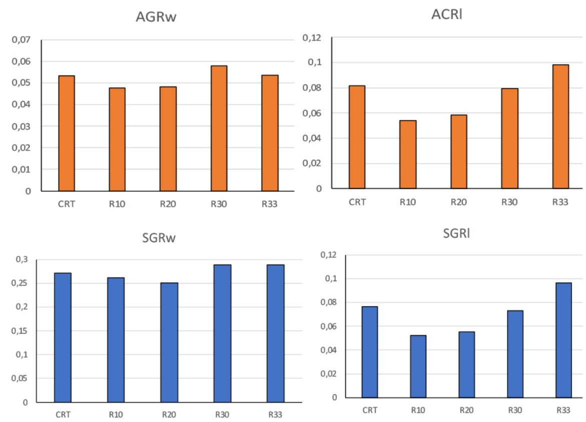

Fig.2 Absolute and Specific Growth rates (AGR and SGR)

Collectors r33 showed the highest AGR: 0.10 mm d-1 and SGR: 0.10%, both determined by length. The AGRw, instead, was higher for mussels from treatment R30 (about 0.06 d-1), while SGRw was higher in both R30 and r33 individuals with values close to 0.29% (Fig. 2).

LWRs showed allometric growth in all treatments, with values of the coefficient b between 0.27 and 0.34 and with correlation coefficients between 0.80 and 0.95.

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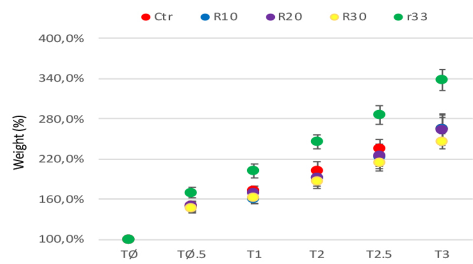

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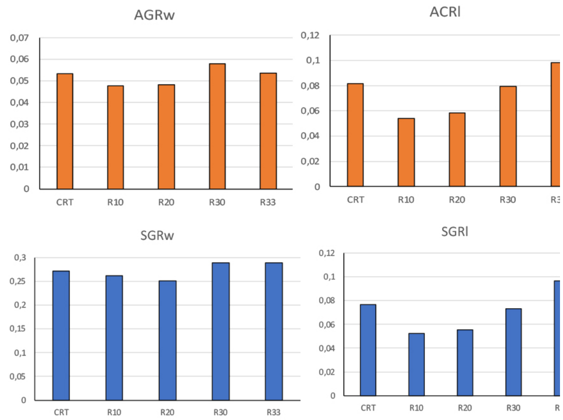

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THE POTENTIAL OF SELECTIVE BREEDING TO ELIMINATE UNFAVOURABLE SCALY PATTERNS IN AMUR MIRROR CARP

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The two main scale patterns of common carp are scaly and mirror phenotype. However, the pleiotropic effects of genes for mirror phenotype significantly reduce mirror carp's performance (growth, survival) compared to scaly ones. Therefore, the Amur mirror carp (AMC), a new Czech mirror carp breed established in 2014, has been genetically approved to overcome this problem. The breeding approach to establish the AMC was based upon the introgression of Amur wild carp - AC (*Cyprinus rubrofuscus*) genes into the Hungarian synthetic mirror carp (HSM; *Cyprinus carpio*) to improve the non-specific resistance of mirror carp stocks. Since then, AMC has become the primary mirror carp used in Czechia's hybridization and selection program. Despite its better performance, genes of AC significantly affect the general appearance of AMC, whose scales are distributed diversely over the body and a high ratio of AMC individuals do not display a typical and demanded mirror phenotype. Therefore, this study aimed to evaluate the possibility of improving unfavourable scaly patterns in AMC by selective breeding.

Table 1: Heritability (± standard error) estimates (diagonal) in bold and diagonal, phenotypic (below the diagonal) and genetic correlations (value ± standard errors, above the diagonal) for scaly pattern phenotypes and market size performance traits

	Scale_Bin	TotalSC_A	Rel_SC_A	Scale_NO	BW	FC	Fat	Res_Fill	Surv
Scale_Bin	**0.54±0.09**	0.95±0.02	0.96±0.02	0.94±0.02	-0.23±0.14	-0.33±0.12	0.29±0.14	-0.01±0.15	-0.29±0.18
TotalSC_A	0.66	**0.78±0.09**	0.96±0.01	0.94±0.02	-0.10±0.14	-0.27±0.12	0.23±0.14	-0.04±0.14	-0.18±0.27
Rel_SC_A	0.67	0.94	**0.79±0.09**	0.95±0.01	-0.29±0.13	-0.27±0.12	0.17±0.14	-0.03±0.14	-0.11±0.28
Scale_NO	0.63	0.90	0.91	**0.62±0.09**	-0.28±0.13	-0.28±0.12	0.24±0.14	-0.01±0.14	-0.25±0.28
BW	-0.06	0.02	-0.18	-0.11	**0.56±0.08**	0.50±0.10	0.07±0.14	-0.40±0.13	-0.02±0.27
FC	-0.15	-0.18	-0.19	-0.18	0.36	**0.89±0.09**	-0.14±0.13	-0.25±0.13	0.42±0.25
Fat	0.11	0.08	0.01	0.04	0.20	0.01	**0.63±0.09**	0.29±0.14	-0.29±0.27
Res_Fill	-0.04	-0.09	-0.08	-0.09	-0.04	-0.04	0.28	**0.46±0.08**	0.15±0.28
Surv	-0.01	-0.01	0.01	-0.02	0.04	0.01	0.03	-0.03	**0.06±0.02**

(Continued on next page)

The experimental stock of AMC established out of 20 dams and 40 sires was reared communally until market size (three years) under semi-intensive pond conditions. After the second summer, the stock was harvested (n = 1,864), and each fish was photographed to further determine scaly patterns and body area. To measure the number and total area of scales and body area without head and fins, two dedicated deep learning models were trained based on a cellpose 2.0 fine-tuning cyto2 model. The resulting images were quantified using an ImageJ macro. The scaly pattern phenotypes were scaliness (Scale_Bin: 0 = standard mirror, 1 = other), total scale area (TotalSC_A), relative scale area to body area (Rel_SC_A) and scale number (Scale_NO). Fish were also phenotyped at market size (n = 1,572) for survival (Surv), body weight (BW), Fulton's condition factor (FC), muscle fat content (Fat) and fillet yields (Res_Fill). Heritability and genetic and phenotypic correlations were estimated with DMU software using an animal model and pedigree record.

The heritability estimates of scaly pattern phenotypes were very high (0.54 – 0.79), suggesting a great potential to change the unfavourable scaly patterns of AMC to a typical mirror phenotype using a selection program (Table 1). Moreover, high genetic correlations among the scaly pattern phenotypes show a reduction of technical issues among the different ways to phenotype the scaly pattern in AMC. Hence, the easiest selection based only on the scaly appearance of fish (Scale_Bin) might be applicable in a breeding program. The genetic correlations between scaly pattern phenotypes and performance traits were, in many cases, insignificant. Only the correlations with BW (-0.28 – -0.29), FC (-0.27 – -0.33) and Fat (0.23 – 0.29) were found at the edge of significance, but they were low. Thus, including the scaly phenotype trait in the AMC breeding program should not affect the effort for further genetic improvement of the new and prospective mirror carp breed.

GROWTH OF RAINBOW TROUT *Oncorhynchuss mykiss* IN A ZERO-DISCHARGE RAS WITH PASSIVE WATER TREATMENT FIELD

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Discharge management in recirculating aquaculture systems (RAS) plays a crucial role for environmental friendliness, but also for economic viability. Low-cost solutions such as woodchip bioreactors and wetlands have been explored and adopted in RAS. A small-scale RAS accompanied by a passive water treatment field consisting of a woodchip bioreactor (50 m^3), a vertical flow constructed wetland (45 m^2), and a sand infiltration unit (650 m^3) was constructed at the Laukaa research station (Natural Resources Institute Finland). The RAS included four 5 m^3 raceway tanks, drum filters, fixed-bed bioreactors, air diffusers, and low-head oxygenators.

An intensive experiment was carried out where RAS water was pumped to the water treatment field and back to the RAS. Sludge from the RAS was thickened with polyaluminium chloride, and the supernatant was pumped to the water treatment field. Relative water renewal rate from the lake was 190 L kg^{-1} feed whereas 476 L kg^{-1} feed was pumped through the water treatment field. Sludge was removed at a rate of 106 L kg^{-1} feed, indicating a water loss of 84 L kg^{-1} feed either through evaporation or small leaks. Water quality remained good in the RAS, where mean nitrate, total ammonia nitrogen, and nitrite values were 49, 0.85, and 0.28 mg L^{-1} respectively. Rainbow trout (*Oncorhynchuss mykiss*) were reared in four fish tanks and showed excellent growth and low mortality (Table 1). This experiment showed that RAS effluent can be treated so efficiently that it can be reused without any harmful effects on the fish. With improved sludge treatment, even small amounts of fresh water are required for efficient aquaculture.

Table 1. Rainbow trout growth, feed conversion ratio (FCR), specific growth rate (SGR), and mortality in RAS with passive water treatment field. Values are means from four fish tanks.

Parameter	Start weight (g)	End weight (g)	Days	FCR	SGR	Mortality (%)
Value (± SD)	61 ± 1	2283 ± 126	274	1.06 ± 0.02	1.32 ± 0.02	4.2 ± 0.8

PAST, PRESENT AND FUTURE OF MUGILIDAE CULTURE: A REVIEW

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Marine aquaculture sustainability relies on finding suitable alternatives to dietary fish oil (FO) and fish meal (FM) for aquafeeds, due that most marine fish species cultured are placed high in the trophic chain, contrary to freshwater ones. In this sense, mullets are great candidates for marine aquaculture diversification due to their euryhaline, eurythermal and low-trophic nature, allowing more sustainable culture practices to be implemented.

The Mugilidae family comprises up to 71 species distributed in temperate regions worldwide. Its cultivation has been done for centuries by different cultures, in many cases by taking advantage of their migrating behaviour from the sea to coastal lagoons or estuaries and vice versa, facilitating them to be stored in confined areas. This adaptability to different salinity environments has also allowed the cultivation of many mullet species from freshwater to high-salinity water.

These attributes have increased the interest in mullets in recent years, and numerous studies from different regions have addressed some Mugilid's gaps in knowledge around the world. The state of the art in Mugilidae reproduction, larval rearing, and fattening is summarized below:

Reproduction

Mullets are oviparous bisexual teleosts with external fertilization and development. The females have group synchronous ovaries and usually only spawn once yearly. Mullet species with the same habitat distribution usually have different spawning seasons throughout the year.

Like other marine teleosts, some mullet species present reproductive dysfunctions in captivity, which has produced that mullet aquaculture has traditionally been supported by wild fry collection. However, in recent years, the breeding of Mugilidae has been developed through the improvement of hormone-therapy protocols (for instance, LH-RH analogues and dopaminergic inhibitors) and culture management protocols, allowing the obtention of viable eggs and larvae at a commercial scale in some species.

Larval rearing

Compared with well-established aquaculture species, there is still a vast knowledge gap related to larval rearing, ontogeny, and nutrition requirements of grey mullets. Size at hatching, yolk sac and oil globule size, reabsorption patterns, moment of mouth opening and mouth size, etc., vary significantly among mullet species, affecting the different rearing protocols that must be implemented and studied for each species to optimize producers' cost and effort.

Fattening

Mullets are very adaptable species; therefore, cultivation conditions and feed formulas are very adaptable to a wide range of conditions. For instance, salinity and dietary lipid sources can be efficiently modulated as a tool to optimize growth, feed utilization and lipid metabolism to improve the synthesis of long-chain polyunsaturated fatty acids such as ARA, EPA and DHA.

Overall, although much research in different fields still needs to be done, mullets are promising species in the prospect of blue growth and sustainable aquaculture, which assessment must be done with a multi-species and multi-areas approach.

DATE SEED MEAL AS SUSTAINABLE AQUAFEED INGREDIENT. POTENTIAL USE IN JUVENILE MULLETS

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Introduction

Due to Mullets ability to bio-digest and bio-synthesize, novel raw materials can be used as Aloe Vera and its by-products in diets for these fish (Quirós-Pozo *et al*., 2021). However, there are few works or studies that evaluate the use of vegetable derivates or by-products in diets for *Liza aurata* or the purpose of adding ingredients for improving fish health and quality.

In aquaculture world, date seed meal is notable for its high monosaturated fatty acids concentration, mainly oleic acid, and it is a good source of lipid-soluble antioxidant compounds like phenols, tocopherols, and phytosterols (Mrabet *et al*., 2020). Also, this fruit contains bioactive compounds, even insoluble fibers, which may benefit the animal's health and well-being (Kari *et al*., 2022).

Material and Methods

Fifty fish per tank (5±0.33 g) were randomly placed in 200 litres fiberglass tanks with open-flow system and fed three times a day, six days a week with the experimental diets. The control diet was formulated according to Quirós-Pozo *et al*. (2021) and four diets with date seed meal were prepared with defatted meal with and without microwave processing (10RDS, 10MRDS, 15RDS, 15MRDS).

After the feeding trial, lipid composition (Folch *et al*, 1957), fatty acid profile (Christie, 1982; Izquierdo *et al*., 1990), and histopathology (Martoja & Martoja-Pearson, 1970) of the liver and the intestine was evaluated.

Results and Discussion

It is demonstrated that 10 MRDS diet results the most productive diet even respect to the control diets. Microwave may improve the utilization of date seed, as used under a simple method improve the response of the fish at 10% dietary inclusion.

Date seed meal has the potential to become an effective feed ingredient given the results observed in diets treated with microwaves and a 10% inclusion. The disparity it shows with the results of its 15% inclusion counterpart indicates that more detailed research is needed on the inclusion levels that could be given for this novel diet.

Bibliography

Christie, W.W.(1982). Lipid Analysis, vol. 207. Pp 207. Oxford: Pergamon Press.

Folch, J., Lees, M., and Stanley, G. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226 (1), 497-509.

Izquierdo, M.S., Watanabe, T., Takeuchi, T., Arakawa, T. & Kitajima, C. (1989). Requirement of larval red seabream Pagrus major for essential fatty acids. Nippon Suisan Gakk., 55, 859–867.

Kari, Z. A., Goh, K. W., Edinur, H. A., Mat, K., Khalid, H. N. M., Rusli, N. D., Sukri, S. A. M., Harun, H. C., Wei, L. S., Hanafiah, M. H. B. M. Ali., Rahman, M. M., Razab, M. K. A. A., Wee, W., Ariff, N. S. N. A. & Dawood, M. A. (2022). Palm date meal as a non-traditional ingredient for feeding aquatic animals: A review. Aquaculture Reports, 25, 101233.

Mrabet, A., Jiménez-Araujo, A., Guillén-Bejarano, R., Rodríguez-Arcos, R., & Sindic, M. (2020). Date seeds: A promising source of oil with functional properties. Foods, 9(6), 787.

Quirós-Pozo, R., Ventura-Castellano, A., Ramírez-Bolaños, S., Roo-Filgueira, J., & Robaina, L. (2021). Evaluation of Aloe vera by-product against cereals in feeds for golden mullet (*Liza aurata*). *Aquaculture Reports, 20*, 100659. DOI: 10.1016/j.aqrep.2021.100659.

FEEDING BEHAVIOUR RESPONSE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* TO A PALATABILITY ENHANCER ASSESSED BY AN AUTOMATED VIDEO TRACKING SOFTWARE

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In commercial shrimp farming, feeding is one of the main costs. On the other hand, slow localization and ingestion of pellets by the shrimp lead to nutrient leaching and uneaten feed, causing a reduction in diet's nutritional quality and environmental contamination. The attractability and palatability of commercial feeds can be improved through additives containing specific chemosensory cues that can be rapidly recognized and initiate ingestion by the shrimp. Shrimp feeding behaviour can play an important role in the assessment of these novel sensory feed additives. Therefore, the development of automated protocols using tracking technologies is beginning to gain attention as an important tool for monitoring behaviours associated to feeding. The objective of this study was to evaluate shrimp feeding response to a commercial feed additive (Luctamax FPE AQUA, Lucta S.A.) via an automated video tracking software, benchmarking with diets of variable levels of attractability and palatability.

Litopenaeus vannamei juveniles (5.50 ± 2.20 g) were sourced to assess behaviour and feed intake responses to three control diets; basal (BC; without additives), negative (NC; BC + 0.07M Quinine-HCl) and positive (PC; BC + 3% Krill Meal) controls, and two diets containing the functional palatability enhancer (FPE) at two different concentrations, BC + 0.1% (FPE 0.1) and BC + 0.2% (FPE 0.2). Non-moulting and 24-hours fasted shrimp were individually video recorded during 20 min, in consecutive days. Rectangular test arenas (78 x 20 x 20 cm; L x W x H) were equipped with a chamber where shrimp were acclimated 10 min before starting the recording, and a feeding area at the other end where 0.5 g of one of the test diets was dispensed. A video camera was setup directly above the test arena to record the entire system. Thirty-four videos were recorded for each test diet and analysed using EthoVision XT 17. A set of behavioural units were analysed by the software, generating metrics related to the attractability and palatability of each test diet. Besides, uneaten pellets were collected at the end of each recording and compared to the initial number for feed intake assessment. Statistical analysis was done by Generalized Linear Mixed Model, considering diet as a fixed factor and shrimp ID as random factor.

The PC and FPE 0.2 diets showed enhanced palatability, indicated by increased time spent on the feeding area (Fig. 1a), and attractability levels, suggested by reduced exploratory activity around the test arena, in comparison with the other diets. An increased number of pellets eaten was also found in PC and FPE diets (Fig. 1b). Results from this study highlight the improvement of attractability and palatability levels using 0.2% Luctamax FPE AQUA as a feed additive in *L. vannamei* diets and the feasibility of implementing automated shrimp feeding behaviour protocols for the industry.

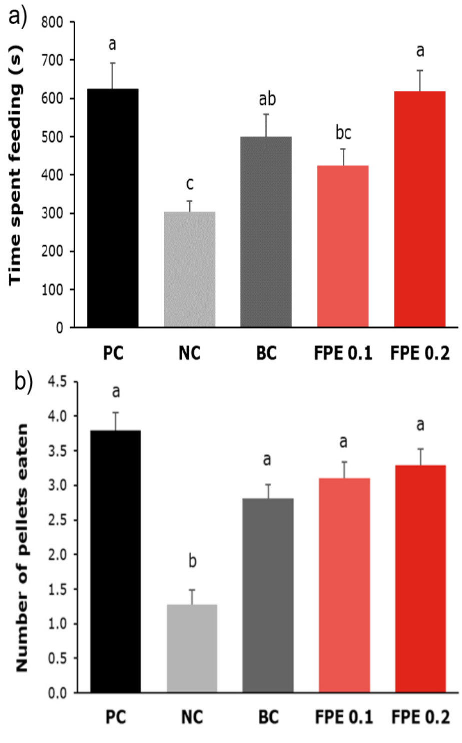

Figure 1. Differences in (a) time spent feeding and (b) number of pellets eaten in relation to test diet. Mean ± SEM, n=34. Different letters show statistical differences ($p < 0.05$).

USING DRIED FISH POWDER TO FILL NUTRTIONAL GAPS IN RURAL zAMBIA: NUTRITION ANALYSIS AND SENSORY PANEL RESULTS OF COMPLEMENTARY FOOD FOR AFRICA+DRIED FISH POWDER

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Child malnutrition due to deficiencies in macronutrients (e.g., high-quality protein, DHA) and micronutrients (e.g., iron, zinc, vitamin A) during the complementary feeding stage (ages 6-24 months) are major global health burdens in low-and middle-income countries such as Zambia. Micronutrients are needed in small amounts, but deficiencies cause serious health conditions such as stunting and anemia. Deficiencies in high-quality protein negatively impact most bodily functions, including micronutrient absorption. Multiple micronutrient powders (MNP) – internationally regulated sachets of vitamins and minerals produced by a handful of global suppliers – have helped reduce global stunting and anemia, but have had less impact on protein malnutrition. Yet even when consumed in small quantities, dried pelagic small fish (~10 cm long) add high-quality animal-sourced protein, fat, essential micronutrients, and multiple vitamins.

In order to promote fish consumption among women of reproductive age (WRA) and infants and young children (IYC), we piloted a locally produced low-cost, high-quality protein/micronutrient blend, *Complementary Food for Africa+Dried Fish Powder* (ComFA+Fish), among 42 Zambian WRA (Sensory Panel I) and 42 IYC (Sensory Panel II) to confirmed palatability of ComFA+Fish complementary porridge and other fortified dishes. We conducted a nutrient analysis of dried fish powder (see Table 1) and collaborated with a Zambian multi-sector food processor that exports to European markets to develop and pilot ComFA+Fish Plain Instant Porridge among 40 key stakeholders and ComFA+ Fish Vanilla Instant Porridge among 38 of these stakeholders (Sensory Panel III).

We conducted a Scaling Readiness Exercise among these stakeholders to inform our next ComFA+Fish activities. These stakeholders were recruited from villages in Zambia's Southern Province and included WRA, Community Health Workers, entrepreneurs, and local- and district-level Department of Health and Department of Fisheries personnel. We discuss results of our four activities and how they inform our next set of scaling-related ComFA+Fish activities.

Table 1. Dietary Reference Intakes (DRIs) and percentages of DRIs met for infants, children, and women per serving of Kapenta dried fish powder.

Nutrients	Kapenta dried fish powder (100g prepared)	Infants 7-12 months		Children 1-3 years		Women 19-50 years	
		DRI value	% DRI met (10g prepared)	DRI value	% DRI met (20g prepared)	DRI value	% DRI met (30g prepared)
Energy (kcal)	284.6	Variable		Variable		Variable	
Protein (g)	71.16	**11**	65%	**13**	109%	**46**	46%
Total Fat (g)	13.33	30*	4%	–	–	–	–
Omega 3 Fatty Acids							
DHA (g)	9.19	–		–	–	–	–
DPA (g)	1.037	–		–	–	–	–
EPA (g)	5.13	–		–	–	–	–
Choline (mg)	365	150*	24%	200*	37%	425*	26%
Minerals							
Calcium (mg)	2860	260*	110%	**700**	82%	**1000**	86%
Iodine (μg)	16	130*	1%	**90**	4%	**150**	3%
Iron (mg)	10.6	**11**	10%	**7**	30%	**18**	18%
Magnesium (mg)	158	75*	21%	**80**	40%	**320**	15%
Potassium (mg)	1390	860*	16%	2000*	14%	2600*	16%
Selenium (mg)	0.12	20*	–	**20**	–	**55**	–
Sodium (mg)	309	370*	8%	800*	8%	1500*	6%
Zinc (mg)	12.8	**3**	43%	**3**	85%	**8**	48%
Vitamins							
Vitamin A (μg)	1260	500*	25%	**300**	84%	**700**	54%
Vitamin B12 (μg)	11	0.5*	220%	**0.9**	244%	**2.4**	138%
Vitamin D (μg)	42.6	10*	43%	**15**	57%	**15**	85%
Alpha tocopherol (μg)	1.3	5*	3%	**6**	4%	**15**	3%

Note: Recommended Dietary Allowances (RDAs) are presented in bold type and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*).

UNVEILING THE LINK: UNDERSTANDING THE RELATIONSHIP BETWEEN TECHNICAL EFFICIENCY AND FINANCING GAPS

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Farmers in developing countries struggle to invest in modern equipment and quality inputs that can improve outputs. This can lead to lower productivity and eventually, reduces farm level income. This paper aims at determining the precise amount of credit needed to achieve a targeted increase in productivity based on data collected through direct interviews of 452 prawn farms in Bangladesh. This issue has been overlooked in the discussion of farm credit literatures. The study is

The output distance function is used to estimate the farm level technical efficiency (TE), and quantity of output needed to reach a target efficiency level based on current production and TE. Further Harod-Domar growth equation is used to calculate the finance needed to achieve this target efficiency, and to determine the financing gap. The probit regression calibrated with Heckman's two-stage regression is used to identify the determinants of access to credit and its impact on credit size.

It is found that it is possible to estimate the actual amount required to enhance farm level productivity to specific TE levels linking credit amount to production efficiency. Access to credit is influenced by factors like interest rates, education, credit process duration, land ownership, and asset value.This study shows that the Harrod-Domar theory can also be used in defining the relationship between financing needs and productivity.

The practical implications are that farm level TE significantly influences the amount of credit needed to bridge financing gaps and boost productivity. The study guides that tying credit grants to specific production increase targets to reduce mismanagement and indiscipline in credit use especially agricultural credits schemes in many of the developing countries like Bangladesh.

The study is unique in its approach; using TE and applying the Harrod-Domar equation at the farm level to estimate the exact credit amount required for specific production increase, thereby identifying a threshold beyond which mismanagement may occur.

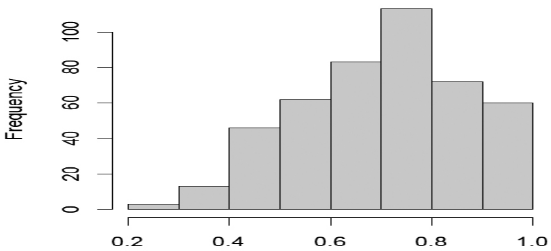
Table 1 Descriptive statistics of the variables of TE estimates

Variables	Mean	Min	Max	Std.
Income prawn (USD)	5227	1740	15680	1979
Income Rice (USD)	424	0.03	2750	346
Labor Cost (USD)	728	142	2043	377
Seed Cost (USD)	1210	275	5610	685
Feed Fertilizer Medicine (USD)	1333	342	5918	711
Fixed cost (USD)	398	20	3827	378
Operating &Trans (USD)	148	0.02	1247	156
Age Year	0.52	0.24	0.67	0.11
Education Year	0.10	0.01	0.18	0.04
Experience Year	0.19	0.02	0.32	0.09
Fam Size_Hectare	0.05	0.02	0.13	0.02
Number of Gher	0.03	0.01	0.28	0.03

Table 2 Summary statistics of financing gap estimates

Variables	Mean	SD	Min.	Max.
Technical efficiency	0.7	0.16	0.24	1
Current Output (USD)	3021	3800	204	76019
Output at target TE (USD)	3453	3886	389	85096
Amount needed to produce at target TE (USD)	1882	2633	882	30283
Personal savings (USD)	1140	3646	-3017	73499
Financial gap (USD)	742	1033	506	40216

Figure 1 Technical efficiency output maximization



IMPACT OF CREDIT CONSTRAINT ON FINANCIAL PERFORMANCE OF PRAWN FARMS: INSIGHTS FROM A PRMARY SURVEY

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Limited access to capital and credits hinders small farms from utilizing resources effectively for optimal performance. Empirical literatures argue that various factors, such as price, risk, transaction and rural financing accessibility contribute to these constraints. To understand this issue, this study examines the impact of capital and credit constraints on Gher based prawn farms in Bangladesh, focusing on factors influencing these constraints and their effects on farm performance. We used econometric methods in analysing the primary survey data obtained from direct interview of 452 gher to estimate two key relationship. First the relationship between credit constraints and variaous socioeconomic and demographic factors, including access to information perceived farm risk as well as the marginal likelihood of being credit constrained. Second we investigated the impact of credit constraints on financial performance. For these analyses, we used to main techniques. Firstly, we employed a probit model with credit constraints as the dependent variable and various factors influencing credit constraint as independent variables. Secondly we applied propensity score matching (PSM) estimator to approximate and quantify the impact of credit constraints on the financial performance of small farms. It is found that limited access to capital, influenced by price, risk, transaction factors, and access to finance, hampers small farms' ability to optimize resource use. Results further show that off farm work, land size, farm specialization significantly affect credit constraints. It is observed that financially constrained small farmers experience lower aggregate income from off farm activities than that of unconstrained farmers, potentially resulting difference in aggregate household income. Furthermore, this study highlighted that specific reasons for credit constraints, such as the inability to obtain agricultural loans at the required.

Table 2: impact of credit constraints on farm

Outcome variable	Treated (Constraints)	Control (Unconstraint)	Std. Mean Diff.	Var. Ratio	eCDF Mean
Aggregate Expenditure HH) (USD)	1847	1909	-0.054	1.346	0.033
Aggregate Income HH (USD)	2901	3146	-0.146	0.107	0.011
Income P & S (USD)	5084	5051	0.020	0.801	0.043
Feed Fertilizer Medicine (USD)	1251	1288	-0.065	0.916	0.022
Income Non Farming (USD)	1245	1581	-0.279	0.054	0.024
Income other Fish_Rice_Veg (USD)	428	416	0.034	1.197	0.015

	Control (Constraint)	Treated (Unconstraint)
All	224	221
Matched	221	221
Unmatched	3	0
Discarded	0	0

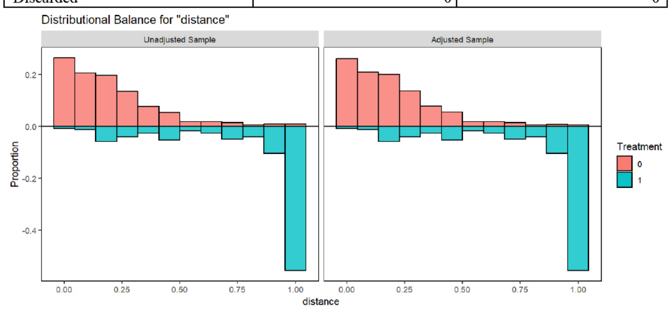

Table 1 Results of Probit regression and marginal effects of the determinants of credit constraint

	Estimate	z value	Pr(>|z|)	Margl
	2.312	0.014	0.989	
Difficult Process.	-2.050***	6.610	0.000	
Long_credit_process	-1.057***	4.012	0.000	
Lack_Cr._supplier	0.471*	1.036	0.300	
Dont know where_to_apply	-0.342	-1.042	0.298	-
No need (Resource available at HH)	-3.637	-0.022	0.982	-
High Interest Rate	-0.179*	-0.881	0.379	-
Collateral	-2.661	-0.016	0.987	-
Bank account	-0.189	-0.429	0.668	-
losing_collateral	0.300*	1.250	0.211	
fear_rejected	-0.413*	-1.671	0.095	-
Dont like to be indebted	0.115*	0.445	0.656	
loan rejected	-1.112**	2.800	0.005	
Too indebted	-1.337**	3.975	0.000	
Age	-0.012	-1.425	0.154	-
Education Yr	-0.031	-1.368	0.171	-
Enterprize diversity index (OGI)	0.121	0.172	0.864	
FarmSize_hect	-0.061	-0.260	0.795	-
ExPerience	0.027**	2.624	0.009	
Aggre_Income_HH	-0.002*	-0.350	0.727	
Income NonFarming	0.000	0.217	0.828	

DIVERSIFICATION ECONOMIES AND EFFICIENCY IN PRAWN GHER FARMING: A STUDY IN BANGLADESH

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Gher farming is a unique fish farming systems in coastal region of Bangladesh that combines freshwater prawn, fish, rice and vegetables. This study aims at examining the system's performance by examining economies of diversification and scale among these complementary enterprises and technical efficiency of the whole farm. The stochastic output distance function is used to measure economies of diversification, scale and technical efficiency based on the data obtained through direct interview of 452 gher based prawn fish farms. The finding indicates that there is an existence of significant technical inefficiency in gher farming, which implies that there may be opportunities expand gher outputs by eliminating technical inefficiency. Furthermore, there exist diversification economies in gher prawn farming, particularly in the prawn with other fish and with vegetable combination. Scale diseconomies (0.708) are also found in gher farming. The estimated technical efficiency level is 74% suggesting that there is potential to recover a substantial amount of output (26%) by reducing inefficiencies. Diversification among enterprises leads to significant efficiency gains. Moreover, farmers' education, experience, access to credit and plot size positively impact efficiency. These results suggest that promoting diversification, especially prawn-rice combination, is beneficial for gher farming systems.

Figure 1 Diversification efficiency scores

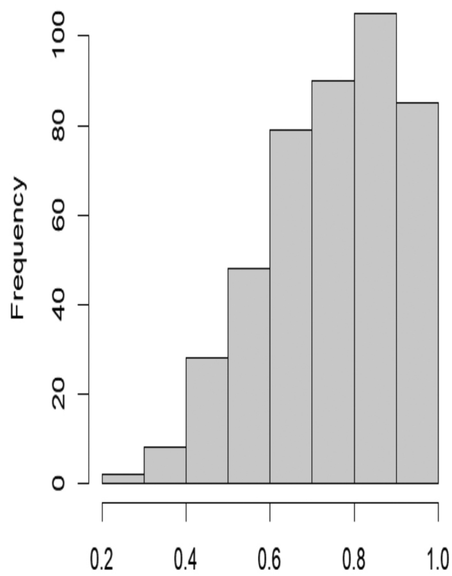
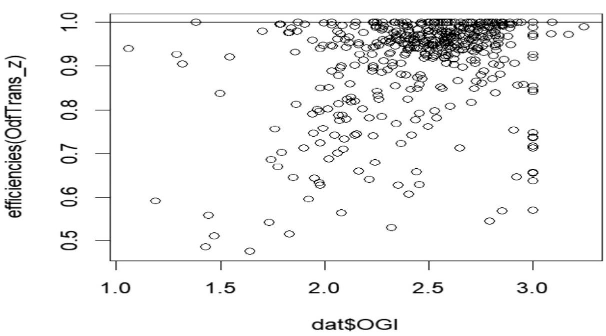


Table 2 Economies of Diversification

Enterprise combination	Coefficient	S.E.
Prawn with Rice	-0.297	0.4
Prawn with Other Fish	0.14	0.6
Prawn with Vegetable	0.098	0.23
Other fish with vegetable	0.012	0.43

Figure 2 Diversification index (ogive index) and efficiencies



INTEGRATING WATER TREATMENT WITH NUTRIENT UTILIZATION IN INTENSIVE AQUACULTURE BY A NEW MICROAEROPHILIC MEMBRANE ASSIMILATION REACTOR SYSTEM

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Aquaculture is fast growing due to increasing demand for fish (FAO, 2022). Its most expensive input is fish feed, which contains 30-50% protein, of which only 20-30% is transformed into fish biomass. The rest is excreted as toxic ammonia and solids. Biofloc technology (BFT) was suggested several decades ago as a potential method of removing total ammonia nitrogen while simultaneously producing protein-rich flocs. Up to date, BFT has resulted in low water quality with a high energy demand to support aeration in the fishpond. Therefore, it was unsuccessful or limited to specific fish species, mainly in small-scale trials. Yogev and Gross (2019) introduced a novel microaerophilic bacterial assimilation process that suppresses nitrification in favor of biomass production. This was done by using a side reactor in which excreted ammonia, fish solid waste, and external carbon sources are assimilated into protein-rich biofloc.

An obstacle in the integration of BFT into the suggested RAS was the relatively high level of total suspended solids (TSS) that averaged ~127 mg/L. To address the issue of high TSS, a hollow-fiber ultrafiltration membrane treatment was introduced (Fig. 1), and its effect on the fish tank water quality and performance of the cultured fish (barramundi, *Lates calcarifer*) was tested. The experiment setup of the pilot scale RAS included a fish tank, and a drum filter for solids collection, which were directed into the micro-aerophilic assimilation reactor. Conditions in the reactor included dissolved $O_2 < 2$ mg/L; C: N ~15 (with semolina as external C source). A hollow fiber ultrafiltration membrane system was integrated, receiving the effluents from the bioreactor. Barramundi stock density of ~20 kg/m^3 was used with daily feeding of 2% of the total stocking biomass.

A 100% fish survival rate and FCR of 1.5 were observed. Nearly 45% of the feed N that was not recovered by the fish was assimilated into microbial (biofloc) protein, demonstrating significant waste reuse. Furthermore, water quality has shown considerable improvement, and TSS was reduced by 74% to 33 mg/L. These outcomes underscore the system's potential for nutrient recycling, waste reduction, and improved water quality towards the development of efficient assimilation-based intensive aquaculture systems.

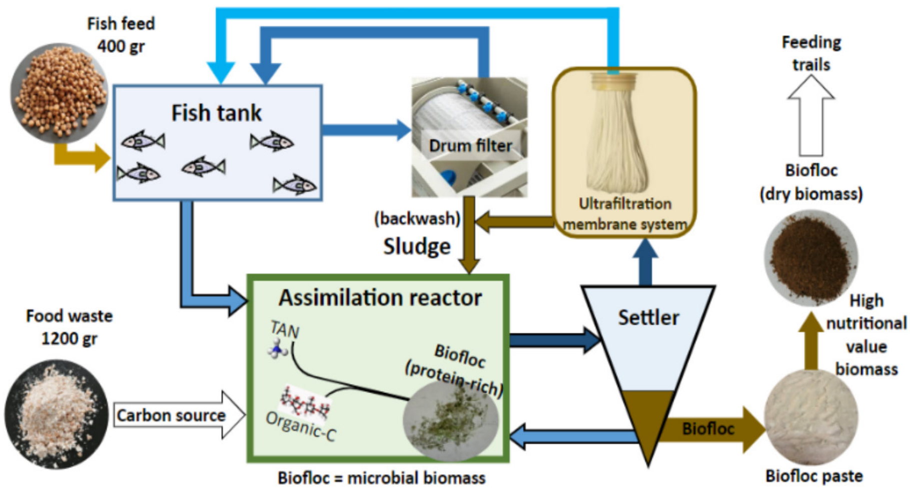

Fig. 1 Novel near zero waste RAS in which recovered N excretions and fish solid waste are assimilated by microbes into protein-rich microbial biomass (biofloc).

GENDER PARTICIPATION IN SUSTAINABLE SHRIMP FARMING AND VALUE CHAINS IN THE CLIMATE VULNERABLE AREAS OF BANGLADESH

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This research broadly explores women's role in shrimp farming and processing industries in areas vulnerable to climate change. It specifically examines how women's participation affects their livelihoods, whether there are any wage disparities and the intricacies that arise regarding the decision-making process and empowerment of women. A survey of 100 respondents from shrimp value chains in Khulna district, known for shrimp production but vulnerable to climate change, was conducted. Half of the respondents were producers, and half were from processing plants.

The study found that women aged 25-35 actively contribute to the shrimp industry through cultivation and processing. Their increased participation has also led to a significant shift in their livelihood status. This change becomes all the more noticeable during seasons susceptible to climatic changes when male members migrate for off-farm activities. Consequently, women take on all aspects of shrimp farming operations, showcasing their resilience and adaptability to environmental challenges.

Women in the shrimp industry face social barriers that limit their access to opportunities despite having decision-making power, specifically in times of worst climatic hazards. Male and female workers in the industry face wage disparities ranging from 10 to 20 percent (Table 1) in areas such as collecting shrimp seeds, farm labor, deportation work, and processing activities. Informal payment methods exacerbate the problem. To achieve pay equity between male and female workers, the study recommends a minimum 15 percent wage increase.

The WorldFish report reveals that about 80 percent of the casual workers in shrimp processing factories are women. However, less than 1 percent of the managers in these factories are women. This study emphasizes the required policy changes to promote gender equality and ensure fair participation of women in the shrimp farming and processing sectors, even in changing climate conditions. Strategic policy frameworks should prioritize women's empowerment, equitable access to opportunities, and the elimination of wage inequities to achieve sustainable and inclusive growth in coastal shrimp farming communities.

TABLE 1: Gender role and equity status in shrimp industry

Gender role	Average income (BDT/month)		Gender Equity Status
	Male	Female	
Seed collection	3500	2500	10% wage gap
Farm laborers	4300	3100	Women are paid 12% less
Deport workers	5000	3000	Not maintained (20% gap)
Processing plant workers	8400	7000	Moderately maintained (14% less).

Source: Authors estimation from survey data

DAPHNIA AS A MONITORING TOOL FOR AQUACULTURE SYSTEMS

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Traditional water monitoring is typically performed with the use of sampling methods or commercially available sensors. While both of these methods provide a reliable dataset for environmental monitoring, they are limited in terms of either the speed or robustness of the data collected. A novel method is proposed, where the environmental data is collected with living organisms. By reading the animal's reaction to its surroundings, we can gain knowledge of the state and stability of the water body. We combine living organisms with artificial parts in the concept of a "biohybrid entity" to increase the robustness of aquatic monitoring. This methodology increases significantly the feasible duration of the monitoring missions and allows them to run continuously. This early-warning system is beneficial to aquaculture systems as it collects a high volume of water quality data and its various aspects.

An informed choice of the organisms of interest is needed to optimize the robustness of the environmental readings of the biohybrid. Through an extensive literature search, *Daphnia magna* was selected as a good bioindicator for the biohybrid approach. This species is highly sensitive to various changes in the environment and presents a unique set of behaviours as stress reactions. By reading these behaviours with an automated image analysis, the state of the water can be reliably estimated.

A field setup was designed to host and harvest data from Daphnia. This biohybrid module includes a flow-through cage restricting the swimming area of the animals. The cage is observed with a Raspberry Pi camera plugged into a Raspberry Pi Zero microcontroller. A controllable LED ring light allows the Daphnia to be readable by the camera.

This system presents an opportunity for extended water monitoring and early detection of toxins in aquaculture systems. It aids in mitigating the effects of a contaminant, disease outbreaks, rapid worsening of the water quality and other damaging factors. With its easy application and low production cost, it is an attractive monitoring tool for aquaculture production.

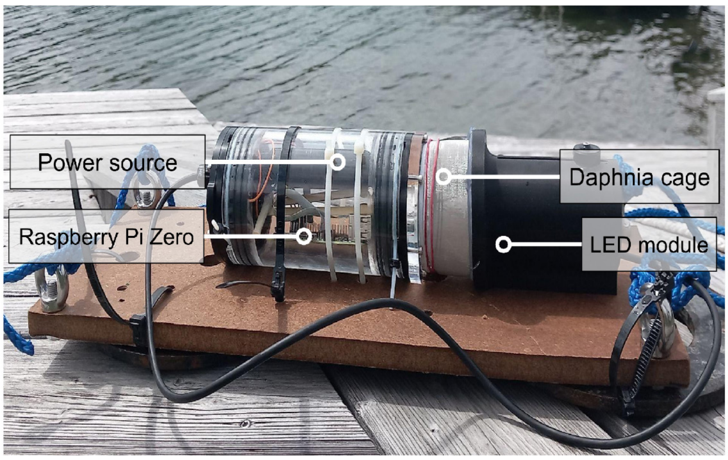

Figure 1. The Daphnia module with a Daphnia cage, a power source, a Raspberry Pi Zero computer and an LED light source.

IMMUNO-MODULATORY EFFECTS OF DIETARY MACRO- AND MICROALGAE ON TURBOT (*Scophthalmus maximus*) BREEDERS: NON-LETHAL ASSESSMENT THROUGH HUMORAL IMMUNE PARAMETERS AND SKIN MUCUS PROTEOMICS

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Europe's bioeconomy responds to escalating food, feed, and bio-based product demands by utilizing renewable resources from terrestrial and marine environments. Aquaculture sector, particularly marine aquaculture, plays a crucial role in ensuring sustainable food security. However, it faces significant challenges due to rapid global population growth and increasing protein demands, creating the need for innovative solutions. This study delves into turbot (*Scophthalmus maximus*), a prized flatfish for human consumption, predominantly farmed in Southern European countries, Iceland, and Norway. Turbot farming faces challenges, particularly in maintaining high and consistent larval survival rates, which are largely dependent on the breeder quality. Aligned with the bioeconomy concept, the study explores solutions by developing new feeds for turbot breeders, incorporating sustainable marine compounds such as microalgae and macroalgae.

The study was conducted at Flatlantic, a commercial aquaculture facility, where turbot breeders were fed two experimental diets. The diets included a control group with the baseline diet and an ALGAE diet enriched with a blend of macro- and microalgae. The ALGAE diet aimed to enhance the immune status of turbot breeders during the breeding season. A holistic less invasive and non-lethal sampling techniques were performed for the collection of blood and skin mucus to study humoral parameters and proteomics in skin mucus.

Results from the four-month trial revealed significant discrimination between experimental diets and gender, primarily influenced by IgM levels in skin mucus and peroxidase, bactericidal, and lysozyme activity in blood plasma. Although no clear differences were observed in plasma humoral parameters, a tendency for higher lysozyme activity in fish fed the ALGAE diet, especially in female breeders, was noted. Additionally, higher bactericidal activity was observed in male breeders, particularly those fed the control diet. Interestingly, IgM values in skin mucus increased in fish fed the ALGAE diet, particularly in female breeders.

The study concludes that incorporating a blend of micro- and macroalgae in turbot breeders' diets has no adverse effects on their performance, while demonstrating a modulatory effect on their immune status. Notably, the increase in IgM values in skin mucus suggests a potential enhancement in the immune response, indicating that dietary supplementation with micro- and macroalgae could contribute to improving the overall health and resilience of turbot breeders against pathogens. The proteomic analysis of the skin mucus corroborated the differences between experimental diets and gender. A total of 3394 proteins were identified in all treatments. Overall, only 144 out of the total were significantly different between gender, and 126 out of 3394 contributed to the differences between CTRL and ALGAE fed fish. Among them immune system related proteins such as calpains, bactericidal permeability-increasing protein, superoxide dismutase [Cu-Zn], were found to be differently expressed. This research offers preliminary insights into the specific proteins and peptides involved in the fish's immune response and their interaction with the ALGAE blend.

This work is part of the BREEDFLAT project (EEA.BG.CALL4.019.2020).

OPTIMIZATION OF FEEDING STRATEGIES WITH NUTRIENT-BASED MODELS: RESULTS OF MODEL EVALUATION

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The OptiFeeSH project (www.sparos.pt/projects/optifeesh/) aims to develop an innovative IT solution that enables fish farmers to select the optimal combination of feeds and feeding rates, tailored to their specific farm conditions. This solution is grounded in the use of nutritional-based models that simulate fish growth and nutrient retention with optimization algorithms that will find the feeding regime that optimizes growth, economic conversion or other KPIs. The models include independent inputs (e.g., feed quantity and composition, temperature) and are formulated by analytic expressions that allow to solve the equations for several optimization targets (e.g., maximum growth, maximum profit and minimum FCR). The evaluation of these models is a crucial step, as they form the basis for data enrichment processes within the OptiFeeSH software. Herein, a nutrient-based model to predict the growth of Nile tilapia (*Oreochromis niloticus)* and gilthead seabream (*Sparus aurata*) is evaluated.

All data used in this work were collected from the scientific literature and from trials generated by Sparos Lda., and its partners in R&D projects. The evaluation process involved both qualitative and quantitative aspects, including error metrics like Mean Absolute Percentage Error (MAPE). The results showed that the model had slightly higher calibration and evaluation errors for Nile tilapia (calibration MAPE = 44.0%; evaluation MAPE = 38.5%) than for seabream (calibration MAPE = 10.8%; evaluation MAPE = 9.7%), although acceptable. Considering only the relevant datasets for production conditions the error metrics are even lower: calibration MAPE = 8.6% for Nile tilapia; calibration MAPE = 8.2%; evaluation MAPE = 5.2% for seabream.

The OptiFeeSH project has the potential to transform fish farming practices through its innovative approach, and future research will focus on expanding the model to include more species and exploring other optimization targets.

This project receives funding from the Eurostars 3 (Call 3) joint programme under grant agreement E!2498. The Portuguese National Authorities or the European Union cannot be held liable for any use made of this publication. The project partners include: SPAROS, Blue Analytics, Integrated Information Systems and Flatlantic.

TABLE 1 – Mean absolute percentage error (MAPE) for the model calibration and evaluation (with an independent data set), for Nile tilapia and gilthead seabream.

Mean Absolute Percentage Error (%)	Calibration		Evaluation	
	All data	Production relevant	All data	Production relevant
Nile tilapia	44.0	8.6	38.5	-
Gilthead seabream	10.8	8.2	9.7	5.2

THE SYNERGISTIC EFFECTS OF FOUR MEDICINAL PLANT SEEDS AND CHELATED MINERALS ON THE GROWTH, IMMUNITY, AND ANTIOXIDANT CAPACITY OF RAINBOW TROUT

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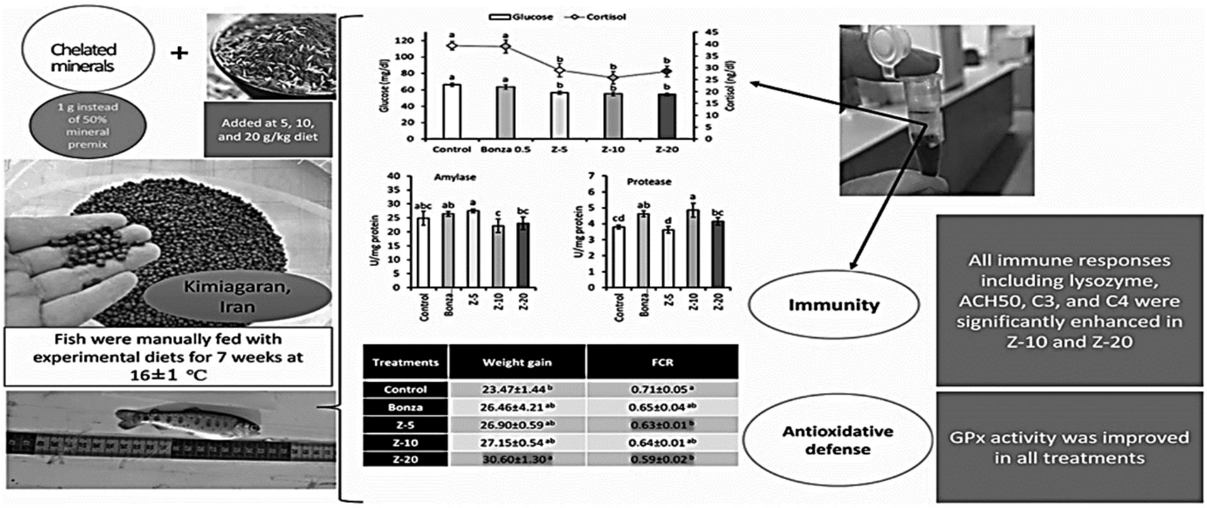
Trace minerals are essential for the normal functioning of all living organisms. In addition, the positive effects of several medicinal plants have been demonstrated in aquaculture. In the present study, we aimed to investigate the effects of a mixture of medicinal plants and test the synergistic effects of medicinal plants and chelated minerals on fish growth and immunity.

Thus, in the present experiment we evaluated the combined effects of a commercial chelated mineral source (BonzaFish®) and a mixture of 4 medicinal plants including caraway, green cumin, dill, and anise. Rainbow trout were fed with five formulated diets, including a control diet (basal diet), Bonza (basal diet + 1 g/kg BonzaFish®), Z-5 (basal diet + 1 g/kg BonzaFish® + 5 g/kg mixture of plant seeds), Z-10 (basal diet + 1 g/kg BonzaFish®+10 g/kg mixture of plant seeds), Z-20 (basal diet+1 g/kg BonzaFish®+20 g/kg mixture of plant seeds) for 6 weeks. In diets including BonzaFish®, 50% of the inorganic mineral premix was replaced by BonzaFish®.

Results revealed that fish receiving the Z-20 diet showed the best performance in terms of growth parameters, followed by the Bonza treatment. The highest protease activity was found in Z-5 and Z-10. RBCs were highest in Z-5, while the highest WBCs and hemoglobin were found in the Bonza treatment followed by Z-20. Stress biomarkers were lowest in the Z-20 treatment. Results showed that Z-20 could elicit the most robust immunological responses of lysozyme activity, ACH50, total Ig, C3, and C4. In conclusion, chelated minerals could be successfully used to replace 50% of mineral premix with no negative impacts on fish growth and together with four medicinal plants, could enhance rainbow trout overall growth performance and immunity.



CO-DIVERSIFICATION OF AN INTESTINAL MYCOPLASMA AND ITS SALMONID HOST

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Introduction

Understanding the evolutionary relationships between a host and its intestinal resident bacteria can transform how we understand adaptive phenotypic traits. The interplay between hosts and their resident bacteria inevitably affects the intestinal environment and, thereby, the living conditions of both the host and the microbiota. We present the gut metagenome from wild Atlantic salmon (Salmo salar), a new wild organism model with an intestinal microbiota of low complexity and a well-described population structure, making it well-suited for investigating co-evolution.

Methods

Sample collections for Atlantic salmon were taken from five locations across the coast of northern Norway, spanning 700 km. DNA extraction was performed using ZymoBiomics DNA miniPrep, followed by sequencing at Novogene (Cambridge, UK). Reference-based mapping and taxonomy annotation were applied to remove unknown eukaryotic contaminants and analyze eukaryotic gut content using MGmapper. Genome-resolved metagenomics was conducted with to infer microbiome taxonomy, functionality and population structu. Population structure analysis was done to investigate co-divergence.

Results

Our data reveal a strong host selection of a core gut microbiota dominated by a single Mycoplasma species. We found a clear co-diversification between the population structure of Atlantic salmon and nucleotide variability of the intestinal Mycoplasma populations conforming to expectations from co-evolution between host and resident bacteria. Our results show that the stable microbiota of Atlantic salmon has evolved with its salmonid host populations while potentially providing adaptive traits to the salmon host populations, including defence mechanisms, biosynthesis of essential amino acids, and metabolism of B vitamins.

Conclusions

The case of Atlantic salmon studied here has not only furthered our evolutionary understanding of this species, but the findings also hold potential for further discoveries towards feed or health optimisation resulting in more sustainable aquaculture practices. We envisage that our study may inspire similar investigations in systems previously investigated using amplicon-based markers to reveal the intriguing functional host-microbiota interactions.

MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE AMONG DIARRHEAGENIC AND NON-DIARRHEAGENIC *Escherichia coli* FROM HEALTHY AND DISEASED FRESHWATER FARMED FISH

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The aim of this study is to understand molecular characterization and antimicrobial resistance of diarrheagenic and nondiarrheagenic *E. coli* from healthy and diseased farm fish from *Cypriniformes* order. A total of 516 healthy and diseased farmed fish samples belong to *Cypriniformes* order were collected from various regions of Punjab and screened for E. coli. Biochemical tests and PCR confirmation were conducted using the 16S rRNA and UspA genes. DEC was examined through PCR using different virulence genes. All isolates were tested for various antibiotics using the disk diffusion method. The phenotypic and genotypic resistances of Extended-spectrum □-lactamases (ESBLs) were investigated. A Chi-square test was performed to compare the prevalence of E. coli, virulence genes, and ESBLs genes with a significance level of $P \leq 0.05$. Out of the total fish samples, 16.66% and 36.57% of E. coli strains were confirmed through biochemical tests and PCR, respectively. The DEC pathotypes were recorded in 30% and 62.02% of isolates from healthy and diseased fish, respectively. A higher rate of 19.37% of EAEC pathogroup was observed in both healthy and diseased fish, while the EIEC pathogroup was only observed in diseased fish at a rate of 10.07%. The significance ($P \leq 0.05$) was observed among the virulence genes. The highest resistance rates among diarrheagenic and non-diarrheagenic strains were observed with tetracycline, amoxicillin, and ceftazidime. A higher prevalence of CTX-M (56.81%) and TEM (43.18%) was recorded in DEC pathotypes, while blaSHV was not detected. The high prevalence of DEC E. coli, and the production of ESBLs, indicate unhygienic practices and the misuse of antibiotics in fish farms.

PHYSIOLOGICAL PERFORMANCE OF *Magallana gigas* JUVENILES UNDER A WARM AND NATURALLY ACIDIFICED ENVIRONMENT

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By the end of the century, climate changes are predicted to occur through increases in sea and air temperature, sea level, water acidification and severe fluctuations in salinity. The worst scenario predicted by the Intergovernmental Panel on Climate Change (IPPC) forecasts a 4°C increase in temperature and a decrease in pH to levels below to what was previously predicted (pH 7.7). Ocean warming and acidification are the direct key stressors of anthropogenic climate change, responsible for causing deleterious effects on marine biological and physiological processes which, consequently, affect bivalve production. Most of the studies have focused mainly on the variation of only one factor of climate change, even though, in the natural environment, organisms are affected by several combined factors. Therefore, this study aimed to address the combined effects of temperature and pH on the physiological and growth performance of *Magallana gigas* spat.

Four treatments with different pH (pH 7.6 and pH 8.2) and temperatures (22 ± 1ºC; 26 ± 1ºC) were tested in triplicate. The treatment pH 8.2; 22ºC was considered as the control group since it represents the regular conditions. Juveniles of *Magallana gigas* (9.29 ± 0.58 mm shell length; 86.63 ± 11.12 mg total weight), were separated into twelve groups of 200 individuals and placed into the experimental tanks for 30 days. Oysters were fed daily with a mixed microalgae diet at a ratio of 6% of oyster dry weight (g) in algal dry weight (mg). Environmental parameters and mortality were registered daily. Condition index, biometric measurements, and biochemical composition (proteins, total lipids and glycogen content) were evaluated every 15 days.

Survival and shell growth were not affected by any of the experimental conditions. However, after 30 days of exposure, the soft tissue weight of spat exposed to higher temperature treatments (26°C) was significantly lower, regardless of the pH. Likewise, oysters exposed to higher temperatures exhibited significantly lower condition index, with the control treatment (pH 8.2; 22ºC) showing the best condition. The biochemical composition did not show significant differences among treatments. The Principal Component analysis (PCA) revealed that temperature had a negative correlation with soft tissue weight (fresh and dry) as well as with condition index, while pH did not significantly correlate with any other variable.

This study highlights that the physiological performance of bivalves is more affected by ocean warming than ocean acidification. Consequently, the increase in the frequency and intensity of heat waves may have severe implications for bivalve aquaculture.

Acknowledgements: Broderick J. House was supported by the Harding Distinguished Postgraduate Scholarship.

A COLLABORATIVE ECOSYSTEM FOR SUSTAINABLE INTEGRATED AQUACULTURE

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New sustainable and profitable Integrated Multi-Trophic Aquaculture value chains in the Atlantic regions were developed within the ASTRAL project.

ASTRAL is a HORIZON 2020 project financed under the Blue Growth programme. The project contributes to the implementation of the Belém Statement and other trans-Atlantic agreements to develop a strategic partnership on marine research, and it will participate in building the All-Atlantic Ocean Research Community.

The project main goal is to increase value and sustainability for integrated multi-trophic aquaculture (IMTA) production by developing new, resilient, and profitable value chains. In IMTA production, multiple aquatic species from different trophic levels are farmed together. Waste from one species is used as inputs (fertilisers and food) for another species. The IMTA concept is used at four 'labs' in Scotland, South Africa, Brazil and Ireland; these sites grow species such as fish, scallops, lobsters, oysters, urchins and seaweed. A prospective IMTA lab will also be assessed for future production in Argentina.

ASTRAL goals include the increase of circularity and the achievement of zero-waste aquaculture systems, as well as the creation of appropriated business models to increasing profitability. Potential climate risks and emerging pollutant (microplastics, harmful algae blooms, pathogens) are assessed, together with the development of innovative technology to monitor the production and the interactions from/to the surrounding environment (specific sensors and biosensors, IoT and AI data analytics), with the final aim to provide monitoring recommendations to policy makers. Sharing knowledge and capacity development are among ASTRAL priorities, to build a collaborative ecosystem along the Atlantic Ocean with industrial partners, SMEs, scientists, policy makers, social representatives, and other relevant stakeholders.

The ASTRAL consortium assembles a multidisciplinary team of experts from different sectors: RTO, academia, SMEs, industrial clusters, intergovernmental organizations and other relevant stakeholders from several countries along the Atlantic Ocean. The ASTRAL consortium includes partners from 10 countries - Norway, Scotland, Ireland, France, Spain, Portugal, Nigeria, South Africa, Argentina and Brazil. Partners includes Research and Technology Organisations, Universities, SMEs, associations/industrial cluster and governmental and intergovernmental organizations.

A summary of the project's results, that will finish at the end of September 2024, will be presented, highlighting the collaborations established between stakeholders and the transfer of knowledge between the two sides of the Atlantic Ocean.

WELFARE IMPROVEMENTS IN SEA BASS *Dicentrarchus labrax* THROUGH ADAPTOGEN AND ENVIRONMENTAL ENRICHMENT

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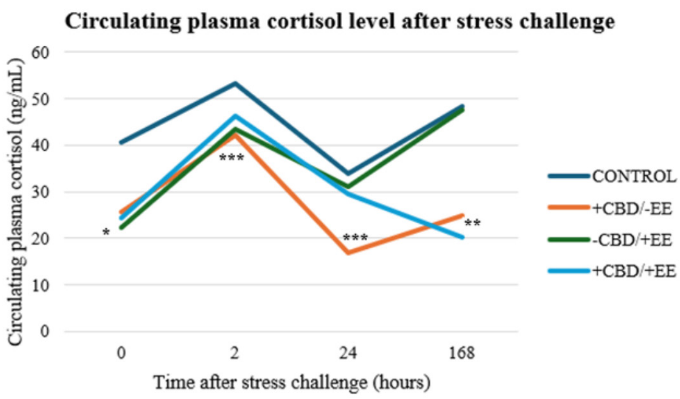
Promoting fish welfare is essential for an effective maintenance of cultured fish health, especially in the current scenario of reduced antimicrobials use. Among the different strategies to improve the welfare of farmed fish is structural and/or environmental enrichment (EE), which has been shown to improve common indicators of fish welfare, such as plasma cortisol. Similarly, the use of adaptogens as functional additives has also been shown to be an effective tool to improve fish welfare. Certain nutraceuticals, such as cannabidiol (CBD), a non-psychoactive derivative of *Cannabis sativa*, has been demonstrated to have anti-inflammatory, neuroprotective and anxiolytic properties. The objective of the present study is to explore the potential effect of an adaptogen containing cannabidiol (CBD) and melatonin (Alchemy CBD oil 40% - Full Spectrum - MCT), as a feed additive, and aeration modification, as environmental enrichment or their combination on European sea bass (*Dicentrarchus labrax*) culture. For this purpose, 4 treatments were assayed; Treatment 2 including CBD in diet at 0.001%; Treatment 3 consisting on aeriation with curtain of bubbles as structural enrichment and Treatment 4 combining both CBD & bubble curtain. (1= control; 2=+CBD/-EE; 3=-CBD/+EE; 4=+CBD/+EE).

Fish were fed for 38 days and subsequently subjected to a confinement stress challenge (2h, 24h and 7d). The stress response was assessed by measuring plasma cortisol levels, lysozyme and bactericidal activities, as well as the relative gene expression of *gr2*, *pomc*, *crh* and *htr1a*□ from telencephalon, hypothalamus and hypophysis. After stress challenge, +CBD fish presented the lowest cortisol levels **(Figure 1)**. Respect to survival rate, -CBD/+EE got the highest one (97.22%) and control got the lowest one (83.33%). In addition, fish subjected -CBD/+EE treatment presented improved SGR (%/day) and RG (%). Combined, CBD and environmental enrichment can improve welfare in European sea bass juveniles.

Figure 1. Circulating plasma cortisol level expressed in ng/mL of European sea bass (*Dicentrarchus labrax*) at different times after the stress challenge.



* Different symbols indicate significant differences (p<0.05) with the control treatment
** Different symbols indicate significant differences (p<0.05) between CBD and EE
*** Different symbols indicate significant differences (p<0.05) between +CBD/-EE and the other treatments

STRAIN-DEPENDANT ANTIVIRAL ACTIVITIES OF HEAT-INACTIVATED LACTIC ACID BACTERIA WHEN FED TO ZEBRAFISH (*Danio rerio*) – A NOVEL DIETARY STRATEGY

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The lack of commercial vaccines and treatments for many fish viral diseases remains a major bottleneck in aquaculture production. There is a continuing need to further develop antiviral prophylaxis strategies. The trial compared the effect and modes of action of different heat-inactivated (HI) *Lactobacillus* species on the gut barrier, intestinal immunity, and anti-viral responses in the zebrafish (*Danio rerio*) challenged with viral hemorrhagic septicemia virus (VHSV). Our hypothesis was that postbiotics were able to modulate the innate immune responses in a species and strain-dependent way and exhibit a type-II like interferon response for removal of intracellular pathogens.

The 31-day study used wild-type adult zebrafish (28 fish/tank) subjected to a VHSV challenge by intraperitoneal injection at day 21. Five treatments were tested in triplicate: 1] non-supplemented basal diet (C1), 2] non-supplemented basal diet, challenged (C2) and 3 diets each supplemented prior cold-press at 6×10^6 CFU equivalent/ g feed of 3] *L. paracasei* H108 (T1), 4] *L. plantarum* HA-119 (T2), or 5] *L. helveticus* HA122 (T3). Each HI bacterial strain (Lallemand SAS, France) was characterized by electronic and atomic force microscopy. Before the viral challenge, 54 fish/treatment (18 fish/tank) were euthanized and intestinal and kidney collected to perform histomorphometry and immune response profiling using gene expression (qRT-PCR) and immunoblotting. At day 21, 30 fish/treatment (10 fish/tank) were challenged with VHSV. Fish survival and viral clearance (qRT-PCR) were monitored for 10 days post-challenge. Differences between control and experimental groups were estimated by a 2-way ANOVA and Bonferroni post-hoc test with significance accepted at $P <$ 0.05.

The level (immunoblotting) of TNF-α, IFN-γ, IL-10, and alkaline phosphatase was higher in intestine and kidney compared to that of control with T1, T2 and T3. Interestingly antiviral response genes, such as *ifnγ, ifnγ1, mx,* and *cd8α* were up-regulated in both intestine and kidney (except *mx* in intestine) by the diet supplemented with T2. A better numerical (T3, P = 0.11) and statistical (T2; $P < 0.01$) survival was observed in postbiotic groups compared to treatment C2, while a trend ($P < 0.10$) of a lower number of viral copies was detected in the T1 group compared to C2. As a result of higher cumulative percent survival and augmentation of immune responses, fish fed the T2 diet presented a robust antiviral defense against VHSV. The T1 group also displayed a positive immune response regardless of survival rate.

In conclusion, this study shows that heat-inactivated bacteria can be immunogenic by activating a type-II like interferon antiviral defense. Further research to characterize the bacterial cell wall microbial associated molecular patterns will be useful to understand the exact immune functional role(s) of the inactivated bacterial strains, and to optimize their use in aquaculture.

THE EFFECT OF USAGE OF *Hermetia illucens* MEAL IN SIBERIAN STURGEON DIETS ON GASTROINTESTINAL TRACT MICROBIOTA AND HISTMORPHOMETRIC PARAMETERS

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Siberian sturgeon is an important species in aquaculture due to its use for caviar production and high quality of meat. One of the most important factors that indicates the health of juveniles is gastrointestinal tract functionality. Scientific literature reported numerous data on the impact of fish nutrition on gut health and microbiota diversity. The following experiment was designed to, among others, evaluate the impact of partial fishmeal replacement with low-fat *Hermetia illucens* (BSF – black soldier fly) meal in Siberian sturgeon diets on gastrointestinal tract microbiota composition and histomophometric parameters.

The amino acid composition was analyzed to calculate the conversion factor (Cf) for BSF. Nine diets were prepared: CON – control group, H75 – group with 7.5% inclusion of BSF meal, H150 – group with 15% inclusion of BSF meal, H225 – group with 22.5% inclusion of BSF meal, H300 – group with 30% inclusion of BSF meal, HK75 – group with 7.5% inclusion of BSF meal based on calculated Cf, HK150 – group with 15% inclusion of BSF meal based on calculated Cf, HK225 – group with 22.5% inclusion of BSF meal based on calculated Cf, and HK300 – group with 30% inclusion of BSF meal based on calculated Cf. A total of 100 fish were used in each experimental group, 20 fish per tank and five replicates per group. The experiment was conducted for 60 days. Fry were maintained in a recirculating RAS system. In the last day of experiment four fish from each tank were euthanized and dissected to collect biological samples – gastrointestinal tract digesta and proximal part of intestine. Gastrointestinal tract digesta were analyzed by fluorescence in situ hybridization.

In the case of histomorphometric parameters, for the intestinal villi height, the highest values in the experimental groups were observed in fish from groups H75, H150, H300, HK225 and HK300, although these results did not differ statistically significantly from the control group, but only between other experimental groups ($P< 0.001$). However, higher muscle layer was observed only in fish from the HK75 group ($P<0.001$).

In the case of the gastrointestinal microbiota composition, an increased total number of bacteria was observed in the H150 and HK300 groups compared to the control group ($P=0.010$). The lowest values of pathogenic *Aeromonas* spp. were observed in the case of fish from groups H75, HK75 and HK150. At the same time, fish from the control group had the lowest value of *Enterobacteriaceae* ($P=0.003$). A reduced content of *Lactobacillus* spp. bacteria was observed in individuals from the H75 group ($P=0.039$). Other bacteria groups did not show nutritional effect.

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

THE EFFECTS OF HORIZONTAL SUBSTRATE AND FILTRATION STRATEGY ON PACIFIC WHITE SHRIMP *Litopenaeus vannamei* PRODUCTION AND WATER QUALITY IN RAS

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Shrimp is the most consumed seafood item in Europe, but its production is still inadequate to meet consumer demand and high-quality domestic supplies are lacking. To facilitate intensive farming of brackish-water species, recirculating aquaculture systems (RAS) can be adopted. This allows farmers to control inputs and outputs, helps save water and salt costs, and prevents adverse environmental impact. This project focused on assessing the effects of two different types of RAS (clear-water and hybrid), along with horizontal substrate on intensive Pacific white shrimp production and water quality. Clearwater (CW) RAS is an effective strategy for shrimp production but may have relatively high equipment and operational costs. Simple hybrid systems (HY) have some advantages as external biofilters maintain water quality, but solids filtration is relatively minimal. Artificial horizontal substrate in RAS may help to enhance shrimp production by increasing the surface area for shrimp to graze and facilitating higher shrimp density, thereby justifying more intensive filtration.

In this study, two levels of each experimental factor were used: system type (CW vs HY) and presence of an artificial substrate (WS) versus absence of substrate (NS). This resulted in 4 treatments: HY-WS, HY-NS, CW-WS, and CW-NS each of them having four replicates randomly assigned to 1 m^3 circular tanks. Ozone, a strong oxidizing agent, was introduced in CW tanks. CW tanks had a foam fractionator, a settling chamber, and a biofilter, while HY systems had only a settling chamber and a biofilter. Four layers of horizontal cloth net, each layer covering 0.5 m^2 area, 20 cm apart from each other, and square in shape were installed as substrate in the eight circular tanks with substrate. Shrimp were stocked at 450 m^{-3} in the experimental tanks and reared for 53 days.

The HY-WS treatment had significantly higher individual shrimp weight at harvest, total biomass harvested m^{-3}, survival, and growth rate compared to the HY-NS and CW-NS treatments. The CW-WS treatment was not significantly different than HY-WS with regard to these parameters, but was also not significantly different than the HY-NS treatment. Solids and turbidity were significantly lower in the CW treatments, as intended. TAN and nitrite-N were significantly lower in HY systems and the presence of substrate also resulted in significantly lower TAN and nitrite-N. It is possible that the biofloc particles in the HY treatments and the surface area provided by the substrate both allowed for more nitrifying bacteria in the systems, thereby lowering TAN and nitrite-N concentrations. In further support of HY treatments having more nitrifying bacteria, these systems also had significantly greater average nitrate-N concentrations. Findings from this study suggest that inclusion of horizontal substrate in the intensive indoor shrimp culture system could enhance production and help in biofiltration.

EFFECT OF PROBIOTICS ON PATHOGENIC *Vibrio* BACTERIAL POPULATIONS IN CULTURE PONDS OF *Litopenaeus vannamei* AT MOPHUS BANDER, SRIKAKULAM DISTRICT, ANDHRA PRADESH, INDIA

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The current study was carried out for 147 days to assess the pathogenic *Vibrio* count of *Litopenaeus vannamei* culture ponds and the influence of probiotics from Mophus Bander, Srikakulam District, Andhra Pradesh, India. Three ponds were selected, one is control and other two were experimental (Pond A and B). The physico-chemical parameters of the culture ponds were recorded by adopting standard methods. During summer crop the control ponds were harvested at 24.0 gm on 107th day and at 31.0 gm on 121st day for the year 2022 and 2023 respectively due to the incidence of *Vibriosis* disease. Where as in winter crop during 2023 the control pond was harvested at 5.5 gm on 47th day because of *Vibriosis* but in 2023 the control pond was harvested normally at 32.5 gm on 145th day, as this pond is free of *Vibriosis.* Experimental ponds were harvested normally in summer season at 30.5 gm on 124th day & 31.0 gm on 127th day during 2022 and 34.0 gm on 127th day & 35.5 gm on 127th day in the year 2023 respectively.

Where as in winter season of 2022 the experimental ponds were harvested normally at 28.0 gm on 124th day as well as on 127th day. In the year 2023 winter, the experimental ponds were harvested at 36.0 gm at 146th day and 35.5 gm at 147th day respectively. This study suggests that the probiotics are the key agents which have a great impact on the reduction of total pathogenic *Vibrio* count in culture ponds of *Litopenaeus vannamei*.

DIVERSIFICATION AND AQUACULTURE: TESTING PATHS IN BRETON OYSTER FARMING

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Globally, aquaculture now plays a significant role in aquatic food production, due to high demand and the limits reached by the supply from fishing. Monospecific aquaculture, particularly oyster farming, has faced sustainability challenges over the years with regular crises. Species diversification is proposed as a means to enhance the sustainability of farms, especially to support activities in the context of global change.

In Brittany, located at the westernmost part of Europe, three diversification strategies (see below) are being tested in the oyster farming sector as part of innovation research projects conducted in collaboration between professionals and scientists (financial support from the EU and the Brittany Region). The aim of this presentation is to report on some recent or ongoing work.

New species association. The combination of the abalone, *Haliotis tuberculata*, and the sea cucumber, *Holothuria forskali*, was tested. The latter, being a detritivore, could benefit from the deposits from the abalone's activity. The experiment, conducted over 9 months, demonstrates the potential of combining two species without additional inputs or labor (Figure 1).

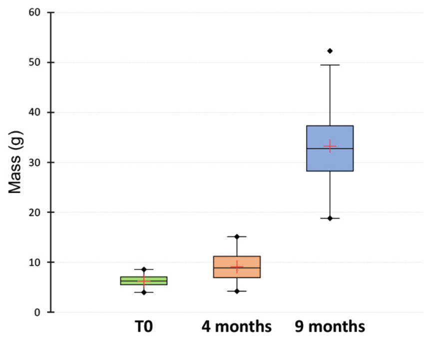

Figure 1. Sea cucumber growth

New spaces. This involves proposing offshore oyster farming (*Magallana gigas*) that is less dependent on continental vicissitudes and requires less labor. Compared to conventional intertidal farming, the survival rate and flesh yield in open sea conditions are significantly higher (Figure 2).

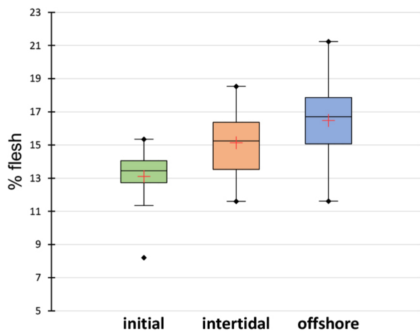

Figure 2. Oyster flesh percentage

New product range. The aim here is to a high-end, small-size product with a high flesh yield and distinctive organoleptic qualities to attract new consumers. To achieve this, the technical route was shortened to reduce mortality, and environmental conditions were selected to optimize weight growth**.**

This work shows the potential for diversification in the oyster sector with the goal of improving the competitiveness of businesses and their resilience to upcoming environmental disturbances.

LARGE SCALE SCREENING OF BIOAVAILABLE TETRACYCLINES AND ARSENIC USING WHOLE-CELL BIOREPORTER FROM PRAWN AQUACULTURE SYSTEM IN SOUTH-WEST COASTAL BANGLADESH

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Tetracycline, a widely used group of antibiotics, has been extensively employed for disease treatment in aquaculture practices in Bangladesh. Arsenic contamination in ground water is very high in southern and upper southern parts of Bangladesh. In this study, a large-scale screening of bioavailable tetracycline (TC) and arsenic (As) was carried out using Whole-Cell biosensor bacteria from the pond surface waters of selected prawn farms in the south-west coastal Bangladesh. Bioavailability of Tetracyclines and Arsenic was detected using biosensor bacteria *E. coli*K12 pTetLux1 and *E. coli*DH5R (pJAMA-arsR), respectively. Among the sampled ponds, 42 were found to contain tetracycline concentrations within the detection limit. The measured tetracycline concentrations ranged from 15.2 to 31.1 μg/l, indicating the presence of bioavailable tetracycline in the aquaculture environment of the south-west coastal Bangladesh. The average age of the experimental ponds was 11.47 years and average depth was 1.2 meters. The tetracycline concentration was highest (31.1 μg/l) in the oldest pond (20 years), while the lowest concentration (15.2 μg/l) was observed in a pond with the age of 14 years. Interestingly, an increase in tetracycline concentration was observed with increasing pond age, indicating a potential accumulation of tetracycline over time within the ponds. Bioavailable As were detected in only 3.7% of all analyzed samples. Detectable As samples were higher in number among the collected samples at Satkhira and higher in percentage at Sathkhira (20%) followed by Khulna (15%), and Bagerhat (10.2%). Though the concentrations of arsenic in groundwater (tube well water) were high in all studied regions, the concentrations of arsenic in pond water were low in every region. The concentration of As in the experimental ponds was neither very high nor in the danger limit. Use of fertilizers, drugs, pesticides, and herbicides possessed with as during fish culture might be the source of as contamination. However, extensive use of antibiotics results in the emergence and spread of antibiotic resistance in the aquatic environment.

ADAPTIVE CAPACITY IN THE SALMON FARMING INDUSTRY: RESPONSES TO SYSTEM STRESSORS AND IMPLICATIONS FOR CLIMATE CHANGE

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Aquaculture is vulnerable to the impacts of climate change including heatwaves, extreme weather events, ocean acidification, deoxygenation, and sea level rise. Vulnerability to climate change is heightened in marine aquaculture operations where fish grown in open net pen cages are exposed to the surrounding environment, and climate stressors can have impacts on production. However, climate change is not the first challenge to be encountered by the aquaculture industry, which has adapted to many different system stressors including disease events, regulatory changes, and supply chain shocks, amongst others. These events provide an opportunity to learn from the capacity of the industry to respond to previous responses and stressors and apply that understanding to climate change adaptation planning.

Through literature review and interviews with key informants, a timeline of significant aquaculture system stressors and responses was developed. This timeline is then used to analyse the adaptive capacity of the industry. Early assets-based theories of adaptive capacity assume that adaptation is a function of access to material and social capital, but these theories do not explain how adaptation is practiced or what is needed to turn adaptive capacity into action. Therefore, this analysis of adaptation in salmon farming considers adaptations to system stressors that have already occurred, identifying both internal and external enablers and barriers to adaptation using a Political, Economic, Social, Technological, and Environmental (PESTLE) framework. Analysis of previous responses to system stressors is then applied to an assessment of adaptive capacity in the context of climate change, and potential barriers and enabling factors for the mobilization of adaptive capacity into adaptation are identified.

Acknowledgements
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MOLECULAR BREEDING IN AQUACULTURE: CHALLENGES AND BENEFITS

Dr. Sharon Reikhav

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Molecular breeding, leveraging DNA testing, plays a pivotal role in facilitating the crossing, selection, and quality assurance processes within selective breeding and production programs. Demonstrated to augment genetic gains while conserving time, resources, and finances, this approach enhances product quality, success rates, and time-to-market across diverse sectors of natural-based commodities. The integration of molecular breeding into aquaculture, similarly to other industries, presents unique challenges. In this presentation, we will delineate these challenges and propose viable solutions, drawing insights from recent endeavors focused on Crassostrea virginica oysters.

RAINBOW TROUT *Oncorhynchus mykiss* ACUTE STRESS RESPONSES INDUCED BY TRANSPORT: A HOLISTIC APROACH

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Improving fish welfare in aquaculture has become a major concern in recent years. This is due to the inherent potential to maximize fish health and reduce the use of chemotherapeutics while minimizing the industry´s environmental impact and economic losses. Fish ability to cope with stress is a major health trait that can determine fish susceptibility to opportunistic pathogens and disease. The present work aimed to assess the effects of transport-induced acute stress on stress resilience as well as immune and oxidative stress responses of rainbow trout (*Oncorhynchus mykiss*) juveniles.

A trial with rainbow trout juveniles was devised to evaluate fish stress response after 2 h of transport. Fish were randomly distributed in triplicate groups of 40 fish (approx. 18 g) that were hand fed to satiation 2 times a day with a commercial-like diet. Following an acclimatization period, one group of fish was immediately sampled to serve as a non-stressed control. Additionally, two groups of fish were transferred, each, to 3 plastic containers (≈25 kg/m^3) with an O_2 saturated atmosphere and transported for 2 h. Upon arrival, one group of transported fish were immediately sampled while the remaining fish were accommodated in the original system and sampled 24 h post-arrival. Blood samples were collected for hematological procedures while plasma was used for cortisol, metabolites and humoral immune parameters. Liver was sampled for the analysis of metabolites and oxidative stress responses. Skin mucus and water were sampled both for proteomics and transcriptomics (miRNA) analyses, while skin tissue was collected solely for RNAseq analysis.

Results showed significantly increased plasma cortisol and glucose levels with a concomitant decrease of hepatic glycogen and glucose immediately after transport. Plasma immune parameters showed a significant increase of nitric oxide generation immediately after acute stress and higher hepatic lipid peroxidation and catalase activity in the recovery phase (24 h after transport). Regarding lipid metabolism, triglycerides increased in plasma whereas lactate decreased in liver 24 h after stress. Results suggested a stress induced response with clear primary and secondary stress response patterns characterised by increased plasma cortisol and the immediate mobilization of energetic resources from hepatic carbohydrate reserves, but also through the production of glucose using lactate as a non-hexose precursor in gluconeogenesis. Higher plasma triglyceride levels 24 h after stress might reveal an energy substrate shift from carbohydrates to lipids as a longer-term strategy by the organism to cope with stress.

Ongoing high throughput data analysis will allow a better understanding of stress and health biomarkers using minimally and non-invasive techniques.

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BLUE FEED INGREDIENTS FOR BLUE FOOD - CAN BLUE MUSSELS BE A SUSTAINABLE RAW MATERIAL IN FISH FEED?

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The use of blue mussels as an ingredient in fish feed is proposed as one of the solutions to increase access to sustainable raw materials and is especially relevant as a source of marine protein. In Norway, blue mussels may be one alternative to increase the amount of locally produced ingredients for fish feeds. Blue mussels can be cultivated along the Norwegian coastline, and the production potential is considered to be higher than what is relevant for human consumption.

The production of blue mussels has a very low carbon footprint, and the use of blue mussels as a raw material may therefore contribute to an overall lower footprint from the feed. This is also dependent on each processing step to make a feed grade raw material. Processing blue mussel meat into silage is of interest as a method with a lower footprint. However, initial short-term studies have shown that the treatment of the blue mussel silage prior to and during feed production can influence the feed utilization and bioavailability of minerals, which will also have an environmental impact. The present study is the third experiment in a series of studies investigating the use of blue mussel silage in feed for Atlantic salmon.

A six-month feeding study was done with Atlantic salmon (start weight ~600 grams) to determine long term effects of using blue mussel silage alone or in combination with blue mussel meal in the feed. The reference feed was designed as a commercially relevant salmon diet containing 10% fish meal. Three experimental diets were made replacing up to half of the fish meal with two levels of blue mussel silage, as well as one feed with a combination of blue mussel silage and blue mussel meal. During the experiment the fish approximately tripled the weight, and no differences were seen in feed intake and weight gain between the experimental groups. There was however a somewhat higher feed conversion ratio (FCR) in the groups given the feeds with the highest inclusion of blue mussels. The results will be presented with a focus on production parameters including feed intake, growth and digestibility, as well as nutrient uptake and welfare parameters. In addition, the environmental impact of the raw materials and feed will be evaluated.

MAARA MOANA (OCEAN GARDEN): AN INDIGENOUS APPROACH TO GROWING SEAFOOD IN NEW ZEALAND

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Howard and Robert are elders from the Ngati Wai (Water people) Aotearoa New Zealand.

Our indigenous charitable trust is establishing an Ocean Garden to restore the wild stock and to provide food for the local community.

We will be restoring the wild garden surrounding our Hanging Garden, establishing four restorative Reef Systems below and a Hanging Garden above.

All of the above will be protected by a Rahui, a protective order by the community, with a buffer zone of 500 meters surrounding the garden.

We are utilising both Western Science and traditional Maori science as the foundation of growing taonga species such as abalone, mussel, scallop, spiny lobster and seaweed.

Local knowledge and time has given us a gift to restore the ocean and to share this knowledge.

They say if the ocean is healthy the people are healthy..

THE NATURAL INFESTATION OF ATLANTIC SALMON SKIN WITH CALIGUS (*C. rogercresseyi*) IN A MARINE FARM SHOWS A SEASONAL-DEPENDENT EFFECT ON THE TRANSCRIPTOME AND ITS METABOLICALLY-ACTIVE MICROBIOME

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Chile is the second largest worldwide producer of Atlantic salmon (*S. salar*). This industry has to deal with sanitary issues causing significant economic losses, such as sea lice (*C. rogercresseyi*, henceforth called caligus) infestations. Caligus is an ectoparasite that infests the skin of Atlantic salmon. Despite its relevance, few studies have focused on evaluating the health status of the skin of Atlantic salmon reared in sea cage farms. In addition, environmental fluctuations such as water temperature can influence the fish skin's health status and the resident microbiota. For this reason, we evaluated the Atlantic salmon skin response and resident microbiota in fish infested with caligus in different seasons of the year.

For transcriptomic analysis (RNA-Seq), total RNA was obtained from the epithelial skin tissue. Differential gene expression analysis between the infested and non-infested salmon was obtained based on the negative binomial distribution using the DESeq2 package. Pathways enrichment analysis was performed using the Gene Ontology Consortium database, STRING, and Reactome. Metatranscriptomics analysis of the metabolically-active microbiome was performed using Kraken2 software and the PlusPFP database.

Enrichment analysis showed the preference of the extracellular matrix process for upregulated DEGs during summer. 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. In autumn, the downregulated DEGs were associated with processes like riboflavin transport and mitochondrion organization. Conversely, downregulated DEGs during summer showed no enrichment. The composition of the metabolically-active microbiome in summer non-infested samples was found to have a greater bacterial diversity (33%) than in infested samples (22%); in autumn, the bacterial diversity of the non-infested was slightly higher (26%) than in infested fish (24%). The most abundant phylum in the bacteria domain was Pseudomonadota, followed by Actinomycetota and Bacillota. These results suggest that the presence of the Caligus parasite could be associated with a change in the salmon microbiota, whose representation would be modulated in a seasonal-dependent manner.

Our analysis revealed that sea lice infestation does not appear to dominate the differential expression profile in summer. This suggests that infested and non-infested fish are more concerned with seawater environmental adaptation to high temperatures. On the contrary, during autumn, the infested and non-infested fish show a differential expression profile and biological processes more associated with the response to sea lice. Taken together, the presence of Caligus affects the skin health of salmon and can alter their microbiome in a seasonal-dependent fashion, which could influence their growth, well-being, and, ultimately, Atlantic salmon production.

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IMPACT OF MONOSODIUM PHOSPHATE (MSP), MONOAMMONIUM PHOSPHATE (MAP) AND MONOCALCIUM PHOSPHATE (MCP) ON PHOSPHORUS AND PROTEIN DIGESTIBILITY AND PERFORMANCES IN JUVENILE *Litopenaeus vannamei*

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Inorganic feed phosphates (IFP) are used in shrimp feeds to meet animals' phosphorus (P) requirements, that may be impacted by minerals dietary concentration (P, Calcium (Ca), etc.) and digestibility. In the present study, performances and IFP digestibility were determined in juvenile whiteleg shrimp (*L. vannamei*) during growth period (until 23 g of individual weight).

Shrimps were fed plant-based diets supplemented with different IFP: monosodium phosphate (MSP), monoammonium phosphate (MAP) and monocalcium phosphate (MCP). Total P level in test diets was 0.79 %. A negative control diet was also distributed, with similar formulation but without IFP supplementation (0.47 % total P). Chromium oxide was added in all diets as a marker.

The negative control showed signs of P deficiency, leading to a significant reduction of shrimp performances compared to IFP supplemented diets ($P < 0.05$) (Figure 1). Performance of shrimp fed IFP diets had a final body weight between 22.8 – 23.3g and a survival rate between 87 – 91 %, without difference among these 3 treatments ($P > 0.05$).

IFP supplementation resulted in higher crude protein Apparent Digestibility Coefficient (ADC) (Figure 2) and diet P digestibility compared to negative control ($P < 0.05$).

The phosphorus ADC of IFP sources have then been calculated: 88.2% MSP, 84.2% MAP and 79% MCP ($P>0.05$) (Figure 2).

To conclude, as MCP and MAP, MSP is suitable for juvenile shrimp feeding, having an optimized feed efficiency, plus a numerically higher P digestibility.

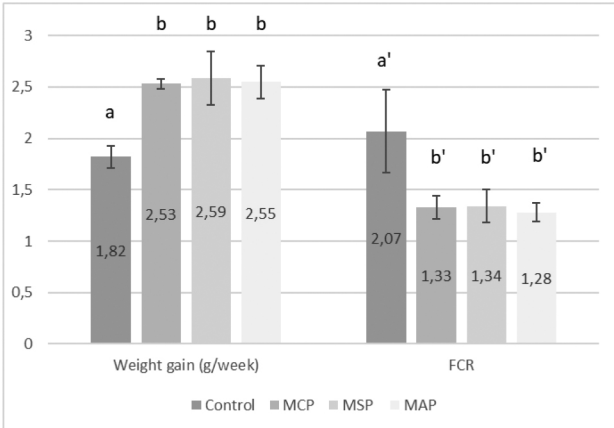

Figure 1. Shrimp performances (Different letters indicate statistically significant differences (P≤0.05))

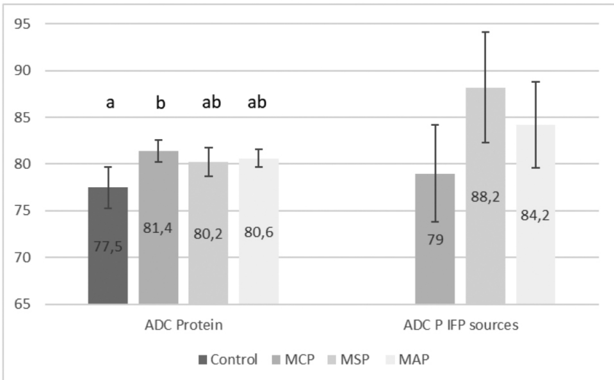

Figure 2. Apparent Digestibility Coefficient (ADC) results (Different letters indicate statistically significant differences (P≤0.05))

ECONOMIC UPGRADING: AN ANALYSIS OF TILAPIA FARMING IN BRAZILIAN MIDWEST AND NORTHEAST BETWEEN 2018 AND 2024

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Fish farming corresponds to approximately 90% of the Brazilian aquaculture production, which in 2022 reached 739,376 t. Tilapia production alone accounts for approximately 55% of this production and 40% of the commercialized value (IBGE, 2023). Despite being a major aquaculture producer in the world (FAO, 2022), the export rates of the country's production are still very low. In the case of tilapia, historically (2013 to 2022), Brazil exported less than 2.2% of its total production (CIAQUI, 2023).

The purpose of this research is to analyze the trajectory of economic upgrading in tilapia farming units in the Midwest and Northeast of Brazil between 2018 and 2024. The research is theoretically based on the Global Value Chains (GVCs) approach and, more specifically, on its analytical dimension of economic upgrading.

Methodologically, the research is divided into two stages. The first stage will seek to review and update the 2018 economic upgrading indexes [Ribeiro & Pedroza Filho, 2022] in the second half of 2024 using the Delphi method. In the second stage, the 2024 fuzzy upgrading indexes will be constructed and compared to those of 2018. The data for the 2024 indicators will be collected through questionnaires in the first quarter of 2025, on tilapia farms located in three production zones: Submédio Baixo São Francisco (BA/PE/AL), Serra da Mesa and Cana Brava (GO) and Boa Esperança (PI).

As expected results, the research will be able to create quantitative upgrading indexes, as well as establish an initial basis for the development of a continuous monitoring system of the upgrading trajectory in the Brazilian tilapia value chain. In the future, a dashboard could include other tilapia value chain links, as well as other perspectives for upgrading, such as environmental and social.

EFFECTS OF DIETARY CARBOHYDRATES/PROTEINS RATIO ON GUT MICROBIOTA AND METABOLISM OF RAINBOW TROUT FED A 100% PLANT-BASED DIET

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To ensure the sustainability of aquaculture, carnivorous fish such as rainbow trout must change their "menu" from a fish-based diet to one without fish ingredients. Feed manufacturers have typically replaced fish ingredients (oil and protein) with plant-based ingredients. However, there is still a major problem with the growth performance of fish fed with a 100% plant-based diet due to a decrease in feed intake and feed efficiency. In addition, in many fish species fed fishmeal and fish oil, the intake of digestible carbohydrates (CHO) can improve protein utilization for growth (protein sparing effect) and reduce nitrogen waste. The aim of this work was to study, for the first time, the incorporation of digestible carbohydrates (up to 20%) in a 100% plant-based diet, thus modifying the CHO/Proteins ratio in rainbow trout. We carried out 3 experiments to test the effect of the CHO/Proteins ratio on the intestinal microbiota, the trout metabolism either in the long term (after 12 weeks of feeding) or in the short term (3 weeks of feeding). Production of short-chain fatty acids (SCFAs), key molecules in the microbiota-host molecular dialogue was also analyzed. In addition, the cumulative effect of inulin supplementation and variations in CHO/Proteins ratio was studied to evaluate the effects on carbohydrates utilization and immune parameters.

The high carbohydrate/protein ratio had a significant effect on alpha and beta diversity and on the abundance of various lactic acid bacteria, as well as *Bacillus*, in both short-term, and long-term experiments, in the microbiota associated with digesta, while the microbiota associated with mucus was less affected. The change in microbial diversity could be linked to the differences observed in AGCC production which may play an important role in host metabolism. In terms of metabolism, the introduction of dietary carbohydrates did not impair trout growth performance, and was not associated with dysfunctional glucose homeostasis and intermediary metabolism. A decrease in several inflammatory markers was also observed with the high CHO/Protein ratio.

In contrast, incorporation of the prebiotic inulin had no positive effect on carbohydrate utilization.

In conclusion, the incorporation of 20% digestible carbohydrates in a 100% plant diet seems to be a promising way to optimize the use of new aquaculture feeds (without marine resources) in rainbow trout.

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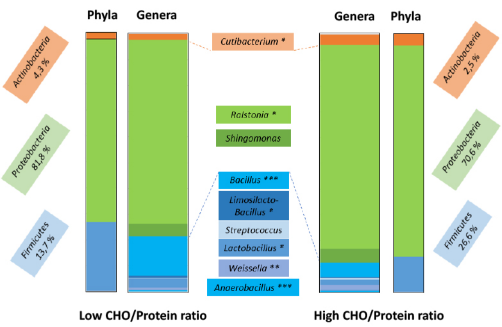

CONTINUED DEVELOPMENT OF INNOVATIVE HATCHERY TECHNOLOGY FOR BLACK GROUER *Mycteroperca bonaci* INCLUDING INTEGRATED MULTI-TOPHIC AQUACULTURE (IMTA) WITH OYSTERS *Crassostrea virginica* AND SEAWEED *Asparagopsis taxiformis*

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Commercial landings of the black grouper (*Mycteroperca bonaci*) from the southwestern Atlantic and Caribbean waters have declined by as much as 94% since 1990 (Figure 1). However, the species remains economically important in the region and in high demand both commercially and recreationally. Therefore, the development of aquaculture technology for this species is important, not only to reduce fishing pressure and supply the seafood demand, but also to potentially contribute to wild stock populations through stock enhancement. Therefore, with support from GSMFC in February 2020, the College of the Florida Keys started the pioneering process of domestication of *M. bonaci* using innovative technology and strategies intended to reduce complications often associated with broodstock collection and maturation for this taxon.

More recently, the project has explored the incorporation of integrated multi-trophic aquaculture (IMTA) using bivalves and seaweed to stabilize water quality in the grouper recirculating aquaculture systems and reduce the labor associated with frequent water quality monitoring and water changes. The focus of this presentation is to provide an update on the progress towards these goals and objectives.

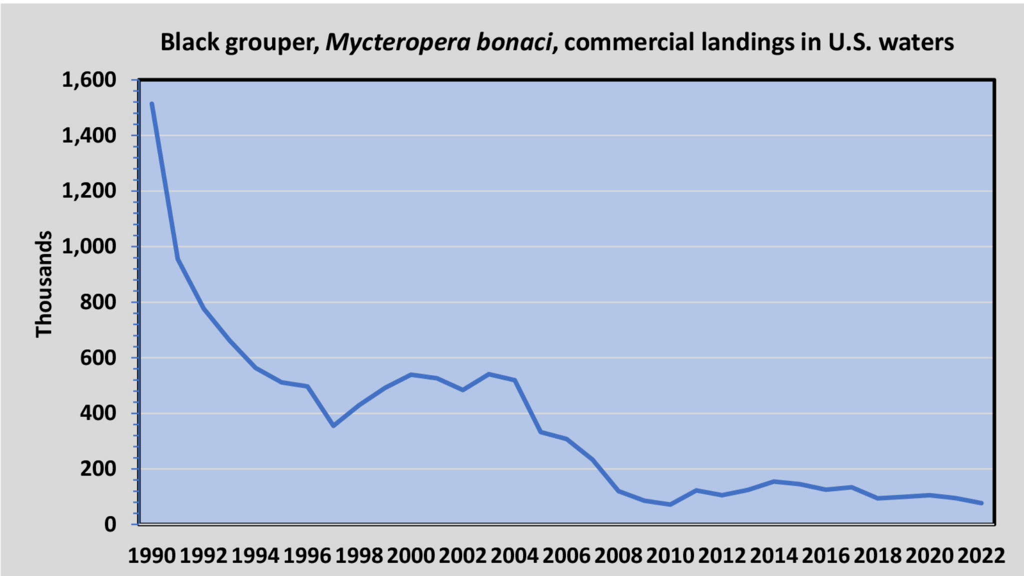

Figure 1. Commercial landings of black grouper (*Mycteroperca bonaci*) in U.S. waters from 1990 -2022 (*Source: Unpublished data from the National Marine Fisheries Service, Southeast Fisheries Science Center*).

SOME LIKE IT HOT: THE DEVELOPMENT OF MULTIPLEX PCR ASSAYS TO AID IN THE RAPID AND COST-EFFECTIVE IDENTIFICATION OF TROPICAL OYSTERS WITH AQUACULTURE POTENTIAL

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The ecology and biology of oysters (Ostreidae) across the tropics is poorly understood. Morphological plasticity and shared characteristics among tropical oysters have resulted in the misidentification of species, creating challenges for understanding basic species-specific biological information that is required for restoration and aquaculture. Genetic barcoding has proven essential for accurate species identification and understanding species geographic ranges. To reduce the costs of molecular species identification we developed multiplex PCR assays using the cytochrome c oxidase subunit I (COI or cox1) barcoding gene for the rapid identification of five species of oysters within the genus *Saccostrea* commonly found in Australia and/or the Indo-Pacific: *Saccostrea glomerata*, *Saccostrea* lineage B, *Saccostrea* lineage F, *Saccostrea* lineage G, and *Saccostrea spathulata* (lineage J).

Multiplex assays were successful in species-specific amplification of targeted species. Non-destructive DNA sampling by extracting oyster pallial fluid was also tested on adult oysters collected from the Noosa estuary in Queensland to assess whether oysters were able to be identified non-destructively. DNA concentrations as low as 1 ng/ µL still amplified in most cases, allowing for identification. The development of these primers has enabled the first successful spawning runs of *Saccostrea* lineage G, a species with high aquaculture potential.

These multiplex assays will be essential tools for species identification in future studies, and we expect that they will be widely applicable after being tested for non-specific amplification of other locally occurring species in other locations. The multiplex assays developed in this study outline easily replicable methods for the development of additional species-specific primer sets for other *Saccostrea* species, which will be instrumental in unravelling the taxonomic ambiguities within this genus in tropical regions.

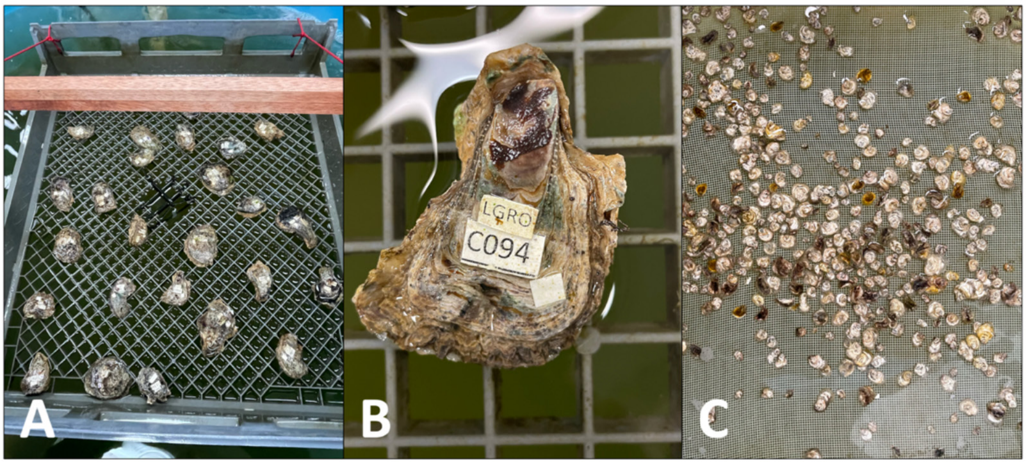

Figure 1. A) Lineage G samples positively identified non-destructively; B) a close-up image of a lineage G oyster; and C) lineage G spat following a successful spawning trial.

A GUT HEALTH SOLUTION TO COMBAT SALMON RICKETTSIAL SEPTICAEMIA (SRS)

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Salmon rickettsial septicaemia (SRS), caused by the aetiological agent *Piscirickettsia salmonsis*, has been the most important infectious disease in the Chilean salmon industry since the 1980's, costing >300 million USD per annum. Despite several initiatives, the industry is still heavily reliant on antibiotic usage to treat SRS. For example, in 2022, 458.6 g of antibiotics were consumed for every ton of Atlantic salmon produced, although this was slightly down compared to 2021. This study was set out to evaluate the efficacy of an enhanced acidifier against *P. salmonsis* (EM-90 Like/ geno group A) in post–smolt Atlantic salmon (*Salmo salar*), in a cohabitation challenge. Three hundred and thirty-six Atlantic salmon were equally distributed into eight tanks (42 fish/ tank), and randomly split into two dietary treatments ($n = 4$); Diet 1 (control) and Diet 2 (control + enhanced acidifier at 2 g kg^{-1}). Fish were acclimated for 18 days before an eight week feeding period where growth performance parameters (feed intake, SFR, weight gain, SGR, TGC) were assessed. After the feeding period, 15 fish per tank were randomly selected as 'shedder' fish, tagged and inoculated with a *P. salmonsis* challenge via intraperitoneal injection (0.2 ml of defined dose, 8.6×10^7 CFU ml^{-1}), before reintroduction into their original tanks. Afterwards, all fish received control feed for the first 10 days to avoid shedder fish access to the feed additive, before reverting to their original feeding regime (Diet 1 or Diet 2), for 55 additional days. After 65 days post infection, the mortality of fish receiving the enhanced acidifier (Diet 2) was significantly reduced (55.0 ± 7.9% vs 72.2 ± 13.9%, respectively; $P = 0.0064$). The relative percentage of survival (RPS) at the end of the challenge was 23.8 and the hazard ratio (Mantel-Haenszel) of Diet 2 was 0.604, compared to Diet 1. These data indicated that the probability for a mortality event in salmon is 39.6% lower in fish fed with the enhanced acidifier, compared to those fed diets without the additive. The benefits observed were not at the expense of growth performance, which remained unchanged during the feeding trial ($P > 0.05$). Compared with commercial trials, these results demonstrate that this enhanced acidifier is not only a useful nutritional tool to improve salmon health and welfare, whilst reducing the reliance on antibiotics.

SIZE MATTERS - ASTAXANTHIN PARTICLE SIZE INSIDE PRODUCT FORMULATIONS DETERMINES PIGMENTATION EFFICACY

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Fillet color is a key quality trait for salmon and trout markets globally. Due to a multiplicity of factors, such as duration of growing cycles and biosecurity, challenges with fish pigmentation have been highlighted over recent years. The formulation of nature-identical astaxanthin, a liposoluble molecule, into a product form is a critical factor for stability in storage, feed application, and utilization by the fish. The digestion and absorption of dietary astaxanthin from the gut is one of the limiting factors in the pigmentation process. Prior to absorption, astaxanthin must be solubilized in the intestinal lumen. Our new research shows that the particle size of astaxanthin within formulations plays a key role in the bioavailability of astaxanthin for pigmentation.

Three test formulations containing differently sized astaxanthin particles were tested for their pigmentation efficacy in rainbow trout. Fish receiving formulations containing smaller astaxanthin particles deposited more astaxanthin than counterpart fish receiving a formulation containing a larger astaxanthin particle size. These results indicate that the size of the astaxanthin particles presented in the digestive tract drives astaxanthin solubilization and absorption by the fish digestive tract. This study highlights the importance of astaxanthin formulation properties for optimal pigmentation.

WHICH ENVIRONMENTAL MINIMUM REQUIREMENTS COULD QUALIFY CERTAIN AQUACULTURE ACTIVITES AS LEGITIMATE NATURE-BASED SOLUTIONS?

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Proposed marine and coastal Nature-Based Solutions (NbS) are presently being implemented across the European Union in various contexts. Yet, what different stakeholders perceive as relevant NbS and their potential impacts and risks may vary greatly, compromising the implementation and effectiveness of NbS. Even worse, it may lead to misuse of the concept, providing a slippery slope for greenwashing either deliberately or accidentally. While general guidelines for NbS implementation exist (IUCN, 2020), several critical uncertainties remain about the detailed interpretation in marine and coastal ecosystems - including aquaculture activities (Riisager-Simonsen et al. 2022). To overcome this, the recently launched EU Horizon project TRANSEATION, will address the risks and challenges facing the different project phases in the development of marine and coastal NbS - including aquaculture. A core deliverable from this project will be a set of environmental minimum requirements which future marine and coastal NbS should align with. This presentation will share the first insights on environmental minimum requirements from several expert workshops, interviews as well as literature and policy reviews. Additionally, we invite Aqua24 participants to contribute with their knowledge and ideas, for how such minimum requirements could be implemented and documented in a relevant way. The aim is to cover the full project cycle from documentation to investors, environmental agencies and beyond.

The final draft minimum requirements for marine and coastal NbS, will be put into public online consultation in the beginning of 2025, before being finalized as guidance for actors ranging from companies, municipalities and capital asset managers towards Hence, improving the implementation, effectiveness and documentation impacts which underpin ecosystem-based marine management.

In the coming years the project will go beyond minimum requirements and draft a marine and coastal NbS building rating system based on the LEED certification scheme.

EFFECTS OF GENOTYPE AND NOVEL FEEDS ON GROWTH, FATTY ACID PROFILE AND E-SENSING ANALYSIS OF EUROPEAN SEABASS FILLET

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Aquaculture has traditionally relied on fishmeal (FM) and fish oil (FO). However, research is rapidly moving towards new and more sustainable ingredients that could reduce or eventually replace the use of FM and FO without compromising farming efficiency. In this context, the use of single-cell proteins (SCPs) in aquafeeds as a replacement for FM has attracted particular interest, as the microorganisms have a high crude protein content, grow rapidly, and are able to grow on a variety of substrates and convert non-food waste materials into high-quality feed. Accordingly, this study investigated the effects of partial replacement of FM with 10% bacterial protein (*Methylococcus capsulatus*) and complete replacement of FO with a mixture of poultry oil (PO) and DHA-rich microalgae oil in European sea bass (*Dicentrarchus labrax*) of non-selected (WT) and selected (HG) genotypes.

Materials and methods

The fish feeding experiment was conducted in the flow-through seawater system of the University of Las Palmas de Gran Canaria (ULPGC). Experimental feeds were produced at the feed pilot plant of Skretting Aquaculture Innovation (Stavanger, Norway). Hatched larvae of genotypes HG and WT were sent from the Ifremer Palavas research station (France) to the ULPGC and reared until they reached the initial experimental size of approximately 30 g. Then, 420 fish were randomly allocated to 12 tanks (6 tanks per genotype), and fed for 300 days with two diets: a commercial control diet (C) based on 20% FM and 5.09 to 7.14% FO, and an alternative diet (SCP) containing 15% FM, 0% FO, 10% SCP and 2-4% PO and 2-3% DHA oil from algae.

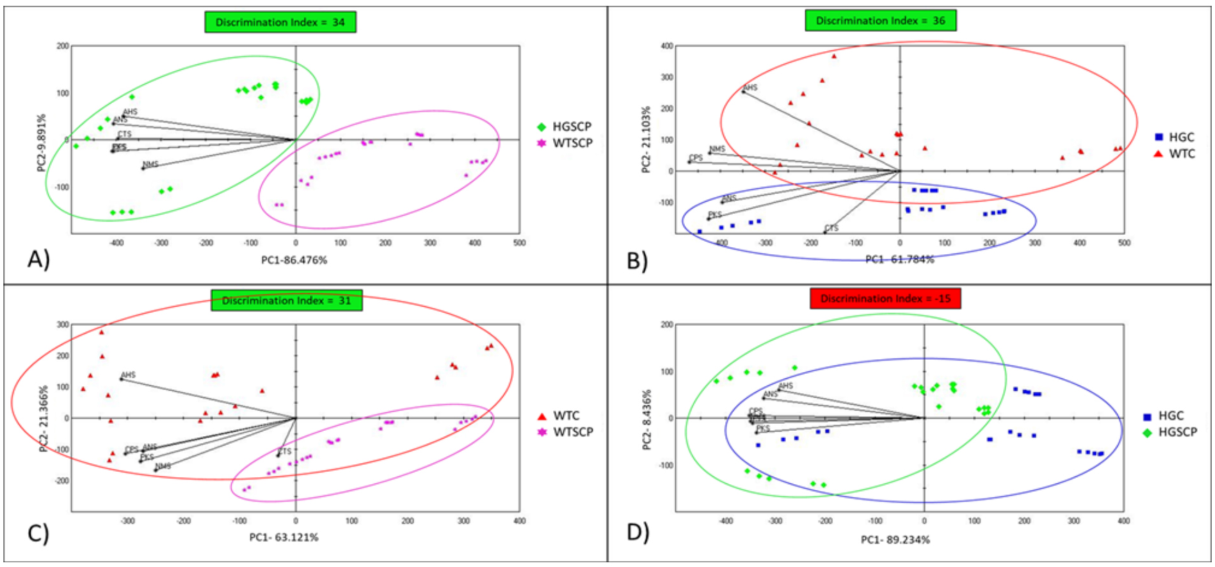

Fig.1. A) PCA diagram of E-tongue (taste map) comparing groups HG-SCP and WT-SCP; **B)** PCA diagram of e tongue (taste map) comparing groups HG-C and WT-C; **C)** PCA diagram of E-tongue (taste map) comparing groups WT-C and WT-SCP; **D)** PCA diagram of E-tongue (taste map) comparing the groups HG-C and HG-SCP.

(Continued on next page)

Results

At the end of the feeding trial, the SCP had no adverse effects on the growth of the fish. The selected HG group showed better growth and feed conversion, but lower levels of major fatty acids (EPA, DHA, n-3 FAs, and n-3 LC-PUFAs) in their fillets. These differences influenced the sensory and qualitative aspects. The electronic sensory analyses showed more pronounced nutritional differences in the WT group, while the E-tongue score was less variable in the HG group (Fig.1). In contrast, the volatile profiles showed no significant differences. A genotype effect was found for fillet chewiness, whereas hardness and adhesiveness were only influenced by diet. An interaction between genotype and diet was found for elasticity and cohesion of the fillet.

Conclusions

The present study showed that SCPs from *M. capsulatus* are an effective alternative to FM for sea bass. The effect of the feed in combination with genetic selection ensured that the fish achieved optimal growth performance. Despite the differences in lipid composition, the results of the E-sensing analysis underlined the ability of the selected fish to better utilize the alternative ingredients. To our knowledge, this is the first sensory profiling study conducted with artificial senses on genetically selected fish. Given these results, the E-nose and E-tongue have proven to be very powerful tools to study the sensory imprinting of fish fed with innovative and more sustainable feeds.

Acknowledgements

The current study was supported by the EU Horizon 2020 AquaIMPACT (Genomic and nutritional innovations for genetically superior farmed fish to improve efficiency in European aquaculture), number: 818367.

IMPACT OF MICRODIET NUTRIENT DENSITY AND FEEDING LEVELS ON SENEGALESE SOLE (*Solea senegalensis*) POST-LARVAE DURING WEANING

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Although weaning was traditionally a bottleneck in Senegalese sole farming, husbandry practices have significantly improved in the last decade. Yet, it is important to continuously improve the nutritional adequacy of microdiets, as well as the quantities fed to fish, and adjusted to the nutrient density of the microdiet during this sensitive stage of development. Too little feed will limit growth, and excess will worsen water quality. This study evaluated how different microdiet nutrient densities and feeding rates affect growth performance and feed conversion ratio (FCR) of sole during weaning.

The trial occurred at SPAROS facilities, where sole larvae were randomly distributed in triplicate tanks at a density of 5000 larvae/m^2. Fish were reared from 20 to 63 days after hatching (DAH) under four dietary treatments. Two microdiets were used with different nutrient densities, 61%:17% and 60%:15%, crude protein (CP) and crude fat (CF), respectively; and fed at two feeding levels: 100% and 80% of apparent satiety. Water temperature during the experimental period was 20.3ºC±0.4.

At the end of the trial (63 DAH), fish fed microdiet 60:15 had a higher wet weight (2W-ANOVA, $p<0.001$) than fish fed microdiet 61:17 (Figure 1), while feeding level and interaction feeding level x microdiet did not show significant differences (2W-ANOVA, $p= 0.10$ and $p=0.35$, respectively). Still, the relative growth rates (RGR) observed for all 4 treatments (ranging from 8.9 to 12.2 %/day) where within the normal range for the species at these ages. Regarding FCR, microdiet 60:15 brought lower FCR (2W-ANOVA, $p=0.001$) than fish fed microdiet 61:17 (Figure 1), while the 80% feeding level led to better FCR ($p=<0.001$), but with a significant interaction feeding level x microdiet ($p=0.017$).

The two microdiets tested lead to high growth in sole post-larvae. Diet 60:15 brought better growth and a better feed conversion than diet 61:17, when fed at apparent satiation. However, the feed conversion benefit disappeared at a slightly restricted (80 % of apparent satiation) feeding level, with both diets with similar FCR. In short, Senegalese sole perform well on different nutrient densities, but both nutrient density and feeding level have a strong impact, and diets may rank different depending on feeding level.

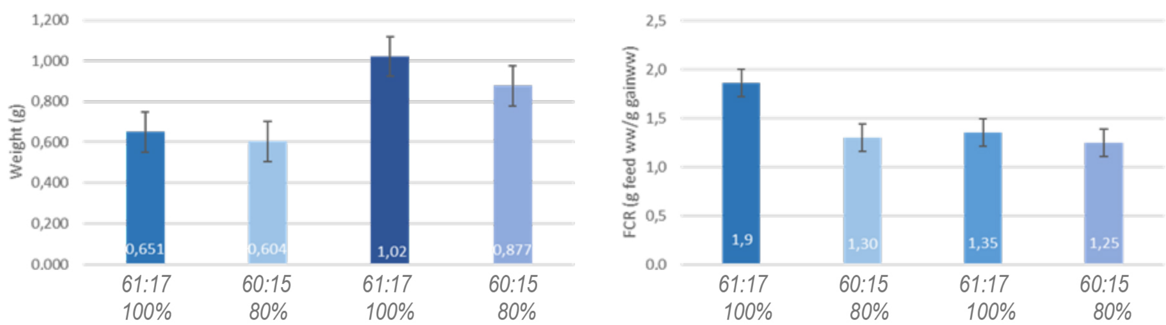

Figure 1. *Wet Weight (Left) and FCR (Right) of sole with 63 DAH, fed with two microdiets with different nutrient densities, 61%:17% and 60%:15% (crude protein: crude fat), and fed at two feeding levels, 100% and 80% of apparent satiety. Results are expressed as means ± SD (n=3).*

LIFE-CYCLE ASSESSMENT OF NEARSHORE VERSUS RECIRCULATING AQUACULTURE SYSTEM (RAS) FOR SEA BREAM FARMS

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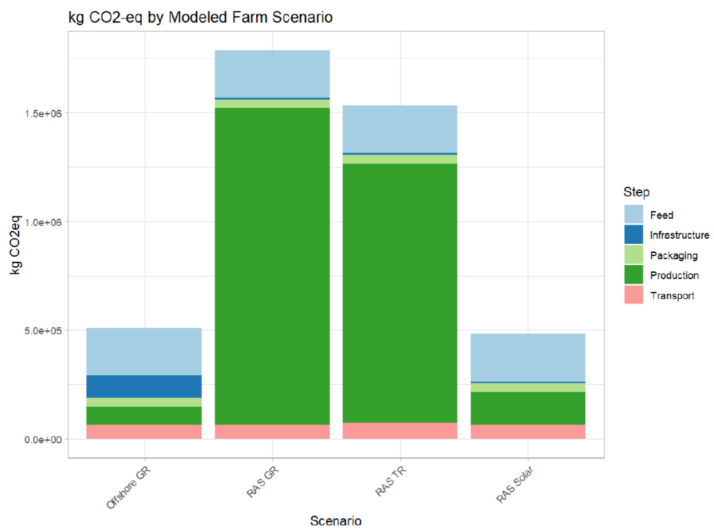
Aquaculture is presently growing to anticipate the significant need for human population growth, healthy foods, and the predicted rise in seafood consumption. Over 90% of market-available sea bream originates from aquaculture farms in the Mediterranean, which might generate substantial environmental impacts. Therefore, it is necessary to compare nearshore farms with recirculating aquaculture systems (RAS) to evaluate environmental footprint, nutrient recycling, and disease control.

This study performs a comparative LCA on a modeled nearshore and RAS farm producing 100 metric tons of sea bream yearly. Using two modeled farms in Greece, the differences in environmental impacts and factors that can contribute to sustainability improvements throughout the sector are highlighted.

The primary contributors to the carbon footprint of the RAS and nearshore sea bream farms are the production of the feeds and the generation of electricity required for the RAS system. The carbon footprint of RAS farms varies with the electricity grid mix, showing higher impacts with the Greek electricity grid than with the Turkish grid, which vastly exceeds the footprint of nearshore farming (see figure). However, powering RAS entirely with photovoltaic produces the lowest carbon footprint across all evaluated scenarios. Comparisons between organic matter, nitrogen, and phosphorus emissions to waterways also show a substantial reduction in discharge amounts from the RAS, lending it to being a superior choice regarding eutrophication impacts in the coastal zones.

The RAS system has a higher carbon footprint with the present electricity mix than nearshore production. RAS only becomes a fully sustainable solution if the electricity comes from renewable energy sources while reducing nutrients released to the environment. These data also provide consumers with greater transparency regarding the environmental impacts of their food, further advancing the movement toward greener farming solutions.



MOLECULAR TRACKING OF *Neopramoeba perurans* FOR IMPROVED MANAGEMENT OF AMOEBIC GILL DISEASE IN ATLANTIC SALMON

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Amoebic gill disease (AGD) is a severe infection of farmed Atlantic salmon (*Salmo salar L.*) caused by the marine amphizoic amoeba, *Neoparamoeba perurans*. Since its emergence in the mid-1980s, the disease has continued to spread and has become a global concern. Currently, we still know little about how *N. perurans* spread between farms, production cycles, regions, and counties. Such an understanding could aid the aquaculture industry to inform fallowing and control measures for salmonids. The genomic analyses required to achieve *N. perurans* molecular epidemiology, however, are frustrated by characteristically high levels of intercellular bacterial contamination. A handful of *N. perurans* genes have been successfully sequenced (18S rRNA, cytochrome oxidase I, ITS, etc) but with insufficient genetic resolution to be beneficial to either farmer or regulator. A draft genome was developed in the Llewellyn lab enabling the development of hundreds of new sequencing markers. A multiplex PCR was developed using 202 primer pairs within a single PCR reaction to amplify loci across the nuclear, and mitochondrial of *N. perurans* and kinetoplast genome. These markers were sequenced directly from gill swab samples at low cost to provide epidemiologically relevant information to genotype *N. perurans* from multiple sites across Norway (n = 9), Scotland (n = 4), and Ireland (n = 1) from 2019, 2021 – 2023 for molecular epidemiological analysis.

To date, there is no effective means of attributing AGD infections to a source or effective prevention of the parasite transmission between farms, production cycles, and the environment. Our work aims to take 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. Further research, aims to relay this information back to individual farm managers of affected sites by developing and implementing a web-based visualisation tool to track genotypes, spread over time to enable targeted and timely approaches to AGD outbreaks.

MARINE PEPTIDE CONCENTRATES BOOST GROWTH PERFORMANCE AND FEED INTAKE IN GILTHEAD SEA BREAM *Sparus aurata*

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Introduction

The composition of marine peptide concentrates varies depending mainly on the raw material, the enzyme type, and the degree of hydrolysis. Also, the inclusion rate in the diet is crucial when evaluating general performance of fish fed protein hydrolysates. In this work, we present two trials: 1) evaluation of the hydrolysis degree to improve feed intake and growth performance in gilthead seabream juveniles (*Sparus aurata*) juveniles and 2) determination of the optimal inclusion level of a peptide concentrate (Pepsea®, Adisseo) selected from trial 1, in the feed for gilthead seabream (*Sparus aurata*) juveniles.

Material and methods

Trial 1: Gilthead seabream of 10.94±0.12 g average individual body weight were distributed in 24 tanks of a marine RAS held at 21ºC. A group of 30 animals were stocked per tank. Six diets including 23% fish meal were evaluated: a negative control (NC, no peptide concentrate included) and 5 diets including peptide concentrates with different degree of hydrolysis (Subt, D1, D2 (Pepsea®), D3 and Phy). The peptide concentrate was included in the range of 1.5% to 3.1% to supply the same amount of functional protein. Fish were fed manually twice per day to apparent satiation 6 days per week. Trial lasted 10 weeks.

Trial 2: Fingerlings of 45.55±1.37 g average individual body weight were stocked in 15 tanks of a marine RAS. Fish were fed 5 diets with a 20% fish meal inclusion in all of them: a negative control (20FM, no peptide concentrate included), a commercial control (2S, including 2% of a commercial salmon hydrolysate) and 3 diets with different inclusion levels (2.3%, 1.4% and 0.7%) of the peptide concentrate Pepsea® (2.3PF, 1.4PF and 0.7PF). Fish were fed automatically distributing the daily ration during 12 hours per day and adjusting the feeding rate every 2 weeks. Trial lasted 11 weeks.

Results & conclusions

Trial 1: The seabream fed diet D2 (Pepsea®), showed the best performance compared to the fish fed the NC diet and compared to the rest of the diets. Feed intake was 17% (highest value) and SGR and FCR were 21% higher and 30% lower, respectively.

Trial 2: Fish fed diet 0.7PF yielded better results than the diet 20FM, with 4% higher feed intake values and 3% better SGR. Feed intake, SGR and FCR of fish fed diet 0.7PF were comparable to the values obtained with diet S2, indicating that with a lower inclusion rate, results are very similar.

The results from these two trials showed the relevance of the degree of hydrolysis in the performance efficacy of peptide concentrates in fish diets and the importance of selecting the appropriate inclusion rate to obtain a cost-effective application.

ASSESSING THE IMPACT OF EXTERNAL SHOCKS ON THE FINANCIAL PERFORMANCE OF NORWEGIAN SEAFOOD FIRMS: A COMPREHENSIVE ANALYSIS FROM 2005 TO 2020

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Norway's seafood sector stands as a cornerstone of its economy, fueled by the nation's expansive coastline and rich marine resources. This paper delves into the profitability dynamics of Norway's seafood firms, examining the impact of external shocks on their financial performance. Drawing upon firm-level data spanning from 2005 to 2020, we employ a dynamic panel data econometric approach to unravel the intricate relationship between profitability and external shocks. Our analysis disaggregates the seafood industry into five distinct segments of the sector supply chain: fishing, aquaculture, processing, services, and trade, offering insights into the vulnerabilities and resilience mechanisms within each segment.

Amidst the backdrop of the COVID-19 pandemic and previous economic upheavals, our findings shed light on the determinants of profitability in Norway's seafood firms. Leveraging the Generalized Method of Moments (GMM) estimation approach, we address endogeneity concerns inherent in panel data analyses, ensuring the consistency and robustness of our results. Our methodology accounts for the complex interplay between lagged dependent variables, predictors, and unobserved individual effects, providing nuanced insights into the sector's response to external shocks.

Through the empirical analysis and validation, this study contributes to the broader discourse on the resilience of export-driven industries in the face of global uncertainties. By identifying key factors driving profitability and elucidating the transmission channels of external shocks, policymakers and industry stakeholders can formulate targeted strategies to fortify Norway's seafood sector against future disruptions.

DIETARY SUPPLEMENTATION WITH *Bacillus pumilus* AND *Bacillus subtilis* IMPROVES PERFORMANCE, MICROBIOTA COMPOSITION AND FUNCTIONALITY IN WHITELEG SHRIMP (*Litopenaeus vannamei*) AND THE CULTURE WATER

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Probiotics are frequently used in intensive shrimp farming to help secure or enhance health and performance. This study assessed the effects of in-feed supplementation with a *Bacillus*-based probiotic on shrimp performance, composition and inferred functionality of the digestive microbiota with the view to screen, if any, the microbial pathways manipulated.

The 8-week (56 days) grow-out trial used whiteleg shrimp (1.2 g) in clear water tanks (290L tanks; 20 ppt; 27°C) fed to apparent satiation. Two treatments were applied in quadruplicate: a control group (CON) fed a non-supplemented diet (BioMAR) and a probiotic group (BAC) fed the same basal feed supplemented with proprietary strains of *Bacillus pumilus* and *Bacillus subtilis* at 6×10^6 CFU/Kg feed (50:50 by CFU; Lallemand, France). Microbiota composition (16S rRNA V3-V4 region) and functional inference (PICRUSt2) were analyzed in the digestive gland (HP) and rearing water at the start and end of the trial.

Dietary treatments had a significant impact on the HP bacterial composition. At the end of the trial, the probiotic group showed a lower prevalence of Vibrionales and a higher prevalence of *Bacillus* genus as well as of members of the Roseobacter clade (*Phaeobacter*, *Ruegeria* and *Paracoccus*) in particular. Shannon diversity was also significantly higher in the BAC group and this index was significantly correlated with the Rhodobacteraceae/Vibrionales (R/V) ratio ($r = 0.76$) (Rodiles *et al*., 2022). Functional inference of the HP microbiota revealed 645 KO differently abundant between groups. Of these, 96% were higher in the BAC compared to the CON group and corresponded to the enhancement of 15 KEGG pathways involved in vitamins and cofactors production, bile acid production, denitrification, fermentation and of serum resistance to pathogens. Some effects on the water microbiota were documented and will be presented.

In conclusion, the probiotic-feed group exhibited a digestive microbiota with enhanced taxonomic and functional diversity. This diversity was associated with specific nutritional and health advantages, as inferred from their functional characteristics. The primary factor contributing to this diversity appears to be a decrease in the prevalence of the Vibrionales taxon due to probiotic feeding. Given that Vibrionales possess limited functional attributes, their reduced prevalence fosters a more diverse and functional microbiota offering both nutritional and health benefits.

Rodiles et al. Relationship between alpha diversity and Rhodobacteraceae/Vibrionales ratio as a potential biomarker of the intestinal microbiota of whiteleg shrimp *Litopenaeus vannamei*. World Aquaculture May 24 - 27, 2022, Merida (Mexico).

IN-FEED PROBIOTICS AND WATER BIOREMEDIATION IMPROVED GROWTH, BIOMASS, SURVIVAL, WATER CHEMISTRY AND MICROBIOTA IN WHITELEG SHRIMP *Litopenaeus vannamei* IN VIETNAM

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The use of probiotics in water (bioremediation) and in-feed are progressively replacing the prophylactic use of antibiotics in the culture of shrimp. This study compared the benefits of probiotic- or antibiotic-based prophylactic strategies and their combination to a non-supplemented control under commercial-like conditions. Shrimp performance and resilience to abiotic stressors were addressed, together with water quality and microbiota to estimate the mechanisms at play and the benefits at farm level.

A 42-day grow-out trial was performed in Vietnam using whiteleg shrimp juvenile (0.5g) reared at high density (150 shrimp/m^3) in 16 outdoor tanks (50m^3), low water exchange using pumped-ashore pre-treated natural brackish water (7 to 14 ppt; 30 to 8°C). Animals were fed to apparent satiation 4 times/day with feeding tray. Four treatments were applied in quadruplicate: Control group (CON) used non-supplemented commercial feed (basal feed; Lion feed, Sheng Long; Vietnam); antibiotic group (ABX) supplemented with Oxytetracycline from day 10 to day 20 (OTC, 5g/Kg feed); Probiotic group (LAL) supplemented with Lalpack Probio and Lalpack Immune (5g/Kg feed each; Lallemand) and rearing water conditioned with Lalsea Biorem (1.2Kg/ha every 4 days; Lallemand); Antibiotic+Probiotic group (ABX+LAL) combined feed and water conditioning. After zootechnical performance analysis at day 42, shrimp from each tank were randomly selected and exposed to an abrupt salinity challenge to assess shrimp robustness (50 shrimp/500L tank; 6 replicates /groups).

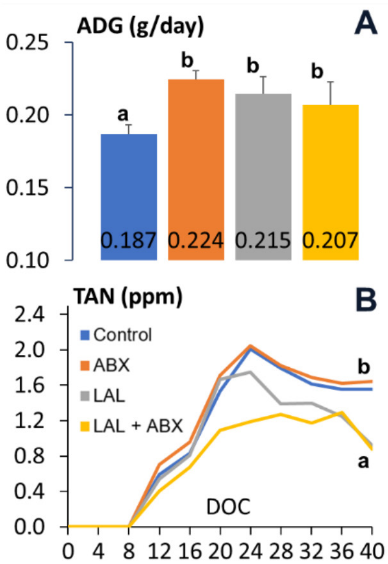

Fig.1. *ADG at day 42 (A); TAN during the trial (B) (P<0.05).*

Results showed biomass gain and average daily growth (ADG) were sig. higher in all suppl. groups compared to CON (**Fig.1A**; $P<0.05$), both ABX groups had a sig. lower condition factor (K). Resilience to the abiotic challenge were higher in all suppl. groups compared to CON, increasing time to 50% mortality: ABX (+6%), LAL (+14%), LAL+ABX (+29%). During the trial, ammonia and nitrite levels remained lowest for both LAL groups (**Fig.1B;** $P<0.05$). Post-intervention levels of lactic acid bacteria (LAB) in water and gut were sig. higher in both LAL groups (+1.0 log), ABX promoted higher total heterotrophic bacteria in water. Water microbiota was significantly clustered by Flavobacteriia (*NS3a marine group*; Bacteroidota) in CON (20%) and ABX (26%), and by *Candidatus Aquiluna* (Microbacteriaceae; Actinobacteriota) in LAL groups (45%). Interestingly, the functional potential of the microbial water modulation revealed, in both LAL groups, a lower prevalence of the N-metabolism enzymes glutamate dehydrogenase (L-glutamate deamination), Nitronate monooxygenase (nitrite-forming), and an enzyme involved in Carbon-fixation (MCEE).

In conclusion, on-growing of *L. vannamei* using an in-feed probiotic and water bioremediation strategy improve shrimp growth, biomass gain and resilience to abiotic stressors to similar or higher levels than that achieved by the prophylactic use of antibiotic. This was associated with a higher LAB prevalence in the gut, as well as with a distinctive modulation of the water microbial community supporting enhanced organic matter and nitrogen-cycling.

INVOLVEMENT OF TUMOR NECROSIS FACTOR RECEPTOR ASSOCIATED FACTOR-2 (TRAF-2) OF CHUB MACKERAL *Scomber japonicus* IN THE CONTEXT OF ANTIVIRAL, ANTI-APOPTOTIC, AND ACTIVATION OF NFKB PATHWAY

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Tumor necrosis factor receptor associated factor-2 (TRAF2) is considered to be one of the most important molecules mediating a wide variety of cellular responses and is mainly considered as a signal transducer for members of the TNF receptor superfamily, including TNFR1, TNFR2 and CD40. TRAF2 regulates several physiological functions, including T and B cell signaling, inflammatory processes and cell survival, by activating the classical and alternative NF-KB, MAPK and JNK activation pathways.

In the present study, chub mackerel (*Scomber japonicus*) TRAF2 (SjTraf2) was identified. The full-length cDNA of TRAF2 consisted of 1,569 bp ORF encoding 523 amino acids comprising of an N-terminal RING-type and zinc finger domain and a C-terminal TRAF domain. The deduced amino acid sequence of TRAF2 from chub mackerel exhibited more than 85 % identity and 90 % similarity with other teleosts and even more than 50 % identity with other vertebrates. Pairwise comparison and phylogenetic analysis suggested TRAF2 from *Dicentrarchus labrax* as the most closely related counterpart of SjTraf2. qPCR results revealed that *SjTraf2* was ubiquitously expressed and robustly expressed in blood, followed by brain and heart. The expression profile of *SjTraf2* in blood was significantly upregulated by immune stimulants, including Poly I:C, LPS, *Vibrio harveyi* and *Streptococcus iniae*. Further assays revealed SjTraf2 enhanced the cell viability of FHM cells under the oxidative stress caused by H_2O_2, which was concentration-dependent (Figure 1). Overexpression of *SjTraf2* in FHM cells reduced the expression ratio of *Bax/Bcl-2* by improving the anti-apoptotic functions. The expression of VHSV proteins, including Nucleoprotein, Matrix protein, Glycoprotein, Phosphoprotein and RNA polymerase were significantly downregulated, and NF-kB pathway-associated transcription factors, including NFkB1, NFkB2, RELA and RELB were significantly upregulated at 24 and 48 h pi in *SjTraf2* overexpressed FHM cells.

Taken together, our findings indicated the prominent role of SjTraf2 in modulating the immune responses during oxidative stress and pathogenic infections.

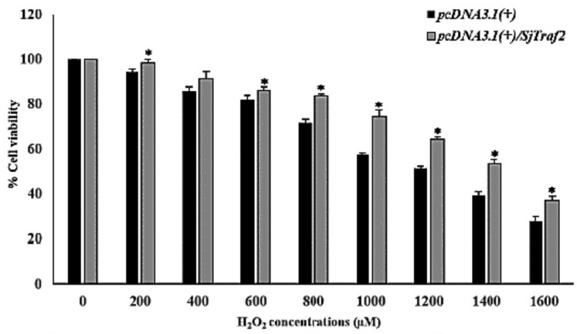

Figure 1. MTT assay showing enhancement of concentration dependant % cell viability in SjTraf2 overexpressed FHM cells during H_2O_2 induced oxidative stress

SENSORY CHARACTERISTICS OF FISH FROM RECIRCULATION AQUACULTURE SYSTEMS

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Introduction

Recirculating Aquaculture Systems (RAS) could help to meet the increasing global demand for seafood given the current environmental challenges (Ahmed & Turchini, 2021). Nonetheless, despite the fact that RAS farms can produce high-quality fish, the occurrence of off-flavors may affect the RAS-farmed fish thus leading to a negative perception in the wake (Moretto et al., 2022). This issue has usually been solely linked to the odor-active compounds geosmin and 2-methylisoborneol, but other compounds may additionally play a role (Jones et al., 2022). Indeed, farmed freshwater fish can contain a range of odorants, commonly described as fishy, grassy, earthy, mushroom-like, moldy, and metallic, all of which are generally considered unacceptable by consumers (Zhou et al., 2024). Consequently, there is a need to comprehensively elucidate odorant compounds in fish reared within commercial RAS facilities. In this project, sensory and analytic instrumental procedures were applied to characterize the aroma profiles and aroma-active compounds in Nile Tilapia and Russian Sturgeon raised in RAS farms.

Methods

The fish samples originated from two recirculating aquaculture farms. All samples were directly stunned, hand-filleted and frozen at -20 °C until use for analysis. The volatile compounds were solvent-extracted (Mahmoud et al., 2017). Briefly, 50 g of the fish fillets were extracted with 100 mL of dichloromethane. Then, the extract was distilled using solvent-assisted flavour evaporation, followed by drying over anhydrous Na_2SO_4, and concentration to approx. 100 µL. Identification of odorants was achieved using a combination of comparative aroma extract dilution analyses (a gas chromatographic-olfactometric technique), gas chromatography-mass spectrometry and two-dimensional gas chromatography with mass spectrometry and olfactometry. Furthermore, aroma profile analysis was performed according to ISO 13299:2016–09.

Results and Discussion

Overall, the odor attributes soapy, fatty, green, mouldy, earthy, fishy and sea water-like/algae-like were selected by the panellists for the description of the two RAS-farmed fish aromas. Through the combined sensory and instrumental analysis, 108 and 114 aroma-active compounds were detected in Nile Tilapia and Russian Sturgeon, respectively. Of these compounds, 77 and 91 were identified by comparison of the respective retention indices, mass spectra and odor qualities with their corresponding reference compounds. The most important substances classes were aldehydes, organic acids, ketones, alcohols, lactones, terpenes and heterocycles. Some of these substances occurred with high flavor dilution factor (FD) in both Nile Tilapia and Russian Sturgeon.

In sum, sensory-instrumental analysis was successfully applied to identify odorant compounds in Nile Tilapia and Russian Sturgeon reared in RAS. In two independent samples, we thereby demonstrated that in addition to geosmin and 2-methylisoborneol, a range of other odorants was present with high FD factors. This study provides a basis to further develop quality control systems for fish reared in RAS by giving insights into the complexity of seafood aroma.

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References

Ahmed, N., & Turchini, G. M. (2021). Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. *Journal of Cleaner Production, 297*. Elsevier Ltd. https://doi.org/10.1016/j.jclepro.2021.126604

Jones, B. C., Rocker, M. M., Keast, R. S. J., Callahan, D. L., Redmond, H. J., Smullen, R. P., & Francis, D. S. (2022). Systematic review of the odorous volatile compounds that contribute to flavour profiles of aquatic animals. *Reviews in Aquaculture*. https://doi.org/10.1111/raq.12657

Mahmoud, M. A. A., & Buettner, A. (2017). Characterisation of aroma-active and off-odour compounds in German rainbow trout (*Oncorhynchus mykiss*). Part II: Case of fish meat and skin from earthen-ponds farming. Food Chemistry, 232, 841–849. https://doi.org/10.1016/j.foodchem.2016.09.172

Moretto, J. A., Freitas, P. N. N., Souza, J. P., Oliveira, T. M., Brites, I., & Pinto, E. (2022). Off-Flavors in Aquacultured Fish: Origins and Implications for Consumers. *Fishes*, *7*. https://doi.org/10.3390/fishes7010034

Zhou, Y., Zhang, Y., Liang, J., Hong, H., Luo, Y., Li, B., & Tan, Y. (2024). From formation to solutions: Off-flavors and innovative removal strategies for farmed freshwater fish. *Trends in Food Science & Technology*, *114*. https://doi.org/ https://doi.org/10.1016/j.tifs.2023.104318

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TEMPERATURE EVALUATION MODEL IN *Litopenaeus vannamei* INTENSIVE CULTURE USING A FUZZY INFERENCE SYSTEM

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Water temperature affects biochemical reactions in aquatic organisms and regulates the maximum dissolved oxygen concentration, so evaluating and monitoring this parameter is necessary. A computational model is presented for assessing water temperature in intensive culture ponds of white shrimp *Litopenaeus vannamei* by studying three fundamental factors: the average temperature, amplitude variation and duration of temperature changes. Through a fuzzy reasoning system, each factor is evaluated using rules, obtaining an indicator of the impact of temperature on the shrimp habitat. The results were compared against the NSF and CCME water quality indices, the most used indices. It shows better behaviour in the daily temperature evaluation, which more appropriately penalizes abrupt temperature changes, which is not considered by the other indices.

The first step in the proposal computational model is the signal preprocessing. The raw data has null samples due to acquisition device errors. The cubic splines are used to reconstruct the signal. Then, a smoothing process is applied to the new signal to remove high-frequency noise.

The smoothed signal is split into 96 segments; every segment corresponds to a whole day from 00:00 to 23:45 hours. The average daily temperature for every segment is calculated as the first fundamental factor temperature average.

Peaks and valleys are calculated in every signal segment to estimate the second and third evaluation factors. Those peak-valley pairs with an absolute temperature difference greater than a threshold °C are selected. The average temperature and the time are calculated as the amplitude variation and duration of temperature changes, respectively.

Once the three fundamental factors are estimated, they are used to feed an inference fuzzy system (FIS) whose output corresponds to the evaluation temperature of water.

The results of the proposal computational model are shown in Figure 1.

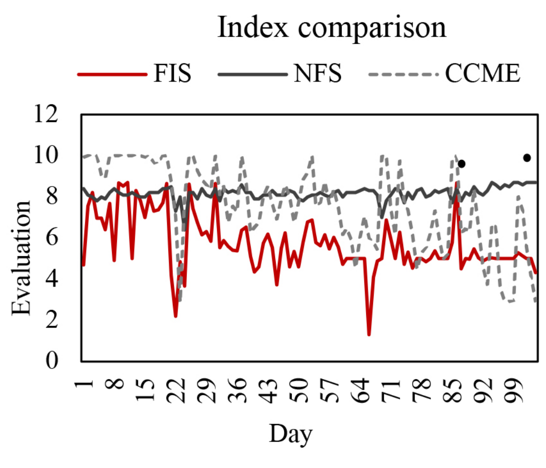

Figure 1. Comparison of the NFS, CCME indices and the FIS proposal results.

THE EFFECT OF MAILLARD REACTION AND PROTEIN CROSSLINKING ON PROTEIN DIGESTIBILITY IN JUVENILE NILE TILAPIA *Oreochromis niloticus*

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High temperatures during the production of fish feed can induce Maillard reaction and protein crosslinking. In mammals these reactions are known to reduce protein digestibility and bioavailability of amino acids. In pigs apparent digestibility of Maillard reaction products (MRPs) ranged between 0 and 30%. In fish such information is lacking. Therefore, the aim was to study the effect of Maillard reaction and protein crosslinking on protein digestibility by determining the apparent digestibility of MRPs and crosslinked amino acids (CLAA) in Juvenile Nile tilapia.

Two diets were formulated, containing either animal-based or plant-based proteins. Fish were fed restrictively and grown for an experimental period of 31 days at 24°C or 32°C. Feed and faeces samples were collected, in which Amino acids, MRPs and CLAAs were quantified using UHPLC-MS/MS. MRPs were present in both diets but the CLAAs were not present in detectable concentrations in the diet containing plant proteins. In the diet containing animal proteins, apparent digestibility coefficients for MRP FL and CLAAs LAN and LAL were approximately 80% to 90%, and thereby in striking contrast to their digestibility in pigs. Moreover, apparent digestibility of MRPs and CLAAs was similar to that of crude protein, suggesting no effect of the Maillard reaction and protein crosslinking on protein digestibility in Nile tilapia. In addition, water temperature did not affect the effect of Maillard reaction and protein crosslinking on protein digestibility. To study the role of gut microbiota in the digestibility of MRPs and CLAAs, samples for compositional analysis of gut microbiota are currently being analysed.

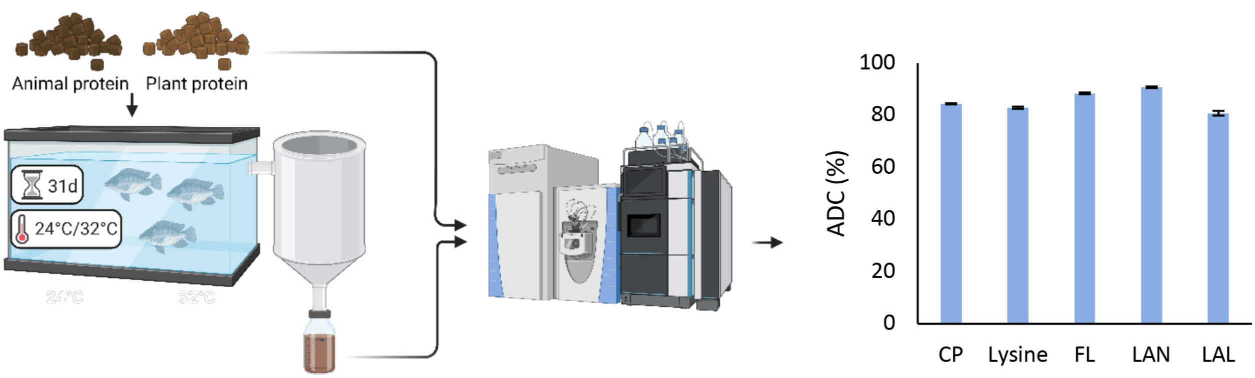

Figure 1. Experimental setup and results on the apparent digestibility coefficients (ADC) from Nile tilapia fed a diet containing animal protein (n=12). CP, crude protein; FL, fructoselysine; LAN, lanthionine; LAL, lysinoalanine. Error bars indicate standard errors.

PROLONGED COLD EXPOSURE NEGATIVELY IMPACTS ATLANTIC SALMON LIVER METABOLISM AND FUNCTION

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In the past decade, several large-scale mortality events have occurred in the winter at Atlantic salmon (*Salmo salar*) sea-cage sites in Eastern Canada and Iceland. In recent lab-based studies, we held post-smolt salmon at ~ 3°C, and ~ 30% of these fish developed symptoms similar to what has been described as 'Winter Syndrome' or 'Winter Disease' (WS/WD) in bream and yellow drum at cold temperatures. In these species, fatty liver disease (FLD) is associated with, and thought to be one of the major causes of, WS/WD. To definitively determine if these salmon developed FLD (which has not been reported previously), we measured hepatic lipid class and fatty acid levels, and the transcript expression of 34 key markers of FLD in fishes and non-alcoholic fatty liver disease (NAFLD) in mammals, in salmon characterized as 'asymptomatic' (healthy) vs. those exhibiting 'early' and 'advanced' symptoms of WS/WD. We also correlated our results with previously reported phenotypic characteristics associated with disease progression in these same individuals. Total lipid and triacylglyceride (TAG) levels increased by ~50% in fish with symptoms of FLD, while sterols and acetone-mobile polar lipids decreased by ~30%. Finally, multivariate analyses revealed that the 3 groups of fish were clearly different, with *saa5* (acute phase protein) contributing the greatest to the difference between asymptomatic and symptomatic fish, and *cyp7a1b* (involved in cholesterol catabolism) contributing the most to the difference between fish with 'early' vs. 'advanced' symptoms. In summary, our results provide very strong evidence that prolonged cold exposure can trigger FLD in this important aquaculture species.

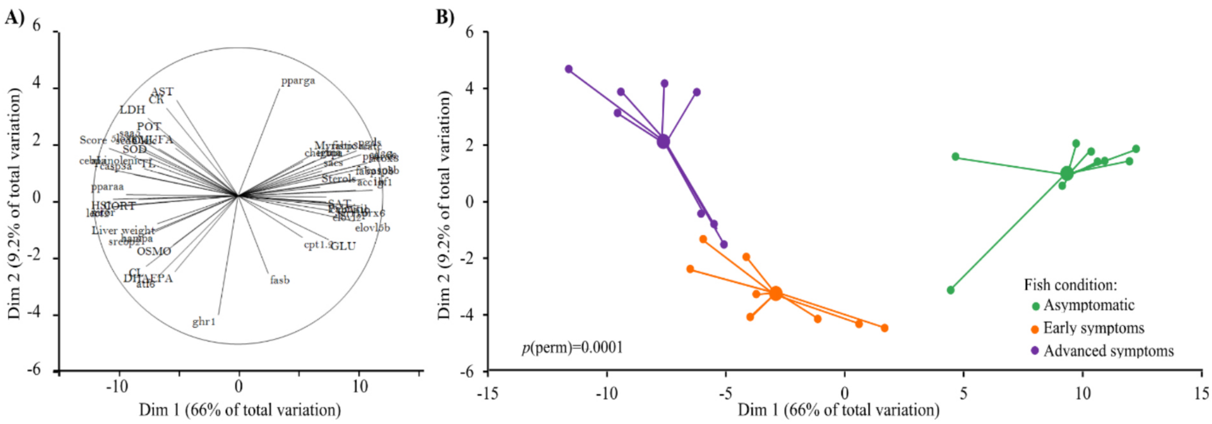

FIGURE 1. Principal Coordinate Analysis (PCoA) between target genes, phenotypic traits, and lipid classes in asymptomatic (healthy) fish and fish with symptoms of liver disease. **(A)** The wagon wheel shows the top 40 factors contributing to the variance in the PCoA. **(B)** PCoA plot illustrates the differential distribution of the individuals in the two-dimensional space connected to the centroid of each group, with overall PERMANOVA result p(perm)=0.0001.The variance explained by each dimension (PCO1, PCO2) is presented as a percentage (%).

FEEDING BEHAVIOUR OF INDIVIDUAL BROODSTOCK *Penaeus Monodon*: OPTIMISING FEEDING AND NUTRITION FOR REPRODUCTION

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The advancement of prawn nutrition research, jointly with breeding and production systems, has supported the expansion of the global prawn industry. Differently from larval and growout prawn nutrition, broodstock nutrition still displays many gaps, including feeding behaviour and potential impacts on reproduction and ovarian development. Accordingly, our research group has developed experimental systems to assess the behaviours of individual broodstock *P. monodon* to further understand basic husbandry practices including feeding. The experimental system consisted of eight aquaria with sand substrates, each equipped with custom computer modules. Individual broodstock prawns (N=60 for feeding and feed preference) were stocked in sand bottom tanks for 72 hours. Animals were fed twice a day fresh-frozen squid and polychaetes. Behavioural responses to different feeds were described and categorised using Behavioural Observation Research Interactive Software (BORIS v8.22.6), and a previously established ethogram for *P. monodon* was utilised, including walking, swimming, digging, feeding, grooming, eye beats, antennal scale flexion and antennae positioning. Time budgets and diurnal behavioural patterns were also developed to determine further baseline information of individuals to contribute to our understanding of individual variability in experimental systems. Feeding and pre-feed behaviours, were examined including recognition of presence of food, time to feed, observation of amount consumed in a single event and the attractivity and preference of type of food offered. These findings establish a baseline understanding of individual broodstock prawn feeding behaviour and provide insights to future research on strategies to optimise broodstock feeding management to contribute to more sustainable methods of reproduction and seed supply globally.

RESEARCH ON NOVEL INGREDIENTS IN AUSTRALIAN AQUAFEEDS

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The search for novel ingredients remains a top priority in the aquafeed industry. This drive is fuelled by the well-described and judicious use of ingredients of marine origin, geopolitical matters, and, more recently, the environmental footprint and credentials associated with these ingredients. In Australia, there is an increasing focus on reducing the reliance on imported ingredients, particularly those with a substantial environmental footprint. The CSIRO aquaculture nutrition research group has evaluated a series of novel ingredients, namely, microbial biomass, mussel by-product meal, insect meals, food waste meals, speciality soybean meals, novel canola meals and concentrates, and fermented plant meals in feeds for several prawn and fish species, in the context of *in-vitro* bioactivity essays, digestibility studies, and growth trials. Careful validation of novel ingredients is paramount to derisking their use for the development of sustainable aquafeed.

Preliminary *in-vitro* results indicate that insect and mussel by-product meals exhibit anti-inflammatory properties that were superior to the levels in comparison to fishmeal and other ingredients. In *in-vivo* trials, mussel by-product meal was well accepted by prawns and enhanced production performance parameters. In terms of juvenile barramundi, food waste streams showed to be a promising source of novel nutrients for aquafeed. Diets based on multiple streams of food waste were not only well-accepted but also supported satisfactory growth performance. Blending multiple food waste streams proved to be a successful approach when utilising highly diverse nutrient sources. The development of canola products, including protein concentrates, showed great promise for use in aquafeeds. Juvenile salmon and prawn effectively utilised these ingredients compared to traditional canola meal and soybean meals. Fermentation of plant meals is another strategy which delivered promising results and was dependent on feed management. The complementary feeding of fermented plant-based feeds via dual-feeding strategies (replacing up to 50% of the commercial feed) achieved performance equivalent to the control treatment fed 100% of the commercial diet in prawns and low trophic fish. In contrast, speciality soybean genotypes developed to have reduced or undetectable amounts of lipoxygenases, altered glycinin profile, and reduced levels of oligosaccharides did not confer any growth and gut health benefits to juvenile salmon when added at high dietary inclusion levels compared to the traditional soybean meal.

Some of these test ingredients are already commercially available, while others are still under development, with the viability of upscaled production yet to be evaluated. Collectively, these findings illustrate the potential and nutrition feasibility of integrating novel ingredients into aquafeeds. The associated challenges and future perspectives will be discussed in detail during the presentation.

NATURE'S ARSENAL UNLOCKED: NEXT-GENERATION SCREENING OF AQUACULTURE-DERIVED BACTERIA TO PREVENT OYSTER DISEASES

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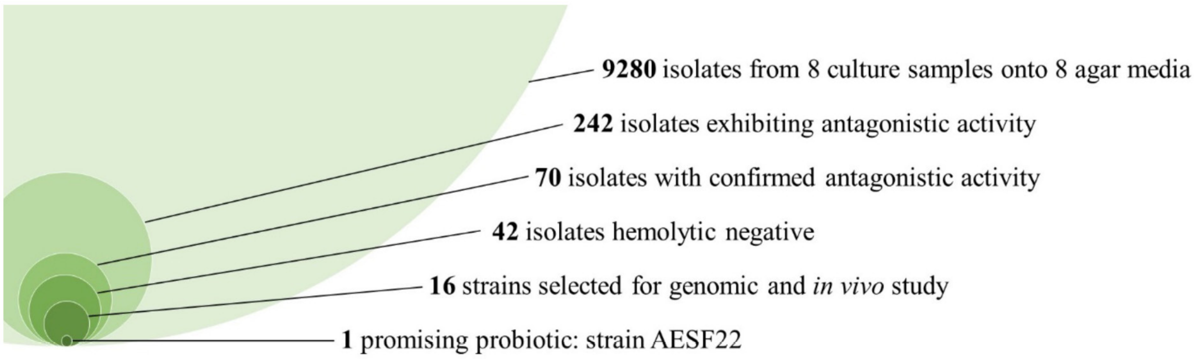
Various *Vibrio* species are known to cause severe mortality events in oyster aquaculture and are a threat to oyster farmers. In France, *Vibrio aestuarianus*, *V. coralliilyticus*, *V. harveyi*, and *V. crassostreae* have been identified as major pathogens causing disease in Pacific oysters (*Crassostrea gigas*). This project focused on addressing these challenges by identifying and characterizing next-generation probiotic bacteria capable of mitigating *Vibrio* diseases at various oyster life stages (larvae, spat, and adult oysters), ultimately enhancing oyster health and welfare. The methodology involved isolating bacteria from oyster tissues and microalgae cultures on a wide variety of oligotrophic media. Sequential screening of bacterial colonies for antagonistic activity against key pathogens (*Vibrio aestuarianus*, *V. coralliilyticus*, *V. harveyi*, *V. crassostreae*, *V. europaeus*, *V. neptunius*, and *V. tubiashii*) was conducted using a replica plating technique. For further characterization, isolates inhibiting the growth of pathogens were tested for hemolytic activity and antibiotic susceptibility. In addition, they were identified via 16S rRNA gene sequencing, and genome analysis (after dereplication). The sequential screening results are shown in Figure 1. A large number of colonies (9280) processed via replica plating technique, yielded 42 candidate probiotic strains exhibiting antagonistic effects against pathogenic *Vibrio* spp. From these 42 candidates, 16 stains exhibiting robust activity profiles and were selected for genome analysis. Ultimately, strain AESF22 emerged as a promising probiotic, demonstrating both efficacy and safety in oyster aquaculture. This research represents a significant advancement in combatting bacterial diseases in oyster aquaculture, promoting sustainable practices within the industry.

Figure 2. The sequential screening used to select probiotics progressively.



EVALUATION OF BLOOD SAMPLING FROM THE CAUDAL VASCULATURE AS A NON-LETHAL METHOD IN ATLANTIC SALMON *Salmo Salar*

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In recent years, there has been a rising interest in medical biochemistry in the fish farming industry to cover the need for rapid, non-lethal methods to monitor fish health and support sustainable management practices. Several challenges need to be solved before non-lethal fish blood sampling and analysis can be routinely implemented in fish health care, such as guidelines to how blood sampling from fish can be conducted in a practical way that safeguards fish welfare. Sampling of blood via puncture of the caudal vasculature involves traversing the needle through several tissue structures like the skin, skeletal muscle, and vasculature. Other important structures found in this region are nerves, in particular the truncus sympathicus and the ventral roots exiting from the spinal cord and vertebrae. During sampling, all these structures are vulnerable to damage. In an effort to evaluate this potential harm and other impacts on fish welfare, we conducted a study involving Atlantic salmon in fresh water (50-100 g) and sea water (250-500 g). The salmon were subjected to sampling of different blood volumes in ambient water temperatures of 5, 12, and 15°C. Anaemia and tissue damage were monitored by different methods. The results revealed significant differences in the ability to regenerate between fish sizes and water temperatures. Here we will emphasize the results from the histology, as we consider such analyses a prerequisite for establishing a best practice for non-lethal blood sampling. Some possible injuries, their impact on fish health and potential for regeneration are described.

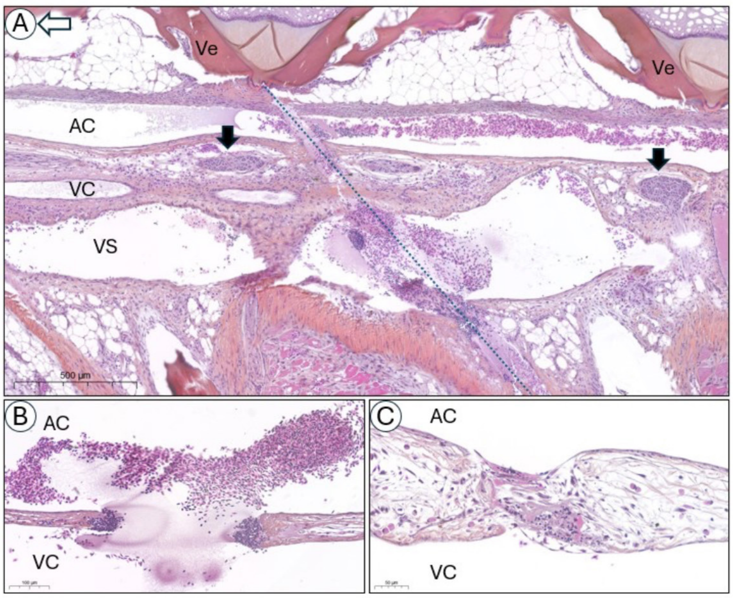

Figure: Morphological study with examples of acute and chronic changes associated with blood sampling from the caudal vasculature visualized in the mid-sagittal plane. **A)** Overview of acute changes, where bleeding and thrombus formation can be seen associated with the injection channel (dotted line) and perforation of the vena subvertebralis (VS) and arteria caudalis (AC). Note ganglia (thick arrows) of the truncus sympaticus embedded in the wall between VS and AC. **B)** Acute changes with thrombus formation after perforation of the septum between vena caudalis (VC) and AC. **C)** Healed septum between the VC and AC post sampling. Ve; vertebra, white arrow in A points in cranial direction.

HOW CAN OPEN ACCESS CONTRIBUTE TO EDUCATION AND TRAINING IN AQUACULTURE

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Introduction
In 2021, in countries of the Mediterranean and the Black Sea, aquaculture production reached 3299000 t mainly obtained in freshwater environment (>70% of the total production volume), followed by marine production (24%) and brackish water production (6%) (FAO-GFCM, 2023).

In the last years, Open Science (OS) has become important as movement to make scientific research and its dissemination accessible to all levels of society in the Blue Growth (Galparsoro et al. 2020). The shortage of scientific, entrepreneurial and managerial skills, needed to solve complex multidisciplinary challenges, is a major barrier to innovation (Muñoz-Tamayo et al., 2022). The topics of Blue Growth, the Artificial Intelligence and Biosecurity are at the base of the ambitions in aquaculture.

In this context, OASIS (Open science technologies Acceleration for a new generation of Student entrepreneurship, Innovation and Sustainable development) Project was performed with the aim to facilitate the adoption of OS technologies in education through practical applications, training and mentoring activities for managing educational and labour-oriented problems and increasing opportunities of work in the Blue Growth in the Mediterranean countries.

Materials and Methods
For the aim, to give opportunities and intercept blue, green and circular economies, an approach based on collaborative platform with an AI engine in a sector, aquaculture, was performed. Meeting and trainings were organized involving students (n. 47-54) enrolled in the field courses of veterinary medicine.

An approach based on working in small groups of students was considered. Every topic was performed using game cards in which topics (biosecurity, farm, environment) and sub-topics (levels of biosecurity, farming techniques, water quality parameters) were reported using short sentences. In a specific case, students were introduced to the use of an open-source software to evaluate the welfare status of intestinal apparatus in farmed fish fed with plant-based proteins. For the aim, the counting of intestinal villi was carried out using a plug-in belonging to the (open source) software IMAGEJ FIJI, USA version 2.9.0, "Trainable Weka Segmentation".

At the same time, the meetings aimed to make known the possibility of subscribing to a platform in which the candidate can find opportunities to work in the Blue Growth field were carried out. The platform was realized according to a methodology based on a mix of surveys, CV analysis skills, adoption of bottom-up tools like Lego Serious Play™ and analysis. PESTLE and ESG's scanned by AI under the Quantum Labour Analysis approach.

Results and discussion
The large part of the involved students (88%, on average) appreciated the OASIS activities because could consider aquaculture as a potential field where to find work as an alternative to a traditional sector of animal production and veterinarian science.

(Continued on next page)

The gamification, known as the process of integrating learning activities by using game elements, encouraged students, working in small groups, to elaborate a pathway of problem solving and purpose a professional plan. At the same time, all the students realized that automation and rapidly changing needs mean the future of employment will probably look very different to now. As concerns the open-source software IMAGEJ, the students were able to train the algorithm to recognize Alcian blue stained vacuoles, excluding areas >20 pixel2, considered too small to be a real vacuole. The importance to promote the OS practices as possibility to improve their skills was demonstrated by the application of open-source software among students able to corroborate their fundings during thesis activities. This approach can help students to continue their studies or provide their skills to the stakeholders in the professional world in the animal production field.

The results showed the possibility to communicate the skills trends and map thus to overcome the lack of awareness, knowledge and skilled workforce for the emerging competencies in the Blue Ecosystem. Specifically, this is relatable to aquaculture both for seafood industry and by-products from blue sector exploitation as key factors for the integration with green and circular economies in terms of effective sustainable systems (Troell et al., 2023). In this situation, education and training activities are very important to fulfil the huge lack of awareness of aquaculture's potential by promoting and valorising a skills-based approach. The shortage of scientific, entrepreneurial and managerial skills, needed to solve complex multidisciplinary challenges, is a major barrier to innovation. In the last years, OS has become an important movement to make scientific research and its dissemination accessible to all levels of society. However, results sharing is more important once the research objectives are met. Sharing can show difficulties and drawbacks to experts which could enhance the efficiency and the time-saving.

Acknowledgements
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References

FAO-GFCM. Strengthening stock assessment in the western Mediterranean. 2023.

Galparsoro I., Murillas A., Pinarbasi K., Sequeira A.M.M., Stelzenmuller V., Borja A., O´Hagan A.M., Boyd A., Bricker S., Garmendia J.M., Gimpel A., Gangnery A., Billing S.-L., Bergh Ø., Strand Ø., Hiu L., Fragoso B., Icely J., Ren J., Papageorgiou N., Grant J., Brigolin D., Pastres R., Tett P. 2020. Global stakeholder vision for ecosystem-based marine aquaculture expansion from coastal to offshore areas. Reviews in Aquaculture 12:1–19.

Muñoz-Tamayo R., Nielsen B.L., Gagaoua M., Gondret F., Krause T., Morgavi D.P., Olsson A.S., Pastell M., Taghipoor M., Tedeschi L., Veissier I., Nawroth C. 2022. Seven steps to enhance Open Science practices in animal science. PNAS Nexus 1:1–6.

Troell M., Costa-Pierce B., Stead S., Cottrell R.S., Brugere C., Farmery A.K., Little D.C., Strand A., Pullin R., Soto D., Beveridge M., Salie K., Dresdner J., Moraes-Valenti P., Blanchard J., James P., Yossa R., Allison E., Devaney C., Barg U. 2023. Perspectives on aquaculture's contribution to the Sustainable Development Goals for improved human and planetary health. J World Aquac Soc. 54:251–342.

VALIDATION OF THE HEMOCUE HB 801 PORTABLE HEMOGLOBIN ANALYZER FOR FISH BLOOD

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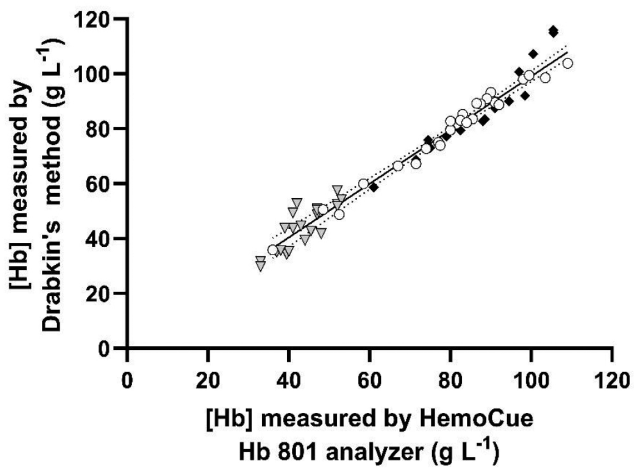
The assessment of hemoglobin levels (Hb) has become a routine measurement for the evaluation of the health and welfare status of farmed animals, including fish. The original method for measuring Hb, best known as the Drabkin method, is not well suited for work outside of a laboratory setting. It is time consuming, contains hazardous cyanide elements and requires specific laboratory material. As an alternative to the Drabkin method, portable analyzers have been developed to measure Hb in human blood. For example, in 1982, the Swedish company HemoCue developed a time-saving analyzer, the Hemocue Hb 201+, that measures human Hb within 10 minutes, using coated cuvettes. While this device was proven accurate and validated rapidly for mammalian blood, it was not until recently that this methodology was validated for fish blood. It has been shown that a correction factor is needed to account for the differences in blood composition between fish and human blood, when using this device to measure Hb in fish blood. In 2019, HemoCue launched a new portable analyzer, the HemoCue Hb 801. In comparison to the Hb 201+, this device uses uncoated cuvettes and takes less than 1 second to analyze Hb, making it even more efficient and suitable for field work.

In this study, the performance of the new HemoCue Hb 801 portable Hb analyzer was compared to the validated Drabkin method in three fish species important for the Swedish aquaculture industry (rainbow trout, *Onchorynchus mykiss*, Atlantic wolffish, *Anarhichas lupus* and Nile tilapia, *Oreochromis niloticus*).

Hb readings were not different between the two methods for any of the three species (Figure 1). Hence, we concluded that this new portable device can be measure Hb in fish blood. Unlike the previous model from HemoCue, the Hb 201+, the Hb 801 does neither need an incubation time nor a correction factor for fish blood. This represents a major gain of both time and precision, especially in settings outside of the laboratory.

Figure 1. Comparison of blood Hb measured by the Drabkin's method and the Hb 801 analyzer for rainbow trout (black diamonds), Atlantic Wolffish (grey triangles) and Nile tilapia (white circles). Curve for all fish (solid line): y = 0.9961x + 0.1012, R^2=0.971



CANDIDATUS SCALINDUA, A BIOLOGICAL SOLUTION TO TREAT SALINE RECIRCULATING AQUACULTURE SYSTEM WASTEWATER

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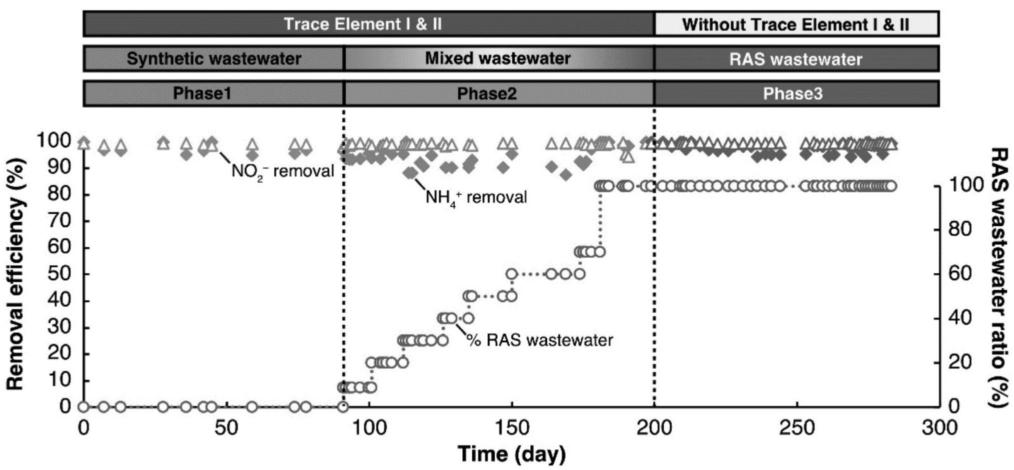
As the aquaculture sector development intensified, concerns regarding the impact caused by the high discharge of nutrients to the environment are rising. Recirculating aquaculture systems (RAS) allow high water reuse alongside a good control of the farming conditions. In RAS, ammonium (NH_4^+) is oxidized into nitrate (NO_3^-) via nitrite (NO_2^-) by nitrifying bacteria in aerobic biofilters. NO_3^- can later be removed from the system through anaerobic denitrification or by regular water exchanges. As an alternative, the anammox (anaerobic ammonia oxidation) process is a cost-effective and environment-friendly way to remove nitrogen compounds from RAS wastewater (WW), where $NH_4^+$and NO_2^- are directly transformed into nitrogen gas (N_2).

We evaluated the potential of the marine anammox *Candidatus* Scalindua to clean RAS WW through a series of experiments in laboratory conditions. A sudden exposure to RAS WW, enriched in NH_4^+ (28 mg.L^{-1}) and NO_2^- (34 mg.L^{-1}), reduced its removal activity for these nitrogenous compounds, without impairing its relative abundance in the granule. To gradually expose *Ca.* Scalindua to RAS WW on the other hand resulted in a successful acclimation of the bacteria (Figure 1), even in the absence of trace element supplementation, while a slight decrease in relative abundance was observed. High NO_3^- concentrations typically encountered in RAS did not affect the removal rate of *Ca.* Scalindua for neither NH_4^+ nor NO_2^- but did reduce its relative abundance. Preliminary results suggest that *Ca.* Scalindua can maintain high removal (> 70%) for both NH_4^+ and NO_2^- rates when exposed to concentrations of NH_4^+ and NO_2^- commonly encountered in RAS (< 1 mg.L^{-1}). We conclude that *Ca.* Scalindua can be successfully used to treat marine RAS WW under laboratory conditions. Future studies need to validate this findings in a pilot RAS.

Figure 1. Anammox performance in the reactor.



SWIMMING INTO SAFETY: MYCOTOXINS IN AQUACULTURE – RESEARCH INSIGHTS AND MITIGATION STRATEGIES IN LIGHT OF EUROPEAN REGULATIONS

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Mycotoxins, secondary metabolites produced by fungi, pose significant risks to animal and human health. In the European Union (EU), stringent regulations are in place to control human and animal exposure to major mycotoxins such as aflatoxins, ochratoxin A, deoxynivalenol, zearalenone, and fumonisins.

These regulations are based on available scientific knowledge. However, until recently, extensive research has focused on mycotoxins' effects on terrestrial livestock and humans, and the impact of these mycotoxins on aquatic species, particularly fish and shrimp was relatively understudied, resulting in general legislation applied to the protection of aquatic species.

In recent years, studies in both fish and shrimp species indicate that mycotoxin contamination, even below the current regulatory guidelines, can lead to reduced growth performance, impaired immune function, and organ system damage such as the hepatic, renal, and reproductive systems leading to reduced productivity, and welfare and increased environmental impact of aquaculture systems. Moreover, mycotoxin exposure may compromise the quality and safety of aquaculture products, thus posing risks to consumers.

Recent survey efforts, aimed specifically at the aquaculture sector, reveal a high prevalence and co-occurrence of regulated and emerging mycotoxins in aqua feeds. Understanding the mechanisms underlying mycotoxin toxicity in aquatic organisms combined with diligent feed surveillance is crucial for developing effective mitigation strategies.

The presented research aims to combine the latest knowledge in the field and to assess whether the current legislation sufficiently protects the aquaculture sector.

CLOSING THE LIFE CYCLE OF EUROPEAN EEL *Anguilla anguilla* IN CAPTIVITY: AN OVERVIEW OF LEPTOCEPHALUS LARVAE REARING

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Background
Glasaal Volendam is a private research company which aims to close the life cycle of European eel *Anguilla anguilla* in captivity. The lengthy life cycle of European eel begins with hatching in the Sargasso Sea. From there larvae drift as leptocephali back to the coasts of Europe, transforming into glass eels. After entering rivers and migrating upstream, once sexually mature they migrate back to the Sargasso Sea to complete the life cycle. Glasaal Volendam made considerable progress in recent years towards closing the life cycle of European eel in captivity, producing high-quality gametes and embryos.

Materials and methods
During the maturation period in RAS, wild female eels are kept at low density (20 kg/m^3), with 36‰ salinity and 18° C. To induce vitellogenesis, females receive weekly injections of Carp Pituitary Extract (CPE) (Kagawa et al., 2005). To induce ovulation, females are injected with DHP (7□,20ß-dihydroxy4-pregnen-3-one) according to Ohta et al. (1996). Male broodstock is obtained from commercial eel farms and receives weekly injections of human chorionic gonadotropin (Perez et al. 2005). Fertilized eggs are incubated for 48 hours before hatching. On day 14 after hatching, larvae are moved to feeding tanks for experiments.

Results and future steps
Currently Glasaal Volendam can produce large numbers of fertilized eggs weekly. Survival rates at the start of the feeding were improved from 0.3% in 2018 to 15% in 2023. In 2022, for the first time, a batch of larvae showed growth. In 2023 larvae reached the 2 cm in length and more than 230 days of age. In 2024 the research is focusing on testing different diets and environmental parameters to evaluate their effects on survival and growth of pre-leptocephalus and leptocephalus larvae. This presentation aims to give an overview of the rearing of eel leptocephalus larvae.

References
Kagawa, H., Tanaka, H., Ohta, H., Unuma, T., Nomura, K., 2005. The first success of glass eel production in the world: basic biology on fish reproduction advances new applied technology in aquaculture. Fish Physiol. Biochem. 31, 193.

Ohta, H., Kagawa, H., Tanaka, H., Okuzawa, K., & Hirose, K. (1996). Changes in fertilization and hatching rates with time after ovulation induced by 17, 20P101 dihydroxy-4-pregnen-3-one in the Japanese eel, Anguilla japonica. Aquaculture, 291–301.

Pérez L, Asturiano J.F., Tomás A., Zegrari S., Barrera R., Espinós F.J., Navarro J.C., Jover (2000) Induction of maturation and spermiation in the male European eel: assessment of sperm quality throughout treatment. J. Fish. Biol. 57(6):1488–1504.

Sørensen S.R., Tomkiewicz J., Munk P., Butts I.A., Nielsen A., Lauesen P., Graver C. (2016b) Ontogeny and growth of early life stages of captive-bred European eel. Aquaculture 456:50–61.

EVALUATION OF FERMENTED SOYBEAN MEAL AS A SUSTAINABLE FISHMEAL REPLACEMENT IN THE DIETS OF EUROPEAN SEABASS *Dicentrarchus Labrax* JUVENILES: EFFECTS ON GROWTH PERFORMANCE, IMMUNE RESPONSE, AND INTESTINAL STRUCTURE

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Fishmeal (FM) is considered to be the ideal protein source for fish production. However, due to its limited availability worldwide and its negative environmental impact, there is a need to focus on sustainable alternatives to reduce the reliance on fishmeal. Recently, fermented soybean meal (FSBM) has attracted considerable interest due to its improved nutritional profile, reduced anti-nutritional factors (ANFs), improved digestibility and associated health benefits. The aim of this study was to evaluate the potential of FSBM as an alternative protein source in the diet of European seabass (*Dicentrarchus labrax*) and to investigate its effects on feed efficiency, growth performance, immune response and intestinal health.

For this purpose, a 13-week trial was performed to investigate the effects of different inclusion levels of FSBM (INOLASA, COSTA RICA) replacing FM. Six isonitrogenous (46.5% crude protein) and isolipidic (16% crude lipid) diets were formulated, a control diet containing 30% FM and five experimental diets replacing FM by 15, 30, 50, 70, and 80%. All diets (3.5 mm pellets) were produced by cooking extrusion.

Juvenile European seabass were obtained from a commercial fish farm and transferred to the HCMR research facilities where they were randomly distributed into 18 cylindroconical experimental tanks of 1,000 L, 35 fish per tank, with 3 replicates for each diet. The average initial weight of the seabass was 30.48 ± 0.14g (SD). Fish were hand-fed to apparent satiation twice daily, and uneaten feed was collected in the waste collector of each tank to calculate daily feed consumption. Similarly, feces were collected and stored at -20 ^{0}C pending digestibility analysis. The average seawater temperature was 20.2 ± 5.6 ^{0}C. At the end of the study, three fish per tank were randomly sampled and tissues from the intestine (anterior and mid) and liver were collected and immediately fixed in 10% buffered formalin for histological analysis. In addition, blood was collected from the caudal vein of the fish, and serum was used for immunological assessments.

This study showed that the tested FSBM could replace at least up to 50% of the FM in the diets of European seabass without affecting growth performance and feed utilization. Digestibility of protein and lipids was found to be lower in the 70% and 80% diets compared to the control. Overall, the FSBM diets didn't induce severe immune responses that would indicate adverse effects on fish physiology. However, the high inclusion rates of FSBM in the 70% and 80% groups significantly reduced fish serum lysozyme levels compared to the control group. Finally, histological evaluation of fish tissues from the anterior, midgut, and liver showed no statistically significant differences between the diets, and no enteritis or signs of inflammation or cellular damage were observed.

DYNAMICS OF AMINO AND FATTY ACIDS IN POND PELAGIC PLANKTON CONSORTIA: LINKS TO TEMPERATURE, PHOTOPERIOD, NUTRIENTS AND COSNUMER GRAZING PRESSURE

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Introduction

Aquatic ecologists or limnologists mostly concern themselves with an elemental view of the aquatic food web, such as nutrient cycles or ecological stoichiometry. Whereas aquaculture nutritionists concern themselves with certain essential biomolecules containing those elements. In temperate shallow-lake ecosystems including European fishponds, primary nutrients and plankton dynamics have a rhythm, closely following the PEG (plankton ecology group) model. There is no such model yet on a biochemical view of aquatic food web. What about the dynamics of biomolecules such as specific amino acids and fatty acids relevant for fish nutrition (?).

We present some analysis, how Earth's physical forcing (temperature, photoperiod), primary ecosystem nutrients (carbon, nitrogen, phosphorus) and consumers' grazing pressure (thermally corrected fish growth, plankton consortia relative trophic position) shape the key amino acids and fatty acids flows in planktonic food web to fish in standing freshwater bodies.

Materials and Methods

A set of six identical (0.2 ha area, 1 m depth), stocked (316 kg ha^{-1} *Cyprinus carpio*), fed (wheat 641 kg pond^{-1}; June to September) experimental fishponds (n=6) in Vodnany, Czechia (49°09′24.9"N 14°09′54.2″E; 404 m a.s.l.) was investigated from May to October 2022. Monthly water quality, climate data, underwater pond temperature data loggers, fish body weight measurements (growing from 0.3 to 2 kg), 'carp gill filterable' net plankton consortia (>200-500 µm mesh) were collected. The net plankton consortia (from beginning of fish feeding in June to cessation of fish feeding in October) were lyophilized and analyzed for amino acids and fatty acids content. To understand grazing pressure (feeding demand), thermally corrected growth (TGC) of fish (n=12 month^{-1} pond^{-1}) in ponds were calculated, with precisely calculated degree days in ponds. The relative trophic position (RTP) of monthly plankton consortia was estimated from the difference of [glutamic acid (−) phenylalanine]. As a forecasting of algal blooming tendencies, phytoplankton phosphorus resource use efficiency (RUE_p) was estimated from [particulate organic carbon/ Total P content in water]. A simple visual trend analysis, followed by Pearson's 2-tailed correlation and 2nd order polynomial regression analysis with a strict alpha-level (p=0.01) and minimum 25% variability explanation (R^2 >0.25) criterion (using OpenAI data analyst 4o and R) was used.

Results

Amino acids: Most critical indispensable amino acid for fish 'lysine' in gill-filterable plankton consortia became deficient mid-season (August) in fishponds, but not 'methionine'. A strong relationship could not be established with Earth's forcing (temperature and photoperiod) or ecosystem nutrients (C, N, P). A relationship with grazing pressure (RTP) could be documented. Plankton consortia lacking higher trophic level organisms such as zooplankton (over-grazed by carp due to extraordinary TGC >2.5 units in preceding months) became deficient in lysine. Missing lysine and lowered RTP also coincided with peaking of algal blooming tendencies in fishponds (RUE_p).

Fatty acids: saturated fatty acids (SFA) in planktonic food, that is a symbol of energy reserve in food web and easiest metabolic fuel for mitochondrial β-oxidation in fish, progressively declined from the beginning (June) till end of season (October). A strong relationship with decreasing photoperiod (<14 hours), air temperature (<20°C), carbon accumulation (>10 mg dissolved C l^{-1}) and consumer grazing pressure (fish TGC >2.5 in preceding months) was documented for SFA deterioration. The long-chain (>C20) polyunsaturated fatty acids (LC-PUFA) is upregulated in food web due to a strong relationship to cold response (air temperature <19°C, photoperiod ≤12 hours), but require a nitrogen presence (>1.25 mg dissolved inorganic N l^{-1}) and low consumer grazing pressure (fish TGC <1). Very long chain (>C22) polyunsaturated fatty acids (22:5n-3, 22:6n-3) within PUFAs are only observed during long photoperiod (>14 hours), but not related to temperature or nutrients or grazing pressure.

(Continued on next page)

Discussions

Nutrition from natural food to fish in ponds is not flawless. In fishponds, fatty acids were majorly influenced by Earth's forcing, ecosystem nutrients, followed by some effects of consumer's grazing pressure. Whereas perturbations in amino acids are mostly sensitive to grazing pressure by consumers, and resultant trophic fractionation of amino acids in the pond food web. Perhaps switching-off lysine synthesis at lower trophic level (RTP). Knowing these trends, we could adapt to (complement) the flaws in natural food base. The nutritional quality of European fishponds could be enhanced by balanced pond feeding (nutrition) strategies, appropriate fish stocking densities and artificially creating zooplankton refuges.

Acknowledgment

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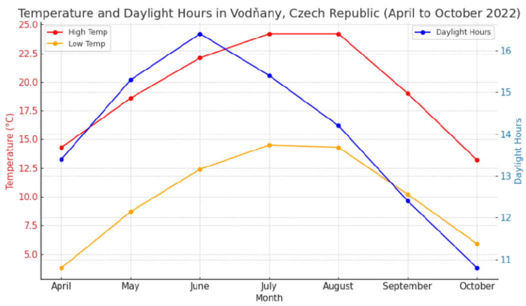
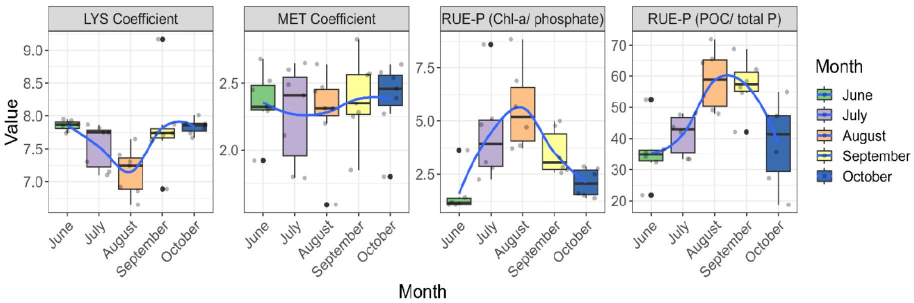
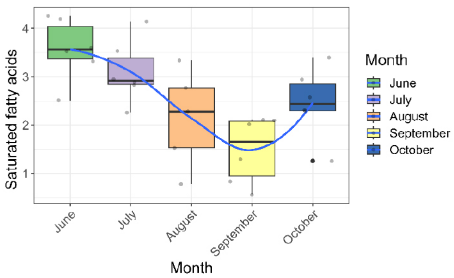
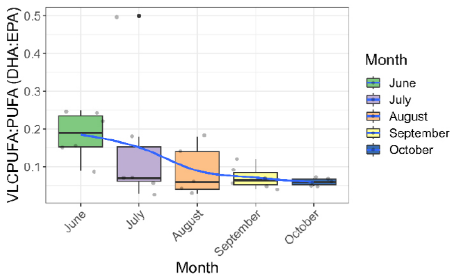




Fatty acids: saturated fatty acids (SFA) in planktonic food, that is a symbol of





MUCOSAL RESPONSE TO A COMBINED CHRONIC EXPOSURE TO NANOPLASTICS AND HIGH SALINITY IN *Sparus aurata*

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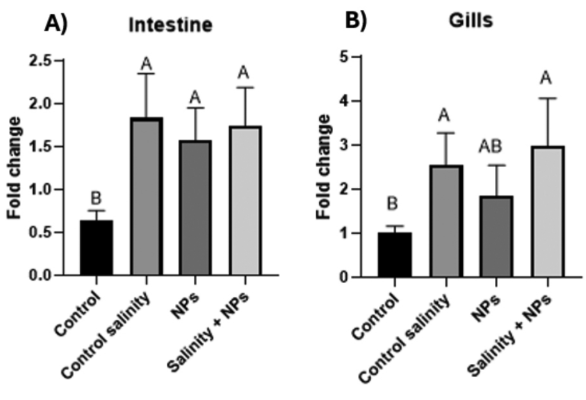
The presence of nanoplastics (NPs) has been reported in aquatic systems all over the world, making them one of the emergent contaminants of greatest concern to the scientific community. In addition, predicted climate change scenarios are expected to lead to considerable fluctuations in environmental parameters, such as increases in salinity in certain coastal regions. The impact of variations in salinity and waterborne exposure to nanoplastics has been studied individually in a variety of aquatic species. However, given the fact that fishes are likely to encounter a combination of stressors in real-life scenarios, it is of utmost importance to investigate the possible synergistic effect of commonly occurring stressors. Furthermore, gilthead seabream (*Sparus aurata*) is one of the most economically important species for the Mediterranean aquaculture industry, and climate change in combination with nanoplastic contamination might cause deleterious effects on the performance of the sector. Therefore, the present study aimed to investigate the effects of increased salinity levels and waterborne exposure to nanoplastics both alone and in combination, on the mucosal health of *S. aurata*.

To this end, juvenile gilthead seabream (14.2 ± 0.8 cm total length) were subjected to a 28-day challenge under four distinct experimental conditions, namely, Control (35 ppt, no NPs), Control salinity (55 ppt, no NPs), NPs (35 ppt, 1000 µg/L NPs), Salinity + NPs (55 ppt, 1000 µg/L NPs), using artificial seawater (AquaForest) and 44nm polystyrene NPs (PSNPs, Bangs Laboratory). Following exposure, fish were humanely euthanised and gills, gut and skin were sampled. The collected organs were processed to obtain cDNA from extracted RNA, and qPCR were performed to investigate the relative expression of genes related to the general stress response (*gr1, mr, hsp70*), oxidative stress response (*sod, cat*), lipid metabolism (*pparα*), and immune response (*tnfα*, *il10, il1β*).

The results suggest a slight effect of increased salinity on the investigated endpoints, showing an activation, to some extent, of the primary stress response, antioxidant mechanisms and immune response, with little to no effect of NPs alone. However, in some cases the combination of both stressors appears to have a synergistic effect on the expression of the studied genes. Overall, the data indicates tissue-specific responses to these stressors, with skin being the least impacted organ, and gills the most.



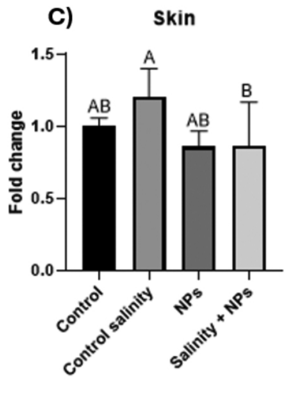


Figure 1: Relative expression of *gr1* in *Sparus aurata* following a 28-day long exposure to increased salinity levels (55ppt) and polystyrene nanoplastics (PSNPs), alone and in combination.

IMMUNOENDOCRINE GENE EXPRESSION RESPONSE OF RAINBOW TROUT SUBJECTED TO REPEATED HYPOXIA

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In this work we have assessed the gene response of the three main mucosal surfaces of rainbow trout *Oncorhynchus mykiss*, skin, gills and gut, when subjected to a repeated hypoxia exposure. The aim of the work was to determine whether a hypoxia-sensitive fish species could habituate to repeated episodes of hypoxia, a circumstance that can occur more often provided under the situation of climate changes and global warming.

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, and H2 and H3 which received 2 and 3 hypoxic exposures respectively. 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 N2 into the system. After 1 hour hypoxia, fish were sampled at 1,6 and 24 post exposure. We analyzed a set of endocrine stress genes (CRH, GR1 and MR), immune-cytokines (IL1β, TNFα, IL10 and TGF β) and hypoxia-associated genes (HIF1, MYO).

The results showed that the response varied depending on the mucosa. Skin showed an alteration of both hormonal stress genes and cytokines that did not recover control levels, whereas hypoxia-related genes showed a ransient decrease followed by a recovery.

In gills, stress genes did not show relevant changes, cytokines showed a transient overexpression followed by a recovery for IL10 and TGF but not recovery for IL1 β and TNFα. Regarding hypoxia-related genes, MYO was overexpressed after 1 and 2 hypoxias and then recovered, whereas HIF1 was overexpressed only after 3 hypoxias.

In intestine, stress genes downregulated at H1 and H2 hypoxias and recoved at H3. Citokine genes showed a significant alteration at H1 but recovery at the subsequent H2 and H3 except for TNFα that was maintained downregulated and hypoxia-related genes showed an altered trend with a significant overexpression of HIF1 and a downregulation of MYO.

Overall the results show alterations in the gene expression after repeated hypoxia, with some degree of adaptation. Thus both hypoxia-related genes and cytokines show significant changes at H1 and H2, but also recovery at the third hypoxic repetition, whereas skin showed maintained downregulation in skin, but minor alterations in gills and intestine.

EFFECT OF DIFFERENT DIETARY LEVELS OF FAT, FATTY ACIDS, AND ZN ON GROWTH, HEALTH, AND FILLET QUALITY OF SALMON IN SEA CAGES

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The composition of the feed for Atlantic salmon in commercial aquaculture is constantly changing over years. The fat levels have increased whereas levels of the omega-3 fatty acids EPA and DHA have decreased. In addition, it has been shown that both the levels of omega-3 fatty acids and fat levels can affect the utilization of minerals.

The purpose of this experiment was to increase the knowledge on how different dietary levels of fat, omega-3 fatty acids, and zinc affects the growth, health, whole body fatty acid retention, and muscle quality of Atlantic salmon in sea cages. The fish grew from an initial weight of 1.5 kg to a final weight of 4.5 kg.

Salmon fed the highest dietary level of EPA + DHA,11% of total fatty acids, had significantly higher final weight than salmon fed 6.5% of these fatty acids. There were no major differences in welfare scores and survival rates between the different diet groups. However, the different diets affected muscle quality. 11% EPA and DHA in the diet improved the red colour in the fillet when compared to the other diet groups, while low dietary Zn resulted in softer texture of the muscle. Higher fatty acid retentions, including EPA retention, were seen in the first growth period in the fall of 2021 than in the second growth period in the spring 2022. The fatty acid composition of tissues largely reflected the fatty acid composition of the feed. The lowest liver fat content was observed in the 11% EPA+DHA group compared to dietary groups fed 6.5% EPA and DHA of total fatty acids.

Overall, these results highlight the importance of the omega-3 fatty acids EPA and DHA and Zn for salmon health and fillet quality.

EFFECTS OF DIETARY INCLUSION OF VISCOSE-RAYON MICROFIBERS ON EUROPEAN SEA BASS PERFORMANCE AND TRANSCRIPTOMIC TRAITS

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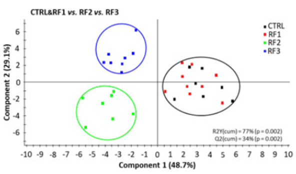
The high prevalence of synthetic or modified microfibres (e.g., cellulosic microfibres) has been reported in aquatic environments, including those supporting aquaculture. However, their potential adverse effects on farmed fish remain poorly explored. This study investigated the impact of dietary exposure to viscose-rayon microfibres (RFs) on the performance and tissue-specific transcriptomics of European sea bass *(Dicentrarchus labrax*). A commercial-based diet (CTRL) was formulated without the addition of RFs. Additionally, three diets with RFs (from a 100% viscose-rayon blouse) were prepared using the same formulation: RF1 (0.001 g/kg), RF2 (0.01 g/kg), RF3 (0.1 g/kg). Groups of 60 fish (~5.8 g) were grown up for 68 days (May-July) in triplicate 500 L tanks in a flow-through marine system, following the natural rise in day-length and temperature (20-27 ºC). Fish were fed three times per day with automatic feeders near to visual satiety. Water [O_2] was always kept above 80% saturation. By the end of the trial, all fish were individually weighed and measured. Additionally, samples of liver, white skeletal muscle, head kidney, and anterior intestine were taken for the simultaneous gene expression profiling with customized qPCR-arrays of selected tissue markers (29 per tissue, 92 in total), covering Gh/Igf growth regulation, lipid and energy metabolism, muscle cell proliferation and differentiation, protein turnover, and humoral and innate immune defence.

All fish in the study grew fast (SGR, 2.40-2.43) and efficiently (FCR, 0.95-0.96), and any impact on growth performance was found with the addition of RFs in the diet. RFs were only detected in the water and fillets of RF2 and RF3 fed fish. Liver weight increased progressively with the RF addition, varying the hepatosomatic index from 1.98 in RF3 and 1.8 in RF2 to 1.49 in CTRL&RF1 fed fish. In line with this, hepatic lipogenic markers (*scd1b*) and components of the negative feedback loop of *de novo* lipogenesis (*elovl5*) were only markedly upregulated in RF2 and RF3 fish, while the opposite was found for hepatic lipolytic markers (*cpt1a, atgl*). According to this, both in liver and the other analysed tissues, the number of differentially expressed genes with a discriminant value (VIP ≥1) in a Partial Least Square-Discriminant Analysis (PLS-DA) was limited to extreme groups (CTRL&RF1 *vs* RF2&RF3). However, when all data and tissues were put together, the PLS-DA was able to differentiate three main groups (CTRL&RF1, RF2, RF3). Such separation was driven by 43 discriminant markers, and its clustering heatmap disclosed a clear over-representation of downregulated genes by RF exposure in head kidney, while the upregulation was the dominant response in liver, muscle, and intestine. All this suggests that the impact of RFs on fish performance could be initially considered slight, although the transcriptomic multi-tissue target approach served to envisage the occurrence of a wide range of potential long-term alterations.

This work was supported by the TNA programme (PID: 22708 and 25209) within H2020 AQUAEXCEL3.0 project (871108) to R. Matias for accessing to IATS-CSIC facilities. Financial support from FCT to R. Matias, through a PhD fellowship (2022.10421.BD) is acknowledged.



COMPARATIVE STUDY OF MICROALGAE VERSUS PROBIOTIC BACTERIA ADDITION ON *Penaeus vannamei* PERFORMANCE IN BIOFLOC TECHNOLOGY (BFT)

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Introduction: Currently, research studies are being focused on microalgae and bacterial probiotics inclusion in Biofloc Technology (BFT). Both have demonstrated a beneficial effect on *P. vannamei* performance, particularly, the inclusion of *Chlorella vulgaris* reported an improvement in terms of water quality, shrimp growth and health status. Therefore, this study aims to investigate the probiotic effects of including and modulating the growth of *Chlorella sp*. on the production of *P. vannamei* in BFT, and to compare it with commercial probiotics.

Material and Methods: In tanks of 1 m^3 (n=3), a biofloc Control (C) and Probiotic (P) group were reared in dark conditions. Both will be compared with a Microalgae (M) and Microalgae + Probiotic (MP) group under BFT and photoperiod of 12:12h (Light:Dark, respectively). Before starting the assay, microalgae were inoculated to achieve a final absorbance of 0.3 AU (λ= 680 nm) into a biofloc system with a total suspended solids (TSS) of ~150 mg/L. Commercial probiotic (Sanolife PRO-W, INVE) was weekly added at 1g/m^3 dosis. The animals (ϱ= 205 animals/m^2) were fed twice per day with a commercial feed (Le Gouguessant Aquaculture, France), and partial weekly sampling was performed to control the feeding intake ratio and gain weight. After 60 days a final sampling was performed, and the animals were undergoing a density stress test. Density challenge consisted in submitting the shrimp for 6 hours at a density of 2200 animals/m^3, evaluating afterwards their survival at 6 and 24 hours.

Results and discussion: Significant differences in chlorophyll concentration are observed between treatments with microalgae addition (0.200±0.02) and without (0.035±0.01), what demonstrates that was possible to achieve a stable *Chlorella vulgaris* population under biofloc conditions. However, lower growth and survival were registered in microalgae groups (M and MP), likely caused by the high nitrates and phosphates levels present from the outset (Table 1), stemming from the NO_3^- and PO_4^{3-} within the microalgae inoculum. In fact, microalgae was able to reduce NO_3^- and PO_4^{3-}, but not enough to achieve non-microalgae group levels.

There were no differences found in survival for the density stress challenge (~90%), then bacteria or microalgae probiotic effect did not provide a major resilience to environmental challenge.

Acknowledgments: This work was supported by European Union Next Generation-Plan de Recuperación-Ministerio de Ciencia e Innovación-Gobierno de España (TED2021-129272B-C21). J. Brol has a predoctoral grant from Generalitat Valenciana (Programa Santiago Grisolía 2021; CIGRIS/2021/109). T. Cascales contract was supported by European Union Next Generation-Plan of Conselleria d'innovació, Universitats, Ciència i Societat Digital of Generalitat Valenciana (INVEST/2022/434).

Table 1: Concentration increase (mg/L) of nitrates and phosphates (Final concentration - Initial concentration) (below). Different letters in rows indicate statistical differences between treatments.

	M	MP	P	C
NO_3^-	286,49±1,56^a	276,04±58,17^a	181,32±13,12^b	180,09±11,24^a
PO_4^{3-}	20,84±2,63^a	21,19±0,14^b	12,89±2,08^c	13,99±0,70^c
Δ [NO_3^-]	16,64±31,58^a	-1,05±17,04^a	179,68±77,32^b	179,53±105,11^b
Δ [PO_4^{3-}]	-16,87±2,51^a	-17,53±3,99^a	8,83±9,31^b	8,30±2,94^b

OPTIMIZATION OF *Mugil cephalus* PRODUCTION UNDER BIOFLOC TECHNOLOGY: EFFECT OF PHOTOPERIOD AND PROBIOTIC ADDITION

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Introduction: To achieve a sustainable Mediterranean aquaculture production, it is necessary to increase the production of low trophic species, such as *Mugil cephalus*, combined with aquaculture systems with low environmental impact, like Biofloc Technology (BFT). BFT is characterized by its minimal use of water, in which bacteria and other aggregates of microorganisms recycle nutrients, maintain water quality, and provide food for the animals. The objective of the present study was to evaluate the light restriction (24h darkness), use of probiotics, and interaction between these variables on the zootechnical performance of *M. cephalus* reared in BFT.

Material and Methods: For the experiment, 180 animals with an initial weight of 91.73 ± 22.4 grams were randomly distributed in 12 circular tanks of 800L of water (initial:150-200 mg/L of total suspended solids (TSS)). In order to study the light restriction and use of probiotics (Sanolife PRO-W INVE, 1g/m^3 per week), 4 treatments were proved: 12-hour photoperiod (Light); light restriction (24h darkness; Dark); 12-hour photoperiod + probiotic (Light+Prob); and light restriction + probiotic (Dark+Prob). A commercial feed (DIBAQ - 3.5 mm for tilapia) with 30% crude protein and 7% crude fat was provided three times daily at a 2 %/d feeding rate.

Results and discussion: The results of the zootechnical parameters are described in Table 1. The different treatments showed no effect on the survival of *M. cephalus*, which was around 100% in all of them. Moreover, the treatments with the presence of light showed the highest feed conversion ratio (FCR), 12.42 ± 1.80 and 11.04 ± 2.25, and lowest specific growth rate (SGR), 0.18 ± 0.03 and 0.19 ± 0.04 to Light and Light+Prob, respectively.

On the other hand, although no significant differences were found in TSS among light and dark conditions, in general, higher values were registered in dark groups, what may have contributed to the better FCR and SGR of these treatments. Probiotics can stimulate the activity of digestive enzymes and thus promote better weight gain. This aspect, combined with the greater bacterial growth (higher TSS values) due to the darkness conditions, may have contributed to the good performance of the animals in the Dark+Prob treatment.

Acknowledgments: This work was supported by European Union Next Generation-Plan de Recuperación-Ministerio de Ciencia e Innovación-Gobierno de España (TED2021-129272B-C21), and Conselleria d'innovació, Universitats, Ciència i Societat Digital of Generalitat Valenciana (GVA AICO/2021/198). J. Brol has a predoctoral grant from Generalitat Valenciana (Programa Santiago Grisolía 2021; CIGRIS/2021/109). R. Olivares-Perona has a technician contract was also funded by TED2021-129272B-C21. T. Cascales contract was supported by European Union Next Generation-Plan of Conselleria d'innovació, Universitats, Ciència i Societat Digital of Generalitat Valenciana (INVEST/2022/434).

Table 1. Growth performance of *Mugil cephalus* juvenile rearing during 41 days in BFT.

	Light	**Light+Prob**	**Dark**	**Dark+Prob**
Initial weight (g)	96.70 ± 2.40	67.22 ± 38.37	104.48 ± 2.47	70.79±31.33
Final weight (g)	104.89 ± 31.33	99.00 ± 2.98	115.84 ± 31.33	104.03±2.44
FCR	12.42 ± 1.80$^{\mathbf{a}}$	11.04 ± 2.25$^{\mathbf{ab}}$	5.87 ± 1.86$^{\mathbf{b}}$	5.92 ± 1.84$^{\mathbf{b}}$
SGR (% day-^{1})	0.18 ± 0.03$^{\mathbf{c}}$	0.19 ± 0.04$^{\mathbf{bc}}$	0.30 ± 0.23$^{\mathbf{ab}}$	0.35 ± 0.03$^{\mathbf{a}}$
Survival (%)	98.34 ± 2.48	98.92 ± 3.09	96.62 ± 2.56	99.08 ± 2.53

Different letters indicate significant differences between groups (P<0.05) according to Newman-Keuls tests.

VISUALIZING OPERATIONS AND IMPACT ON FISH PRODUCTION THROUGH GRAPHDB

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The aquaculture industry in Norway is growing rapidly facing the challenge of managing vast amounts of data daily to ensure the optimal growth and health of fish stocks. Traditional data management approaches often fall short in handling the complexity and volume of this data, making it challenging for fish farmers to infer insights into their production operations. This work demonstrates the use of graph databases to visualize and analyze daily activities logged by fish farmers in the AKVA Fishtalk software [1], focusing on operations such as transfers, splits, and merges with fish groups. By employing custom algorithms to map these operations from data stored in traditional database management software onto a graph database, this work enables a holistic visualization of operations exerted on fish groups allowing a more comprehensive analysis that aids fish farmers in understanding the effects of various operations on fish product quality.

Our **methodology** centers on visualizing daily records stored by fish farmers in traditional database management software, e.g. the AKVA Fishtalk [1] in a graph database framework so-called Neo4j [2]. This involves the transformation of conventional data entries on stock, growth, feeding, and health metrics into a network of interconnected nodes and edges, representing the complex relationships and operations with fish farming activities. Custom algorithms were developed to identify key operational events, such as fish group splits and merges, and to visualize these within the graph database. *Fig. 1* illustrates the Fishtalk software interface. In this environment, data is typically stored in a table format which is hard to understand and analyze.

Fig. 2 depicts a visualization from the Neo4j interface. This visualization is a result of deploying the custom algorithm that transfers the information stored in Fishtalk into a graph representation. This approach allows for a dynamic representation of the lifecycle and operational interventions for each fish group.

This work showcases the potential of graph databases in visualizing operations exerted on fish groups. Graph-based frameworks can provide fish farmers insights on how to optimize the various factors that influence the fish production process. Future directions include the development of predictive models to forecast outcomes of different operational strategies, thereby further enhancing decision-making processes.

References

[1] AKVA Fishtalk., *Fishtalk*. Accessed: Apr. 04, 2024. [Online].
Available: https://www.akvagroup.no/akva-fishtalk/
e: post@akvagroup.com
[2] Neo4j, Inc., *Neo4j*. Accessed: Apr. 04, 2024. [Online].
Available: https://neo4j.com/product/neo4j-graph-databas

DETECTION OF BLOOD PARASITES *Trapanosoma spp* IN NILE TILAPIA *Oreochromis niloticus* Linnaeus, 1758 IN ZOBE RESERVOIR, KATSINA STATE

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The study was conducted to investigate the prevalence of blood parasites, specifically *Trypanosoma spp*, in Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) in Zobe Reservoir, Katsina State. A total of 108 fish samples were randomly selected comprising male and female. Experimental fish samples were purchased from four major landing sites of the reservoir from July- September; 2023. The fish were transported alive to the fish biology laboratory, Federal University Dutsin -Ma, for the blood parasites collection and examination. Sampled fish were measured for length and weighed. The study employed a comprehensive approach in diagnostic techniques such as microscopic examination of blood smears, to accurately identify and characterize the *Trypanosoma* species present. Microscopic examination revealed the presence of *Trypanosoma* parasites in a significant proportion of the sampled fish. Blood parasites were identified and counted. Blood parasites were different in sizes, shape and staining reaction. Male samples had higher per cent of infestation (6.82**%**) than female samples (5.00**%**). The highest prevalence was recorded in samples location C while the lowest was recorded in sample location B. Experimental fish (*O. niloticus*) samples obtained from samples location A were free from trypanosome this could be due to the absence of bloodsuckers in the samples location. The discoveries of this research contribute to the understanding of the prevalence and diversity of *Trypanosoma* parasites in Nile tilapia populations in Zobe Reservoir.

Table 1: Prevalence of Blood parasite of *Oreochromis niloticus* in relation to sex in Zobe reservoir

Sex	No examined	No of infected	% of infection
Male	88	6	6.82%
Female	20	1	5.00%
Total	108	7	6.48%

Table 2: Prevalence of Blood parasite of *Oreochromis niloticus* in relation to sample location in Zobe reservoir

Location	No examined	No of infected	% of infection
A	36	0	0%
B	36	3	8.33%
C	36	4	11.11%
Total	108	7	6.48%

SELECTION RESPONSE OF LICE RESISTANCE IN ATLANTIC SALMON RAISED IN SEA CAGES

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Sea lice infestation has been an increasing problem in salmon aquaculture, with strict thresholds requiring extensive and repetitive treatment events during sea production phases. An alternative and more sustainable strategy to reduce sea lice infection is through selective breeding. The aim of this study was to investigate the expected reduction in lice infestation and associated treatments in sea pen raised Atlantic salmon selected for high genetic resistance.

Three genetic groups, i.e., 0, -1.5 and -2.5 genetic standard deviations (gSD) of genomic breeding values (gEBVs) for lice resistance were created from Mowi nucleus population and subsequently, transferred to hatchery and nursing units until they smoltified and reached 150 g. In April 2023, 4,500 fish were PIT-tagged and distributed evenly across nine sea cages (5x5x5m^3) located at LetSea AS in Solfjellsjøen, Norway. A latin square experimental design was used where each row and column contained all genetic groups with three replicates per group. Growth and lice count were monitored using artificial intelligence underwater camera units (Bioscope model, OptoScale AS). When lice count approached the treatment threshold, 50 random fish per cage were recorded for body weight and manual lice count and all fish were subsequently deloused using mechanical suction. The lice count data from six delousing events (**Figure 1**) was fitted with a linear model. There was no significant difference in lice count due to row and column in the latin square design. The mean lice count from -1.5gSD (-0.02 lice) and -2.5gSD (-0.32 lice) was lower than 0gSD but it was not statistically significant. In conclusion, we observed no significant difference in lice resistance in groups reared separately with higher and lower gEBVs for this trait. These results support previous findings of little or no difference in average lice numbers per fish between groups divergently selected for lice resistance that were challenge tested in separate tanks. These results could be explained by the ability of lice to find a host regardless of the host resistance level of the fish when there is no other choice and suggests further study of the attraction and attachment dynamics are needed when gEBVs from mixed group challenges.

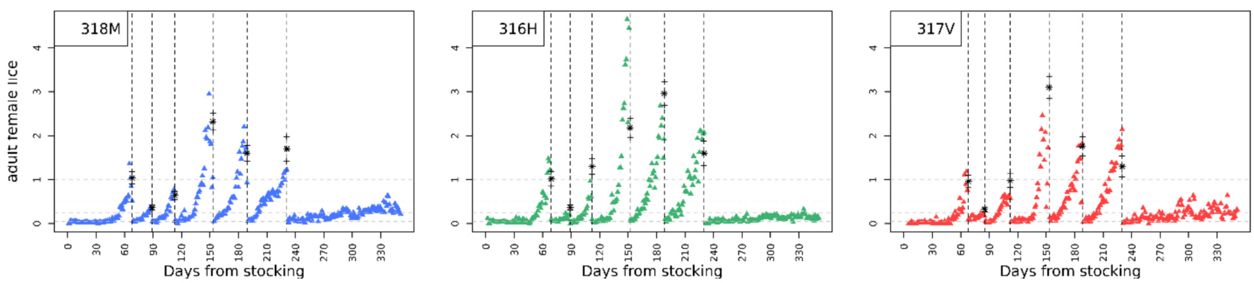

Figure 1 Longitudinal data of adult female lice 0gSD (blue dots), -1.5gSD (green dots) and -2.5gSD (red dots). The broken lines indicate the delousing events.

SKREENING FOR VITAMIN AND MINERAL REQUIREMENTS IN THE ATLANTIC HALIBUT DIET

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Atlantic halibut, the largest flatfish in the Atlantic, is being farmed in Norway, Scotland and Canada. While the production has historically been modest, the Norwegian halibut industry is experiencing significant growth. Overcoming previous limitations primarily linked to larval development challenges, advancements in management strategies have facilitated more efficient production methods. Presently, the focus lies on optimizing the on-growing phase.

However, a notable knowledge gap persists regarding the nutritional requirements of Atlantic halibut. In an effort to address this, we conducted an initial assessment of water-soluble vitamin and mineral requirements.

Retention of the vitamins were analysed in liver, muscle and gills and minerals were analysed in whole fish. Requirements were estimated based on a piecewise linear regression with breakpoint.

This approach is based on the premise that once the fish's dietary requirements for water-soluble vitamins are met, any excess will be excreted. Consequently, there exists a linear relationship between dietary and tissue vitamin levels up to this point. Beyond this threshold, tissue accumulation no longer correlates with increasing dietary levels.

For instance, thiamine requirement was estimated to be 12 mg/kg, although riboflavin estimation is uncertain due to significant variation. Pyridoxine was estimated at 6.2 mg/kg, cobalamin at 0.24 mg/kg, and pantothenic acid at 20 mg/kg. Biotin, vitamin C, and mineral results will be presented and discussed during the presentation.

This method offers the advantage of simultaneously assessing multiple nutrients in a single feeding trial, thereby minimizing the use of animals and resources. However, there is a risk of interaction effects that may influence requirement estimations.

Table: Amount (mg/kg or IU) of vitamin and mineral in each diet. Estimated requirements are in bold.

Vitamin/Mineral	D 1	D 2	D 3	D 4	D 5	D 6	D 7
Thiamine	3,8	5,7	8,8	**12**	16	18	20
Riboflavin	4,6	8,3	12,0	17	22	26	29
Pyridoxin	2,5	3,5	5	**6,2**	7,8	9,5	11
Cobalamin	0,05	0,1	0,11	0,18	0,21	**0,24**	0,51
Folic acid	1,0	4,2	7,3	10,5	13,7	16,8	20
Pantothenic acid	6	8,8	14	**20**	25	30	37
Biotin	0,36	0,36	0,37	0,39	0,45	0,4	0,45
Vitamin C	11	150	310	470	590	760	880
Cu	6,5	13,8	21,0	28,3	35,5	42,8	50
Fe	158,8	232,3	305,9	379,4	452,9	526,5	600
Zn	45,5	104,6	163,6	222,7	281,8	340,9	400
Mn	26,1	32,6	39,1	45,5	52,0	58,5	100
Se	0,9	1,1	1,3	1,4	1,6	1,8	2
I	0,3	0,9	1,5	2,1	2,8	3,4	4

Gracilaria spp. AQUACULTURE IN DENMARK

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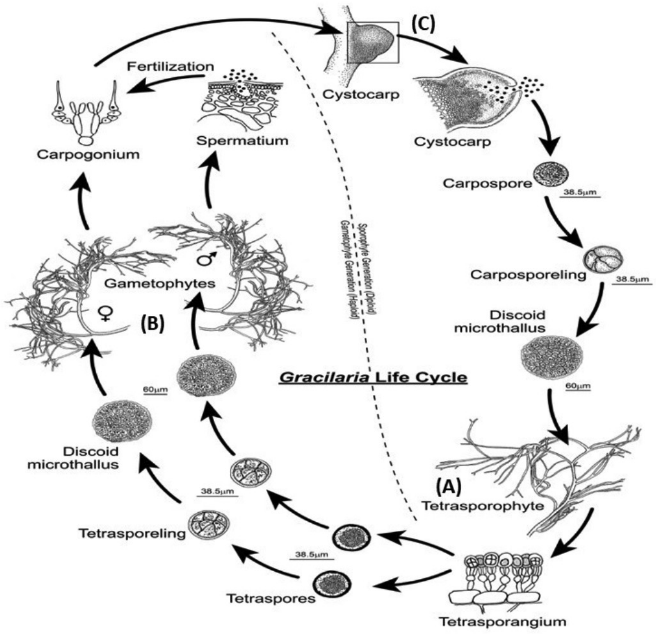
The use of traditional and a new grafting technique to produce seedlings using mature tissue that releases carpospores is investigated, as well as the newer method where germinating gametophytes are used, which can potentially re-establish attachment ("re-attachment"). A risk assessment of the invasive *Gracilaria* spp. spread in Limfjorden is done using DNA analysis technics. The aim is to cultivate in land the native Gracilaria, meaning, inducing cystocarp maturation, cystocarp sporulation, settlement of spores in specific substrate, growing seedlings, maintaining cultures and inducing tetrasporangia growth. Overall, the study is aiming to reproduce the life cycle of *Gracilaria* spp. in land to secure a steady supply of raw material for the development of native *Gracilaria* spp. aquaculture in Danish waters.

The life cycle of *Gracilaria* spp. is shown in Figure 1. The adult tetrasporophyte (A) releases tetraspores into the water and the spores attach to the surface by developing a discoid attachment organ. They then germinate and develop into either a female or a male gametophyte (B). During sexual maturation, the male gametophyte releases its gametes (spermatia) into the water, which is taken up by the female gametophyte through short-lived cellular tubes that carry the spermatia into the egg cell. After fertilization (C), the next generation develops and is called the cystocarp, and is seen as distinct brown knobs on the female gametophyte stems (see cover photo). In the cystocarp tissue, the microscopic carposporophyte forms, which matures and forms many carspospores, which are released into the water and attach to a surface, after which a new tetrasporophyte develops, completing the life cycle (A).



GENETIC SELECTION FOR RESISTANCE TO *Enterocytozoon hepatopenaei* AND WHITE FECES DISEASE IN WHITELEG SHRIMP *(Penaeus vannamei)*

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Infectious diseases present one of the main threats to the expansion and productivity of the shrimp industry. In recent years, Enterocytozoon hepatopenaei (EHP), a microsporidian pathogen, and White Feces Syndrome (WFD), an emergent disease of unknown etiology, have caused severe losses for producers in Asia and America. Although the exact cause of WFD is still unknown, the disease has been reproduced by co-infection of *P vannamei* with EHP followed by *vibrio parahemolyticus* or *vibrio algynolyticus*. This approach mimics what happens in commercial ponds, where the animals first show the presence of EHP, and later, during the grow-out cycle, they develop the white discoloration of the gastrointestinal (GI) tract, and floating white fecal strings characteristic of WFD appear in the pond.

As management of EHP and WFD has proved difficult, selection for resistance to the diseases could be a useful tool, and the use of genomic selection can increase the accuracy of selection, accelerating genetic gain. This study aimed to quantify and characterize the genetic variation in resistance to EHP and WFD in *P vannamei* juveniles. A challenge test was developed at Dr Loc's Tran laboratory in Vietnam, combining cohabitation with EHP-infected animals followed by infection with a *vibrio algynolyticus* strain. This challenge model was initially tested on different lines derived from the Benchmark Genetics Colombia breeding program in 2022 showing significant differences in the survival curves of the lines. In 2023, full siblings of the families from the breeding nucleus were also challenged with EHP+WFD. Animals from all families were challenged at an average weight of 0.6g with both pathogens and followed up for 40 days after infection. The dead animals during the test and all harvested survivors were tissue sampled and genotyped with a ~40K SNP array. Family assignment was performed using the genotype data, and the final survival of the families in the challenge test ranged from 0 to 77% with an average of 32%, based on animals successfully genotyped and with assigned parents. Final survival in the co-infection of EHP and WFD had a moderate and highly significant heritability of 0.41± 0.003.

Furthermore, a low positive genetic correlation between body weight in the growth test in Colombia and survival of the families in the EHP+WFD combined challenge test was observed, implying that genetic improvement of resistance to EHP can be achieved without compromising on growth performance. Additionally, cross-validation analysis showed 0.81 ± 0.003 accuracy of genomic prediction, indicating the major potential of a genomic selection strategy for achieving genetic improvement for resistance to EHP + WFD in *P vannamei*.

THE USE OF FISH ERYTHROCYTES AS CELLULAR TARGETS FOR NOVEL VACCINE PROTOTYPES: FROM IMMUNE SYSTEM STIMULATION TO CELL-TO-CELL COMMUNICATION

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The high prevalence of infectious diseases has made fish vaccination a major ambition for the aquaculture sector. Even though vaccines are one of the most cost-effective means of reducing economic losses due to viral and bacterial infections, their poor immunogenicity is still a concern. Furthermore, species diversity and limited knowledge of the fish immune system are themselves challenges in developing new vaccines. Interestingly, the most abundant cell type in fish blood, the erythrocytes or red blood cells (RBCs), have been implicated in several immune-related functions, such as antiviral response, phagocytosis, or cytokine-mediated signaling, and can even halt viral hemorrhagic septicemia virus (VHSV) infection. As part of our contribution, our next step was to evaluate rainbow trout RBCs as cellular targets of a recombinant protein subunit vaccine (referred to as nanopellet, NP) against VHSV with an incorporated ligand to RBCs and interferon-gamma (IFN□) as an adjuvant. Previously, we demonstrated that the ligand-targeted NPs were successfully internalized by rainbow trout RBCs and led to the upregulation of genes associated with the antiviral immune response *in vitro*. In this study, RNA-Seq-based transcriptome analysis revealed the activation of functional pathways related to endoplasmic reticulum stress- and mitochondrial stress-related pathways, as well as uptake, secretion, and transport of vesicles in RBCs after immunization with ligand-targeted NPs. Even more, we successfully isolated extracellular vesicles (EVs) derived from RBCs stimulated with ligand-targeted NPs and we characterized them by confocal and electron microscopy. Further study of the miRNA cargo in EVs from fish RBCs will be an essential step in identifying promising modulating molecules and biomarkers for the development of next-generation vaccines.

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CIRCULARITY ASSESSMENT IN ATLANTIC IMTA – OPEN WATER, LANDBASED PUMP ASHORE AND BIOFLOC SYSTEMS - THE ASTRAL PROJECT

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Integrated multi-trophic aquaculture (IMTA) systems promote the incorporation of circular principles. This study aims to explore the potential principles embedded in IMTA and the existing alternatives to quantify circularity. In line with Chary et al [1], nutrients and resource use, are fundamental pillars embedded in the principles applicable to aquaculture (safeguard and regenerate, avoid, prioritize, reuse and recycle and entropy). Therefore, bioremediation indicators together with the efficiency indicators in terms of feed, water, energy, and infrastructure material used are selected to evaluate the circularity performance of 4 IMTA trials in 3 aquaculture facilities (table 1).

Results were interpreted through a ranking system, where the contribution to circularity is observed in Table 2, according to the intensity of green (less or more intense indicate less or more circularity performance).

This study confirmed the fact that multi-trophic aquaculture systems perform in line with the circular attributes embedded in the essential definition of bioremediation. Metrics on bioremediation would promote standardization of nutrient recycling rates, from which the effectiveness of the systems could be evaluated.

In addition to bioremediation, complementary indicators applied to IMTA provide evidence for the implementation of resource efficiency strategies, which further ensures the alignment of these systems with the circular economy.

Bibliography

1. Chary, K.; Van Riel, A.; Muscat, A.; Wilfart, A.; Harchaoui, S.; Verdegem, M.; Filgueira, R.; Troell, M.; Henriksson, P.J.G.; De Boer, I.J.M.; et al. Transforming Sustainable Aquaculture by Applying Circularity Principles. *Reviews in Aquaculture* **2023**, raq.12860, doi:10.1111/raq.12860.

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Table 1. Scenarios of study

Case study	Monoculture scenario	IMTA conditions
Ireland IMTA-lab	Salmon open water marine production	Salmon marine cages with seaweed longlines and urchin and oysters in baskets
Brazil IMTA-lab	White shrimp in ponds	White shrimp with tilapia and seaweed in closed Biofloc systems
South Africa IMTA-lab	Abalone semi-closed	Abalone and seaweed semi-closed
	Urchin semi-closed	Urchin and seaweed semi-closed

Table 2. Ranking – results of circularity performance

	RESOURCE USE EFFICIENCY					NUNTRIENT MANAGEMENT		
	FCR formulated feed	Linearity	Water	Energy	Infrastructure	N Bioremediation	P Bioremediation	C Bioremediation
Ireland IMTA lab								
Brazil IMTA lab								
SA IMTA lab (abalone)								
SA IMTA lab (urchin)								

Table legend		
	Not information	
	Not aplicable	
	Not circularirty attribute	
	Low performance	high performance

GENETIC AND EPIGENETIC REGULATION OF GROWTH IN THREE DISTANT POPULATIONS OF EUROPEAN SEA BASS

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Epigenetic marks are thought to have a role in local adaptation and short-term evolution. However, information about their effect in animal models is scarce, and it remains a topic of debate. We use the European sea bass, a valuable species for fisheries and aquaculture, to investigate DNA methylation levels and gene expression patterns from three genetically distinct populations: Atlantic (AT), Western (WM), and Eastern (EM) Mediterranean (Figure 1A). The objective is to reveal genomic regions showing differential methylation between populations exposed to the same environment, elucidate their associations with changes in gene expression and the organism's ability to allocate energy towards growth. First, we are generating an atlas combining epigenomic and expression data, providing a reference database for each population in three key tissues: liver, muscle and fin. Second, we are investigating which of three tissues is better fit to investigate differences between populations in regards to robustness. Third, we aim to identify biomarkers of growth within and between populations (Figure 1B). When possible, we will identify metastable epialleles, i.e., environmentally responsive loci showing similar trends in epigenetic changes across tissues of the same individual.

To accomplish this, we use fish produced from AT, WM and EM broodstock which were reared under a seasonal thermal regime representative of the Eastern Mediterranean. Once the fish reached 10 grams, individuals from the three populations were mixed and individually tagged. At one year of age, we sampled 48 individuals (8 fish x 3 populations x 2 sexes = 48 fish; 48 x 3 tissues = 144 samples) and performed DNA and RNA extractions. By creating pools of four individuals, we are processing up to 12 pools per tissue for both gene expression (RNA-seq, 30 M reads per sample) and DNA methylation (whole genome Enzymatic Methyl-seq, at 15 x coverage), resulting in a total of 72 libraries. This study aims to identify robustness markers crucial for assessing population vulnerability and sustainable aquaculture. Additionally, it contributes to the understanding of genotype-environment interactions, local adaptation, and the impact of global warming on sea bass populations. This study is supported by the ANR FishNess project and CRECHE (P120-0001-01-DF).

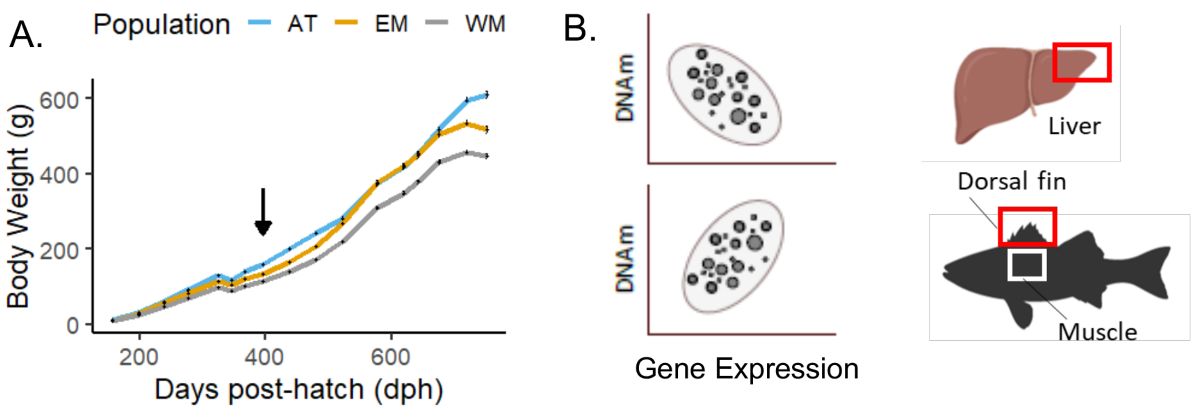

Figure 1. A. Average body weight (in grams) of individuals from three genetically distant populations tracked over the first two years at 17 sampling points. The black arrow indicates the sampling point at 396 days post hatching (dph) **B.** Diagram of DNA methylation (DNAm) and gene expression correlation from the three key tissues selected: liver, dorsal fin and muscle. Created with BioRender.com

VACCINATION AND CHALLENGE AGAINST *Yersinia ruckeri*

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Yersiniosis in Atlantic salmon is caused by *Yersinia ruckeri* (*Y. ruckeri*). Since 2015 it has mainly been a problem in larger fish (> 1kg), causing elevated mortality over 30 % at cage level. The outbreaks occurring in late sea phase have exclusively been caused by *Y. ruckeri* serotype O1b. Serotype O2 has also been found in a few freshwater sites but seems to be less virulent than O1b. So far, there is no evidence of O1a in Norwegian salmon industry.

Norwegian salmon farmers have since 2016 started using injection vaccination of smolts to secure long-term protection also after sea transfer. Efficacy after injection vaccination, assessing RPS, and safety, using the Speilberg scoring system, have been obtained in controlled laboratory studies. Field surveys have been used to collect safety and efficacy data in a commercial setting, with focus on survival and disease detection as well as antibody responses.

Laboratory challenge studies with different *Y. ruckeri* serotypes have been performed in Atlantic salmon, using different administration methods and challenge models. Results from these studies will be presented and discussed.

The data shows high consistency between results from laboratory studies and field surveys. The level of protection against yersiniosis is overall high, including in sites with elevated challenge due to outbreaks in adjacent unvaccinated cages. There have been no registered outbreaks on fish groups vaccinated with this vaccine by injection since the start in 2016. The water-based vaccine gives high level of protection against yersiniosis in Atlantic salmon up to slaughter (18 months post vaccination) and is safe to use in co-injection.

ZEBRAFISH GROWTH PERFORMANCES AND GUT IMMUNITY: EFFECT OF MEAL FROM *Hermetia illucens* REARED ON DIFFERENT SUBSTRATES

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Hermetia illucens (also known as Black Soldier Fly - BSF) is one of the most widely used insect species for producing insect meal and replacing fishmeal (FM) in aquafeeds. It can be reared on a wide variety of biowaste. The nutritional composition of rearing substrate is extremely important for an efficient BSF growth, and it can modulate the insect meal nutritional value. In turn, insect meal nutritional profile may affect fish performance. To this regard, few studies have been carried out. Hence, the purpose of this study was to investigate the effects of partially or totally FM replacement, with larval meal obtained from BSF reared on strawberry by-products (SB) and spent grain (SG), on fish growth performance and gut health.

To this purpose, 375 double transgenic zebrafish Tg(*coro1a:eGFP;lyz:Dsred*) were used as fish model, and macrophages and neutrophils were distinguished by green and green and red fluorescence, respectively. The experimental period lasted 42 days and five isoproteic, isolipidic and isoenergetic experimental diets were used: a control diet (CTRL) containing FM, and four experimental diets (SB25, SB35, SG25 and SG35) where 71% or 100% of the FM was replaced with BSFLM (Black Soldier Fly larval meal) obtained from larvae fed on SB and SG, respectively. At the end of the trial, confocal imaging analysis on the anterior intestine was performed to assess macrophages and neutrophils density (n/ μm^2). mRNA expression analysis of *gata4*, *nfkb1*, *pht1* genes (intestinal inflammation markers) was also performed on the caudal intestine portion.

The results showed no negative effects of BFSLM on fish mortality, feed intake, and feed conversion rate; moreover fish fed BSFLM showed higher growth performance (BWg, SGR and Kf) than the control (Figure 1). No differences were observed also for macrophages and neutrophils density as well as for *gata4*, *nfkb1*, *pht1* expression. However, these genes showed to be downregulated in fish fed on BSFLM, even though not significantly (Figure 2).

However, it may be of interest to better understand the interactions between insect rearing substrate compositions and the possible different uses in aquafeeds.

In conclusion, the results suggested that using *Hermetia illucens* larvae meal for replacing fishmeal in aquafeeds, enhances fish growth performance, regardless of the type of substrate used.

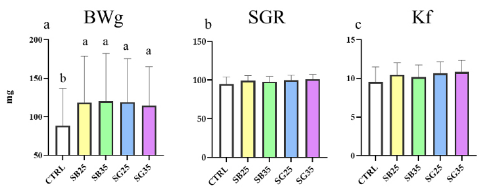

Figure 1: (a) BWg; (b) SGR, (c) Kf (n=75; $p < 0.05$)

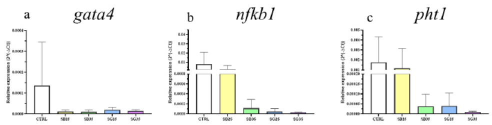

Figure 2: qPCR analysis of (a) gata4, (b) nfkb1b and (c) pht1 genes (n=3; $p < 0.05$)

POTENTIAL OF SOLAR SYSTEMS ON SMALL SCALE SHRIMP FARMS IN BANGLADESH

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The aquaculture industry has expanded significantly over the last decade, leading to higher energy demands that can represent up to 50% of total production costs. Renewable energy options are often incorporated into aquaculture models, and less frequently experimentally into farm-based studies to investigate improvements in sustainability and economic viability of aquaculture sectors. Here, we showcase a pilot study carried out in Bangladesh in 2018, investigating the potential influence of solar panels on the health of cultured shrimp and the associated knock-on effects on the ecosystem within the ponds. Mock solar systems were installed to cover 44% of a 0.12 ha experimental shrimp pond and compared to a control pond of the same size containing only bamboo poles, to control for the effect of periphyton growth. The experimental pond exhibited an increased planktonic and microbial population and higher shrimp production compared to the control pond, potentially due to the shading of the mock solar panels. Energy production was estimated and shown to substantially increase farmers income of about 70%. While this preliminary study was promising a more comprehensive assessment is necessary to corroborate the results obtained and fully understand the impact of solar systems structures on shrimp pond health and ecosystem.

SINGLE-CELL PROTEINS AS A SUSTAINABLE SOURCE OF PROTEIN FOR SALMONID AQUAFEEDS

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Blue foods from aquaculture are essential in bridging the protein gap to feed the human population in the future. 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. Two early prototypes of single cell protein with over 50% protein (named here SCP1 and SCP2) were produced by researchers at the DSM Bioscience Centre (The Netherlands) and were tested over a 12-week period in rainbow trout (*Oncorhynchus mykiss*).

Seven different feeds were produced in a pilot-scale feed mill in Village-Neuf (France). The control diet contained 10% of fish meal and 20% of soy protein concentrate. The two test products were included at 5% (SCP1-5, SCP2-5), 10% (SCP1-10, SCP2-10) and 20% (SCP1-20, SCP2-20) in the diets to replace 5% of the fish meal, 10% of the fish meal and 10% of the fishmeal+10% of the soy protein concentrate, respectively. Each of the seven diets was fed to triplicate groups of rainbow trout (IBW 50g) for 84 days.

Our results show that single cell protein inclusion leads to similar fish performance measured by final body weight as at the end of the trial, performance was similar across the treatments and only the diet formulated with 20% SCP2 showed significantly lower growth (-4.4% compared to the control). The trial's findings demonstrate a high protein digestibility and amino acid profile, as well as health benefits measured through oxidative burst response. Small differences in protein digestion and amino acid digestion and retention were expected and show that fish metabolism adapted to the new formulations.

The present results demonstrate the potential of incorporating SCP in salmonid aquafeeds up to 20% dietary inclusion, as replacers for traditional ingredients such as fish meal and/or soy protein concentrate. With the move towards the use of environmental criteria in raw material selection, novel raw materials such as single cell proteins can deliver lower footprints, water use, land use and limited impact on bio-diversity. In addition, improving the nutrient quality of the novel ingredients by increasing the digestible protein content, together with the commercial scale-up are needed to extend the raw material basket and deliver sustainable raw materials.

DIETARY INCLUSION OF HYDROLYSATES FROM AGRI-FOOD BY-PRODUCTS: EFFECTS ON GROWTH PERFORMANCE, MUSCLE QUALITY, AND METABOLOME OF EUROPEAN SEABASS

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The bioactive potential of hydrolysates in modulating myogenic and protein accretion processes has attracted increased interest in the aquaculture industry. This is due to their capacity to promote fish growth and improve feed efficiency, when included in aquafeeds. This study aims to elucidate the impact of replacing high-quality fishmeal (FM) with novel hydrolysates from local agri-food by-products in the diet of European seabass (*Dicentrarchus labrax*). The study evaluated fish growth performance and nutrient utilization, while also assessing muscle nutritional value, cellularity, and metabolome.

Experimental protein hydrolysates were obtained through the hydrolysis of blue shark skin, fisheries by-products, and swine meat and bones, respectively. A control (CTRL) plant-based diet (with 70% plant proteins) containing 12.5% FM (LT70) was formulated and compared with three experimental diets where 3% of the FM was replaced by one of the previously referred hydrolysates, resulting in three isoproteic diets: SHARK, FISH, and SWINE. Seabass juveniles (initial body weight of 12g) were evenly distributed among twelve tanks and fed the experimental diets three times daily until satiety, with triplicates for each diet. After 86 days of feeding, all fish were weighted and measured. Dorsal muscle samples were collected for determination of nutritional value, morphometric analysis, and primary metabolite profiling by GC-TOF-MS. After the growth trial, nutrient digestibility was evaluated for all diets.

The diets equally promoted specific fish growth rate (1.6), feed conversion ratio (1.1) and nutrient and energy retention efficiencies (% digestible intake). The final condition factor and somatic indexes were statistically increased in fish fed the SWINE diet. Whole-body and muscle composition were similar across groups, and all fillets provided 500 mg of EPA + DHA per 100 g. Regarding the muscle metabolome, the SHARK group exhibited significantly higher levels of glycolytic intermediates and lower levels of glucogenic amino acids (AAs). Muscle cellularity remained unaffected by the dietary treatments, although the number of small-sized fast-twitch fibers (<30 µm) was highest in the FISH group.

The highest somatic indexes in the SWINE group suggest metabolic adaptations in seabass, whilst the muscle metabolome of fish fed the SHARK diet seems to induce protein catabolism to obtain glucogenic AAs. However, these changes were not reflected in fish growth performance and muscle flesh quality, which remained similar among treatments. Results suggest that hydrolysates from agri-food by-products could replace FM in plant-based diets.

Acknowledgments
This work was supported by the Blue Bioeconomy Pact (C644915664-00000026), for the exercise of activities in the WP6 FEED (Pep4Fish project) through international funds provided by the European Union. Luciano E. Santos acknowledges FCT for PhD grant (2023.00947.BDANA).

SYNERGISTIC EFFECT OF BIOACTIVE COMPOUNDS EXTRACTED FROM ALGAE ON EUROPEAN SEABASS *Dicentrarchus labrax* IMMUNITY

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While disease outbreaks continue to occur in European fish farming, different prophylactic strategies are being developed to boost animal resistance. The inclusion of marine algae extracts with bioactive properties in aquafeeds presents as a possible alternative to chemotherapeutics and a complementary strategy to vaccination. The main objective of the present study was to evaluate the innate immune condition and resistance against bacteriosis of European seabass juveniles fed diets containing a combination of algae-derived compounds.

After acclimation, 540 fish (22.7 ± 1.8 g) were randomly distributed in 12 tanks to which the experimental diets were assigned in triplicates. Dietary treatments consisted of a commercial diet (CTRL), two CTRL-based diets with two different inclusion levels of an algae extracts blend (*Tetraselmis* chui aqueous extract, sulphated polysaccharides and phycocyanin, Diet1 and Diet2, respectively) and a CTRL-based diet including sulphated polysaccharides and grape-seed extract (Diet 3). The feeding period lasted for 14 days and was followed by an intraperitoneal bacterial challenge (2.62×10^5 CFU/mL of *Photobacterium damselae piscicida* strain MT415). Samplings were performed immediately before and 6 hours after the bacterial challenge. At each sampling point, fish were euthanized (n=9 per tank) and sampled for blood (haematology), peritoneal leucocytes, plasma, liver (oxidative stress), intestine and head kidney (molecular markers). Cumulative mortality was followed for 14 days in the remaining fish.

Despite no significant differences were observed amongst diets regarding fish survival, fish fed Diet 1 presented an enhanced antioxidant and immune response after the bacterial challenge. The hemoglobin content, intestinal total glutathione and catalase activities and the expression of genes related to innate immunity were increased in infected fish fed Diet 1 when compared with those fed CTRL. Results clearly point to Diet 1 immune-enhancing properties and further investigation should be focused on the optimization of the feeding regime.

IMMUNOMODULATORY POTENCIAL OF *Euglena gracilis* BIOACTIVE COMPOUNDS: AN IN VITRO APPROACH

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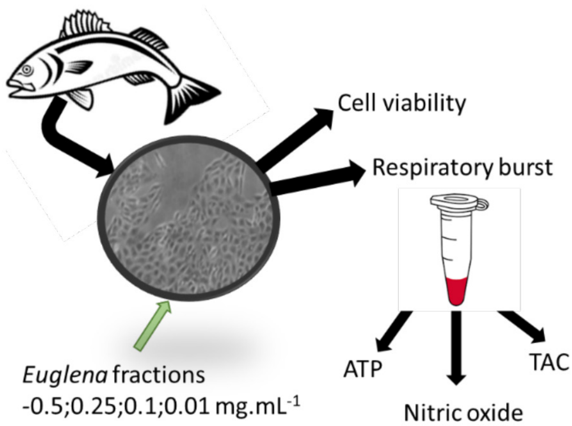
Aquaculture industry is growing at a fast pace owing to the world population increase and general acceptance by consumers of farmed fish. However, diseases are a recurrent problem in aquaculture due to the inherent fish habitat. Therefore, the scientific community is continuously looking for sustainable solutions that can boost the immune system of the fish, while reducing the pressure on wild fish populations. Owing to several bioactive compounds in its composition, microalgae have been suggested for this purpose. *Euglena gracilis*, a microalga of the Euglenales family, produce high amounts of algal β-1,3-glucan, omega-3 fatty acids, protein, and vitamins C and E, which may stimulate the immune system when consumed. Consequently, diet supplementation with *E. gracilis* has the potential to support immune function. Therefore, this study aimed to uncover the potential of *E. gracilis* bioactive compounds as immunomodulators using European seabass (*Dicentrarchus labrax*) head-kidney primary cells.

Four different concentrations (0.5, 0.25, 0.1, and 0.01 mg.mL^{-1}) of fractions obtained from *E. gracilis* (strains G0, G1, J0, J1, J2) were tested in European seabass head-kidney primary cells to assess cell viability. Afterwards, all fractions were used at 0.5 and 0.01 mg.mL^{-1} concentrations for 24h to assess their effect on cell viability, ATP, total antioxidant capacity, nitric oxide, and respiratory burst after stimulation with inactivated *Photobacterium damselae piscicida* MT1415 for 4h.

Results showed that the European seabass head-kidney primary cells viability, ATP production and TAC were not affected by the fractions, as no significant differences were detected, regardless of the concentration. The nitric oxide was significantly lower in fractions G0, and G1 upon stimulation with inactivated bacteria when compared to the placebo, whereas in fractions J0, J1, and J2 an increase was observed.

Further analysis focusing on gene expression will be assessed in European seabass head-kidney primary cells stimulated with inactivated bacteria to determine whether *E. gracilis* bioactive compounds exert a consistent immune modulatory effect.



NATURAL ANTIOXIDANTS FROM INGREDIENT TO FISH

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The use of natural antioxidants (NAOX) for preventing fish feed oxidation and reducing oxidative stress on fish is gaining popularity in the fish feed sector. Nowadays, NAOX have been proposed as a sustainable alternative to synthetic antioxidants such as the banned ethoxyquin or the highly questioned BHT and BHA. Hence, it is imperative to study the behavior of those compounds with natural antioxidant activity (e.g., polyphenols) during the whole fish feed production (from ingredients to final feed) and fish organs. This project studied the effects of a commercial ingredient containing NAOX from two different sources, added at two levels to a commercial fish feed. Thus, the objective was to analyze quantitatively selected polyphenols in various stages of a fish farming process for salmon. Simple extraction methods for the selected polyphenols were developed for different matrices: antioxidant ingredient, fish feed and fish tissue, and further quantification by LC-MS/MS was conducted, allowing the monitoring of the content of polyphenols from the antioxidant ingredient to the fish tissue. Additionally, for studying the effect of the NAOX in the process, several oxidation and oxidative stress indicators have been measured; in feed ingredients, in feed at different stages of production and storage, and fish fed the two diets, compared to a control diet.

Selected polyphenols from four stages (ingredients, before extrusion, before drying and final feed) of the fish feed production cycle and from salmon organs (liver and muscle) were analyzed (Figure 1.) The main polyphenols quantified in fish feed ingredients, fish diets at different production stages, and fish organs were hydroxytyrosol, carnosol, gallic acid and carnosic acid. Additionally, other related components, presumably 12-O-methyl carnosic acid, rosmanol and rosmarinic acid, were detected and semi-quantified by using the standards that closely resemble their chemical structure. The NAOX's content was not stable, an important loss of polyphenols was evidenced, especially during the extrusion process. However, the analysis of liver and muscle samples, dissected from salmon fed the final feed of the production process, revealed that two polyphenols were transferred into these tissues, namely, carnosic acid, methyl carnosic acid.

Interestingly, and despite of the loss of NAOX, results from oxidative parameters suggest thast dietary NAOX affected MDA and glutathione metabolism in both the muscle and liver of Atlantic salmon: MDA was lowered already at 0.01% NAOX in the muscle, while 0.05% was necessary to significantly lower liver MDA. This could indicate that dietary NAOX prevented in vivo lipid oxidation in the fish tissues.GSH and GSSG were lowered in the liver while Eh was constant. In the muscle, GSH increased while GSSG was constant, leading to a more reduced Eh. This shows that redox metabolism was affected by dietary NAOX. No effects of the dietary NAOX on growth, condition factor, body indices or cataract were detected. The results obtained were promising and further studies are needed for advancing in the understanding of NAOX in redox biology of fish.

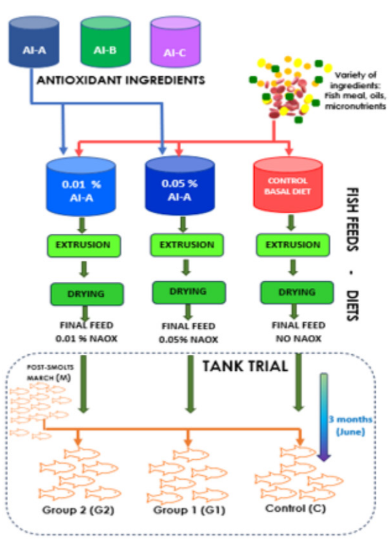

Figure 1. General scheme of study of effect of NAOX in aquaculture of salmon

USE OF DIETARY PHYTOGENIC FEED ADDITIVES AS AN EFFECTIVE APPROACH TO REDUCE *Vibrio anguillarum* INFECTION INCIDENCE IN EUROPEAN SEA BASS *Dicentrarchus labrax* JUVENILES

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Sustainable development in aquafeed production involves reducing reliance on marine and high CO_2 footprint ingredients in favor of eco-friendly alternatives. However, reducing dependence on marine raw materials may induce variable effects on fish nutrient metabolism and mucosal health. To address this challenge, the application of complementary tools to mitigate adverse effects and facilitate the incorporation of alternative ingredients while maintaining fish performance, health, and disease resistance is critical, especially in a global context of reducing the use of chemotherapeutics. Functional ingredients, such as phytogenic feed additives (PFA), are effective immunomodulators to enhance fish disease resistance in farmed animals. Nonetheless, it is complex to elucidate a precise mode of action of PFAs and to determine the potential effects modulated by the bioactive compounds present, especially since synergies and antagonisms may occur. Thereby, the aim of the present study was to determine the role of a PFA prototype based on a mixture of citrus fruits, Asteraceae and Labiatae oils (AL) on European sea bass (*Dicentrarchus labrax*) disease resistance, host-pathogen interactions, and implications on oxidative stress- related processes. Experimental diets, reflecting commercial standards, were formulated with AL at two inclusion levels, 200 (AL200) and 1000 ppm (AL1000). Fish (iBW: 9.4±0.1g) were fed for 60 days, and then challenged with *V. anguillarum* (ip; 10^5 cfu/fish). Plasma and tissue samples were collected at 0, 2 days and 7 days post-inoculation for analysis.

Fish fed both AL dietary levels presented an improved ($p<0.001$) disease resistance against *V. anguillarum* after 60 days of feeding (Figure 1). This improvement was correlated with the up- and down-regulation of genes involved in the orchestration of the immune response at the level of the head and kidney during the development of the infection. Besides, fish fed AL1000 diet presented reduced ($p<0.05$) hepatocyte area compared to fish fed the control diet in relation to a lower activity of liver antioxidant related enzymes.

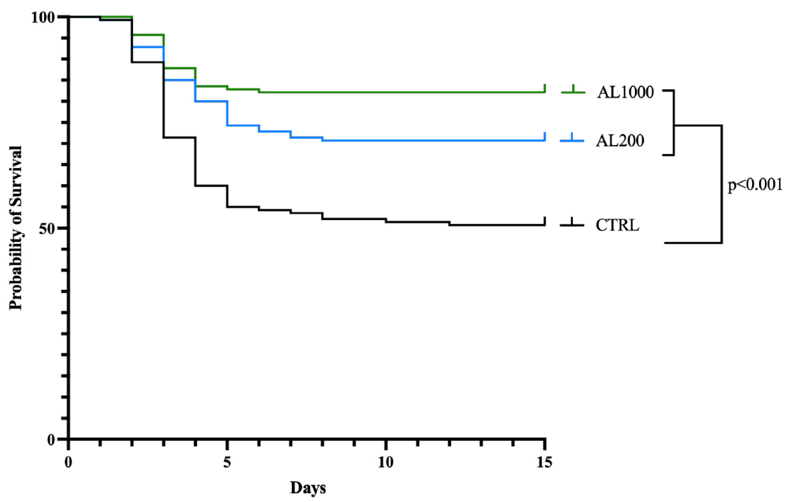

Fig. 1. Cumulative survival (%) European sea bass (*Dicentrarchus labrax*) during the challenge test against *V. anguillarum* fed the experimental diets. AL200= 200 ppm AL; AL1000 =1000 ppm. AL: based on a mixture of citrus fruits, Asteraceae and Labiatae oils.

PROMOTING CIRCULAR ECONOMY BY THE ASSESSMENT AND VALIDATION OF MUSHROOM INDUSTRY BY-PRODUCTS AS SUSTAINABLE INGREDIENTS FOR RAINBOW TROUT *Oncorhynchus mykiss* DIETS

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The European Union produces more than one million metric tons of mushroom per year, with Spain ranking as the third producer country in Europe, contributing approximately 10% of the total production. According to the European Mushroom Growers Group, from each metric ton of mushrooms cultivated and sold as fresh, canned, or frozen, 150 kg of organic residues are produced. The management of the generated waste is becoming an important challenge for the mushroom industry. Under this scenario, the potential use of these agriculture wastes (mushroom stems or not marketable mushrooms) as alternative protein and functional ingredient sources in aquafeeds seems a promising strategy for promoting circularity in this industry. Therefore, the present study assessed the *in vivo* digestibility of mushroom by-products from *Agaricus bisporus* (Ab), *Lentinula edodes* (Le) and *Pleurotus ostreatus* (Po) in rainbow trout (*Oncorhynchus mykiss)* and further correlated to key performance indicators.

Three experimental diets were designed to contain 30% of mushroom meals replacing fish meal and wheat meal (35%CP/12.5%CL). The control diet (Ctrl) was void of mushroom meal (44.1%CP/17.1%CL). Following a 40-day feeding trial in rainbow trout (initial body weight: 120.11 ± 0.30g), fish fed with mushroom diets presented reduced growth performance (Fig. 1A; $p<0.05$; BW_fCtrl = 227.6 ± 0.7 g; BW_fAb = 207.3 ± 1.9 g; BW_fLe = 202.5 ± 6.4 g; BW_fPo = 207.9 ± 2.9 g). Fish fed diets including different mushroom meals grew less than the Ctrl group (-8.9% for the Ab diet, -11% for the Le diet, and -9.0% for the Po diet). The use of mushroom meals slightly increased FCR values in comparison to those fed the control diet (Fig. 2B; $p<0.05$). Growth performance data was correlated with the results of ADC, hematology, digestive enzymes activities and liver and gut morphology. These results may indicate that regardless of the reduction in somatic growth (-9 to -11% in BW_f), mushroom meals may still be an alternative sustainable source of protein in aquafeeds, even though further studies are needed to proper determine their optimal level of inclusion and their functional properties.

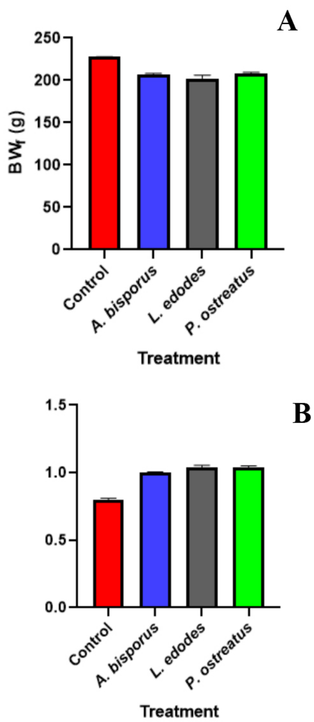

Figure 1. Growth and feeding performance of *O. mykiss* fed with different types of mushroom meals. Final body weight (**A**); Fed conversion ratio (**B**).

MONITORING EARLY-STAGE BEHAVIOR OF ATLANTIC SALMON *Salmo salar* USING A "PING360" SONAR: WHAT HAPPENS TO POST-SMOLTS DURING THEIR FIRST MONTH IN A COMMERCIAL SEA-CAGE?

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Atlantic salmon (Salmo salar) aquaculture production in Norway focuses increasingly on better fish welfare and more sustainable practices. Understanding fish distribution in sea-cages is necessary for optimal farm management, e.g. to feed them adequately, and to better control welfare over time. The task is difficult because of the size of sea-cages on a commercial scale (60 m to 240 m circumference) and the number of individuals per cage (up to 200,000 in Norway). The Ping360 (Blue Robotics Inc), a relatively low-cost single beam mechanical scanning imaging sonar, was successfully used in an aquaculture context to study avoidance behavior of salmon towards intrusive objects in cages. We assumed it also had potential to study fish distribution in a commercial sea-cage if placed to scan vertically. Indeed, the Ping360 enabled us to scan of a complete section of a sea-cage, when most of the sonars and echosounders used in aquaculture nowadays have a limited coverage and are extremely costly.

We followed post-smolt salmon (123,000 individuals, 500 g on average) distribution during a sensitive period of the production: their transfer at sea from the land-based facility to a commercial sea-cage (18 500 m3). The objective was to observe the evolution of their behavior (feeding, diurnal pattern, schooling) with time when adapting to a new environment after the stress of the transport. We collected data with the Ping360 continuously for 1.5 months. It was placed vertically in the center of the cage at 7 m depth. Single 360° scan images were extracted via Python (v 3.8.5) and edited into videos for manual observations and registration of behavioral patterns. Average scans with one hour of data compiled were also computed for easier comparison between days (fig 1).

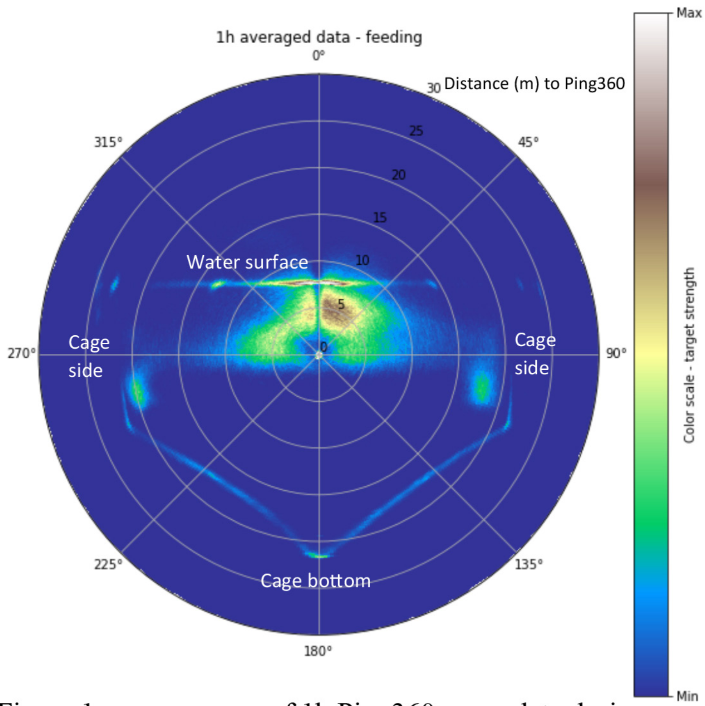

Figure 1: average scan of 1h Ping360 sonar data during feeding. The sonar is at the center of the diagram and each ring represents a 5-meter distance.

The results are still under interpretation but having the cage outline visible on the results allows for a great overview of the vertical and horizontal distribution of the salmon in the cage. Our methodology also has great potential to assess cage deformation due to currents. The first week, salmon mainly stayed in the upper part of the cage (0-7 m depth) and no significant change was observed between day and night. After 3 days a diurnal pattern became clearer. After a week, evident schooling patterns appeared for some hours of the day and fish feeding organization became more structured. Salmon distribution will be correlated to environmental parameters (T°C, DO, current, salinity, light).

MONITORING BACTERIA AND FUNGI ASSOCIATED WITH CULTIVATED SEAWEED TO LIMIT ECOLOGICAL RISKS

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The European seaweed aquaculture sector is developing rapidly. Cultivated kelp is utilized in food, pharmaceuticals, cosmetics, biofuels, animal feed, bioremediation, and industrial chemicals. The up to 90 % loss of seeded kelp poses challenges though, potentially bearing understudied economic and ecological risks. To limit both, the SEASEEDS project aims to improve the attachment of cultivated sugar kelp (*Saccharina latissima*). Therein, we focus on filling the gaps of the unknown potential consequences of detached sugar kelp associated bacteria and fungi. First, we finalized 16S and 18S rRNA gene sequencing protocols to monitor *Saccharina latissima*-associated bacteria and fungi, respectively. Second, we validated these protocols for seeding and harvest material of cultivated sugar kelp in collaboration with the Dutch seeding company Hortimare B. V.. Third, sugar kelp and seawater samples taken throughout the full cultivation cycle of the nearshore Dutch seaweed cultivation site in the Oosterschelde, owned by The Seaweed Company, are used as a case study to examine year-round succession of bacterial and fungal communities in sugar kelp from the gametophyte stage till natural decay (Figure 1). The monitoring results support fundamental understanding to foster early-sector developments towards an economically valuable and ecologically sustainable industry in the Netherlands and beyond.

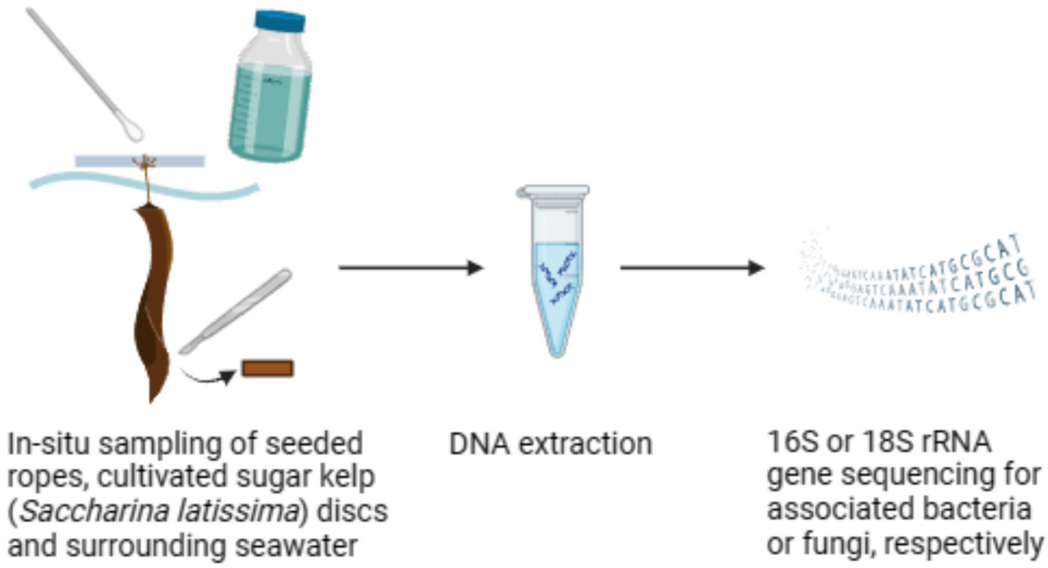

Figure 1: Method development towards revealing the role of bacteria and fungi associated with cultivated sugar kelp (Saccharina latissima). Better understanding of key players seems promising to limit yet understudied ecological risks. Illustration was created by F. R. Schanz using BioRender.com.

EMPOWERING COMMUNITIES: INCITIS-FOOD PROJECT’S TRAINING OF TRAINERS APPROACH TOWARDS SUSTAINABLE CIRCULAR FOOD SYSTEMS IN AFRICAN CITY REGIONS

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The EU-funded Project “INtegrated and Circular Technologies for Sustainable city region FOOD systems in Africa (INCiTiS-FOOD)” aims to bolster food and nutrition security (FNS) across African city regions while concurrently mitigating environmental impacts and fostering circularity within food systems. The project will empower communities by creating opportunities in agri-food supply chains and promote environmental justice through transformative food policies. Embracing an interdisciplinary approach, INCiTiS-FOOD integrates technologies, engages stakeholders, builds capacity, and cultivates Europe-Africa partnerships for collaborative engagement.

Central to the project are eight living labs situated in six African countries (Kenya, Nigeria, Sierra Leone, Cameroon, Gabon, Ghana), led by African partner organizations. They employ a user-centric approach to foster socio-technological innovation through participatory research and development and capacity building for local end-users via training initiatives. Focusing on hydroponics, aquaculture, aquaponics, and insect farming, these living labs adapt circular agri-food technologies to local realities, addressing challenges such as limited material availability and water scarcity. Prototype systems have been established across all living labs, complemented by comprehensive on-site trainings during the summer and autumn of 2023 in Ghana, Kenya, and Gabon. Leveraging a blend of theoretical and practical knowledge, participants gained foundational knowledge through the online edX course “*Aquaponics the circular production system*“ before engaging in hands-on training sessions at hosts’ living labs.

INCiTiS-FOOD’s Training of Trainers (ToT) approach plays a pivotal role in co-creating circular agri-food technologies and practices, equipping individuals with essential skills for sustainable development. The ToT curriculum, co-designed by ZHAW, prioritizes relevance and translatability, ensuring the efficient operation of living labs and the diffusion and adaptation of technologies to local conditions. Technical expertise is imparted through collaborations among consortium partners, covering a spectrum of topics, including aquaponics, aquaculture, and renewable energy. The ToT empowered over 100 participants while fostering gender equality and enhancing the capacities of living labs. Testimonials from Ghana, Kenya, and Gabon underscore the transformative impact of these sessions on skill acquisition and fostering networks critical for the dissemination and adoption of circular food technologies within African city regions.

MARKING THE OTOLITHS OF HATCHLING COD: A METHOD COMPARISON

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Introduction

Restocking programs aim to restore native fish species to waters where they have been overfished or can no longer reproduce. In the Baltic Sea, the eastern Baltic cod population is severely threatened due to a combination of several factors, creating the need for conservation efforts, such as restocking. In order to assess the success of hatch and release efforts, it is crucial to identify released fish larvae during recaptures at a later stage. We therefore investigated the marking success of different otolith labelling techniques, as well as lethal effects of these techniques. The results of this study will give insight into possibilities for marking yolk-sac fish larvae, which is valuable for restocking efforts of fish in early life stages, as well as other release and recapture studies.

Material and methods

Eggs from a cultured broodstock of eastern Baltic cod were kept in a hatchery in recirculating seawater (17 psu, 7 ˚C). Newly hatched larvae were then treated with different approaches to find the most suitable method for labelling. Chemical labeling included the approach of incubating larvae in water containing different concentrations of alizarin complexone, and by incubation in water with high concentrations of strontium. Further, the effect of aquarium salt used in the production facility on the chemical composition of the otoliths was tested. Water samples from the hatchery's water system were taken, as well as water samples from the Baltic Sea. These will be analyzed for their chemical composition and act as a baseline to evaluate how the aquarium salt affects the chemical composition in the water and therefore can create chemical marks in the larval otoliths. Lastly, thermal marking was tested, for which the larvae were incubated at different temperatures on a day-to-day schedule, which potentially alters the appearance of daily increments in the otoliths. From the day of first labelling, survival of the larvae was noted daily for 7 days, until the onset of the first-feeding stage, to allow for comparison of mortality effects between the approaches.

Expected results

With this study, we expect to find a suitable method to label yolk-sac cod larvae. Techniques tested are established for other species, life stages, or in different chemical concentrations. Factors such as strength of created label, lethal effects of treatment and effect on growth will be evaluated. Ultimately, the identification of an effective technique to label yolk-sac larvae is a critical factor in hatch and release endeavors. A suitable method facilitates the recapture of released larvae, allowing for the assessment of the overall success of release efforts.

POO MATTERS

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Nutrition research in aquaculture historically focused strongly on impacts on fish (growth, health, etc.). With the raising importance and awareness of sustainability in aquaculture the attention in research on environmental impacts has increased. Feed is a important factor for the amount of solid and dissolved waste being discharged by aquaculture. On of the reasons of culturing fish in recirculating aquaculture systems (RAS) is its potential to reduce and control the impact on the environment. For a proper functioning of RAS solid waste manage is a key factor. Solid waste accumulation in water is the primary source for suspended solids and contributes to increased dissolved waste. Suspended solids accumulation can hamper fish health, gill functioning and hamper proper functioning of RAS (water purification units). In farms using RAS that apply proper feeding management, faeces is the major source of solid waste produced. Complete and quick removal of faeces from the water is vital in a good faecal waste management strategy being targeted at reducing the amount of none-removed faecal waste from the water. Nutrition is vital in managing faecal waste. Reduction of the amount of none-removed faecal waste can be achieved by reducing the amount of faeces egested (i.e., increasing the nutrient digestibility) and/or by increasing the removal efficiency of faeces from the water. Faecal removal efficiency (by settling and/or filtration) is an interplay between system design and the characteristic of faecal waste. Factors influencing removal efficiency are faecal pellet size, density and stability. Currently there is still a lack of knowledge on faecal pellets with many unanswered questions like: what is a natural faecal pellet and stability; are faeces characteristics different between fish species; which dietary factors determine removal efficiency; can dietary factors improve faecal stability (i.e., reducing disintegration of faecal pellets); are faecal waste characteristic determined by ingredients or nutrients within a diet; are dietary factors determining faecal removal similar between different fish species? Such knowledge is required to formulated optimal feeds for RAS. In this presentation an overview will be given on published data and in house data from studies linking dietary interventions to faecal waste management. With the major focus on factors that determine the removal efficiency as measured in various fish species, like salmon, trout, tilapia, common carp and striped catfish (Table 1). Factors that will be addressed are the impact of: feeding level; dietary ingredient composition; type of carbohydrates; type of protein rich ingredients; dietary starch level; etc.

Table 1. The range in removal efficiencies measuring by settling in different fish species.

Fish species	Range faecal removal efficiency (%)	Source
Nile tilapia	56-80	Amirkolaie et al. (2006)
Sea bass	53-88	Fountoulaki et al. (2021)
Sea bream	21-28	Fountoulaki et al. (2021)
Common carp	68-74	Prabhu et al. (2019)
Striped catfish	30-50	Tu-Tran et al. (2020)
Rainbow trout	55-85	Prakash et al. (2023)
Yellowtail kingfish	40-50	Horstmann et al. (2023)

WHAT'S HOLDING BACK OPEN OCEAN AQUACULTURE?

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The ICES Working Group for Open Ocean Aquaculture has explored the terminology used around the open ocean sub-sector of aquaculture, specifically defining the terms "offshore" and "exposed", providing guidance on when and how to use these terms effectively, and creating indices to qualify these terms and add specificity. We have also explored many of the impacts and trends surrounding the continuums of nearshore vs offshore and protected vs exposed farming sites.

However, much research remains in the arena open ocean aquaculture, highlighted by the ultimate question of "why don't we see more progress in this space"? This talk will review the status and need for further research in 5 key areas that are most necessary to advance an open ocean aquaculture industry and discuss why specifically don't we see more new commercial open ocean farms today.

1. Advancement and adoption of operational support technologies
2. Innovation and value engineering in design of culture systems
3. Confidence in financial model
4. Interactions between farm environment and environmental impacts
5. Clarity and confidence in the permitting process

USING A SUBMERSIBLE PEN TO MANAGE CHALLENGING OCEAN CONDITIONS

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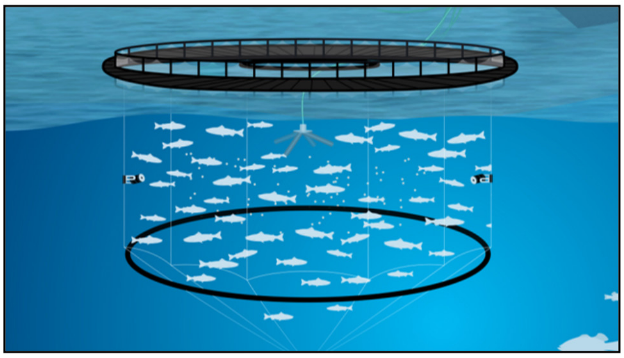
Farmers in many areas of the world are looking at open ocean sites to expand operations, either due to user conflicts for protected sites, or simply the nature of the coast in their country. Open ocean environments hold a lot of promise but experience challenges which require new technological solutions to manage. Wave energy and strong currents are a major concern although submersible pens offer an effective and cost-competitive solution.

This talk will review the experience and status of a farm in the Red Sea installing and operating Innovasea's submersible net pen, the SeaProtean, along with an array of environmental sensors. The focus will be on the environmental parameters at the farm site and how the equipment allows for easier operations and creating an ideal environment for fish.



NUTRITIONAL VALUE OF SORGHUM: AN UNDERUTILIZED PLANT INGREDIENT IN AQUATIC ANIMAL DIETS

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Aquaculture is the fastest growing animal food production system, which reached an all-time high in 2020 with the consumption level double what it was 50 years ago. A large part of this growth has been sustained by shifting the dependence of aquatic species feed formulations from limited and expensive fish meal and fish oil towards abundant and competitively priced terrestrial plant ingredients. To date, corn, soybean, and wheat products have been the predominant plant ingredients included in aquafeeds. However, the growing demand for aquafeed ingredients represents a substantial market opportunity for underutilized plants. Sorghum grain is commonly included in terrestrial animal feeds; however, only limited studies are available regarding the suitability of sorghum grain for aquaculture, limiting its market acceptance as an aquafeed ingredient. Research efforts to expand the utilization of sorghum in aquatic feeds that accurately define nutritional value and optimal inclusion levels of sorghum products for rainbow trout and hybrid striped bass will be presented, as well as progress toward efforts to develop value-added products through novel processing technology and enzyme addition.

CONDITIONING OF THE MEDITERRANEAN SCALLOP *Pecten jacobaeus* L. UNDER HATCHERY CONDITIONS WITH TWO TYPES OF FEED – IMPLICATIONS FOR AQUACULTURE

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In recent years, mariculture has become the largest market supplier of bivalves, but this offer is often limited to a small number of species, so there is an increasing need for introduction of new species into the aquaculture. 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. Greatest challenges for farming of this species are the lack of seed from nature, so to overcome this obstacle, the solution lies in producing the seed in hatcheries.

For this purpose, we conducted a study on the conditioning of *P. jacobaeus* broodstock under hatchery conditions for its possible preparation of spawning. The broodstock, collected from nature, was acclimatized in the hatchery for 10 days at 15 °C and salinity of 35. Afterwards, it was separated in two groups that were fed with two different types of feed in the amount of 3% of their total dry soft tissue mass. First group was fed with live algae *Isochrysis galbana* and the second group with concentrate of frozen algae consisted of species *Tetraselmis sp*., *Thalassiosira weissflogii* and *Thalassiosira pseudonana.* Before and 21 days after feeding started, specimens were collected from tanks for the calculation of physiological indices. Shell was separated from soft tissue, which was further dissected for gonads, muscle, and the remaining soft tissue. Total mass of the shell and soft tissue was measured, in wet and dry weight (g), after processing in the dryer at 60 °C for 48 h. Condition index (CI), gonadosomatic index (GSI) and muscle index (MI) were calculated by following methods:
CI= total soft tissue dry weight (g)/shell dry weight (g) x 100,
GSI= gonad dry weight (g)/ total tissue dry weight (g) x 100,
MI= muscle dry weight (g)/ total tissue dry weight (g) x 100.

The results indicate that the conditioning gives good results with both types of feed. The group fed with live feed had higher CI than the group fed with concentrated frozen feed, which can be prescribed to the fact that the bivalves prefer live feed. The group fed with concentrated frozen feed had higher GSI than the group fed with live feed, which can be prescribed to the fact that the feed which consists of more than one algae species promotes better gonadal development, but also to the fact that remaining soft tissue declined in weight. MI showed a slight decline in both groups, which can be attributed to the utilization of the muscle reserves for supporting gametogenesis.

Obtained results suggest that the best results could be achieved with the live feed that consist of more than one algae species. These results will be improved upon so to induce controlled spawning under hatchery conditions in the future.

INCREASED RESISTANCE TO *Vibrio parahaemolyticus* IN PACIFIC WHITE SHRIMP FOLLOWING DIETARY SUPPLEMENTATION WITH NUCLEOTIDES AND A BIOACTIVE OLIVE FRUIT EXTRACT

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Introduction:
Acute hepatopancreatic necrosis disease (AHPND) is a bacterial disease mainly caused by *Vibrio parahaemolyticus*, which negatively affects the health of Pacific white shrimp (PWS). Dietary nucleotides modulate the immune response, and a particular nucleotide-rich yeast extract, Nucleoforce® (Bioiberica S.A.U., Spain), has been reported to improve survival in AHPND-infected PWS in several studies performed in Indonesia and Vietnam. On the other hand, fruits and leaves of the olive oil tree contain bioactive compounds with anti-inflammatory, antioxidant, and antimicrobial effects. Aquolive® (NATAC Biotech SL, Spain), a bioactive olive fruit extract (OFE) has been proven efficacious in different aquaculture species. The objective of this trial was to evaluate the effects of the combined use of nucleotides and OFE on PWS survival upon challenge with an AHPND-causing *V. parahaemolyticus* strain.

Methods:
A total of 600 PWS (0.57±*0.1*1 g) were used in this trial performed in Vietnam. After acclimatation for 2 days, PWS were classified into 5 groups (4 replicates/group; 30 PWS/90L-tank) and received different diets for 28 days: 1 group with no challenge (NC, Negative Control) and 4 groups challenged by immersion with *V. parahaemolyticus* and supplemented with either 500ppm Nucleoforce® (NU500), 500ppm Aquolive® (OFE500), 500ppm Nucleoforce® with 500ppm Aquolive® (NU500OFE500) or no supplementation (PC, Positive Control). PWS were followed for 10 days post-challenge to quantify and compare survival rate between groups.

Results:
The PC group showed a significantly lower survival rate (30.63 ± 4.70%) *vs* NC (88.29 ± 2.73%; p<0.05). A non-significantly higher survival rate *vs* NC was seen with NU500 (33.95 ± 9.62%) and OFE500 (40.99 ± 11.07%), while NU500AO500 achieved a significantly higher (p<0.05) survival rate (55.80 ± 10.02%) *vs* PC and NU500 (Figure 1).

Conclusions:
The 1:1 combination of nucleotides with OFE has a synergistic effect, reducing PWS mortality upon AHPND challenge. This innovative nutritional intervention could therefore be used to improve PWS health, especially as an alternative to conventional AHPND therapies, such as antibiotics and disinfectants, which often have limited success and negative consequences.

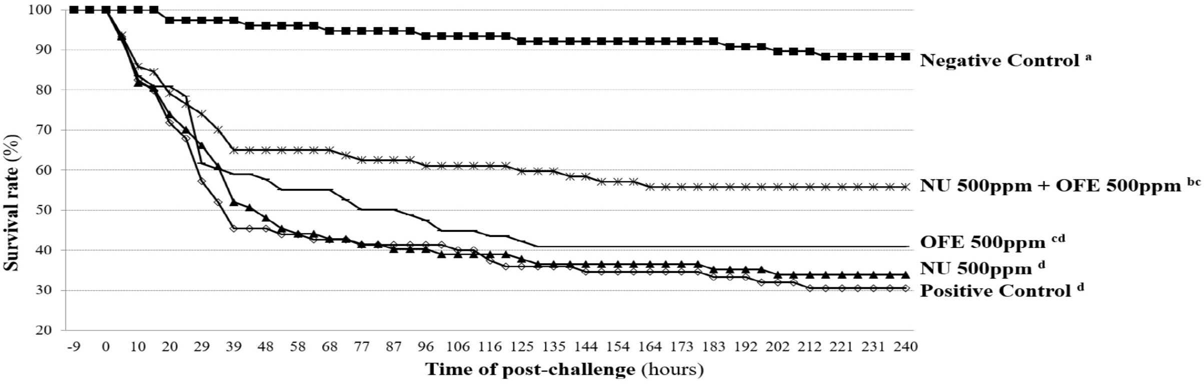

Figure 1. Survival rate of PWS after AHPND challenge. Different superindex letters indicate statistically significant differences (p<0.05).

EFFECTS OF AMINO ACIDS ON THE FEEDING MECHANISMS OF YELLOWTAIL *Seriola quinqueradiata*

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Yellowtail (*Seriola quinqueradiata*) is an important aquaculture species in Japan; it has low palatability for an alternative protein source of fishmeal. For sustainable aquaculture, increasing the feed intake of low- or non-fishmeal diets in yellowtail is necessary. Olfactory and gustatory senses are important in fish feeding; however, it remains unclear how each of these senses stimulates feed intake in yellowtail. Therefore, this study investigated the effects of amino acids on the feeding mechanisms of yellowtail.

Twelve L-amino acids (Ala, Pro, Ser, Asp, Tau, Thr, Gln, Met, Trp, Val, His, and Ile), exhibiting both strong or weak olfactory and gustatory stimulation, were selected for this study. Different amino acid solutions (10 mmol) were added to the rearing water of yellowtail, and the frequency of "search" and "bite" instances were counted (Fig. 1). The addition of strong odor-stimulating amino acid solutions resulted in significantly higher frequencies of "search" behavior than that seen with the vehicle. Compared to that seen with the vehicle, the "bite" frequency was significantly increased by the addition of Pro and Met. Pro was the most taste-stimulating amino acid, whereas Met exerted a very weak taste-stimulating effect. The mRNA response of neuropeptide Y (*npy*), an orexigenic hormone in the olfactory bulb, telencephalon, hypothalamus, and cerebellum of the yellowtail, was measured after adding 10 mmol of amino acid solution to the rearing water. The addition of Ala, the strongest olfactory stimulator, significantly decreased *npy* expression in all brain regions. Fifteen minutes after adding an amino acid solution to the rearing tank, a commercial diet was fed until satiation and feed intake were measured. Compared to that seen with the vehicle, the addition of strong olfactory stimulants such as Ala, Ser, Met, and Gln significantly reduced feed intake (Fig. 2).

In conclusion, yellowtail recognizes amino acids as feed via olfaction and gustation, leading to feeding behavior and contributing to feed intake, primarily by regulating *npy* expression in various regions of the brain apart from the hypothalamus.

Fellows Grant Number JP21J23499, JSPS KAKENHI Grant Number JP21H02286, and a JST Grant Number JPMJPF2117.

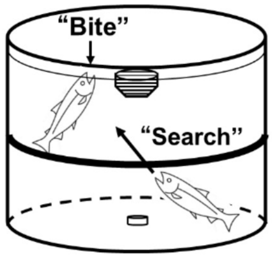

Fig. 1 Experimental tank for observing feeding behavior.

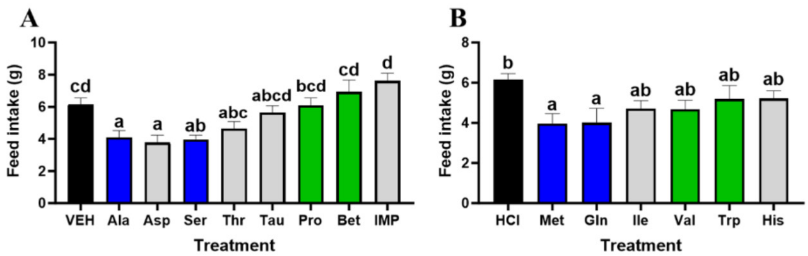

Fig. 2 Feed intake after adding 10 mmol amino acids dissolved in pure water (A) or 0.5 M HCl (B) to the rearing tank. Bars represent mean ± SEM. Different superscripts indicate significant differences ($P < 0.05$). Blue and green bars represent strong odor-stimulating and taste-stimulating amino acids, respectively.

IS SUPPORTING PERIPHYTON GROWTH WORTHWHILE IN PIKEPERCH FRY PRODUCTION?

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The European pikeperch (*Sander lucioperca*) is a promising fish species cultured in European freshwater aquaculture. Despite various technological and breeding innovations, most European pikeperch farmers need more high-quality juvenile material of this species. We hypothesized that promoting periphyton growth in ponds by installing substrates would enhance the availability and quality of natural food, leading to improved growth and survival of European pikeperch fry. Our experiment was carried out in 12 fishponds of the same size using heather and geotextile substrates to support periphyton development. The initial stocking density of pikeperch larvae (5 ± 0.2 mm TL) was 374,000 ind ha^{-1}. Their culture was finished after 47 days. The geotextile treatment yielded the largest advanced pikeperch fry (39 ± 6.8 mm versus 35 ± 5.0 mm TL in heather treatment; Fig. 1), while the heather treatment showed the highest survival rates (26 ± 22 % versus 10 ± 11 % in geotextile treatment; Fig. 2). Cannibalism was observed, particularly in the geotextile treatment, emphasizing the need to consider rearing strategies for optimal growth and survival. Our results show that promoting pikeperch production using artificial substrates is possible under Central European conditions. The experiment was financially supported by NAZV project (QK22020144).

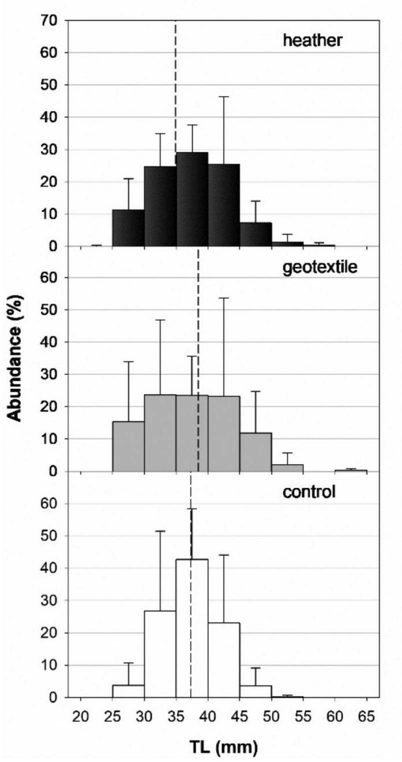

Fig. 1. Histogram of relative frequencies of total length (TL) (mean ± S.D. variability among fishponds) of advanced pikeperch fry after 47 days of rearing in the fishponds. The dashed lines show the mean total length in each treatment.

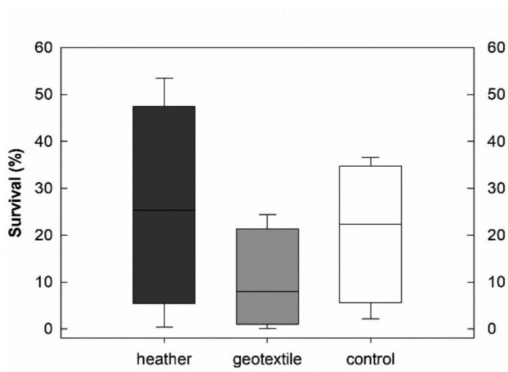

Fig. 2. Survival and its variability of advanced pikeperch fry after 47 days of rearing in the fishponds. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars representing the 10th and 90th percentiles.

DELIVERY OF HEALTHY AND SUSTAINABLE LIVE FEED FOR JUVENILE FISH - DELIFEED

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Trond Rafoss, LANDAKVA
Ansgar Stratmann, W42

The DELIFEED project takes a circular bio-economy approach to advance sustainable aquaculture practices by utilizing waste streams and byproducts as feedstock and develop innovative methods for producing nutritious and immune-stimulating live feed to support the health and growth of juvenile farmed fish. This initiative seeks to address the critical need for high-quality live feed during early fish development, enhancing animal welfare and environmental sustainability while bolstering aquaculture production and profitability.

Key objectives include replacing *Artemia* with terrestrial invertebrates like nematodes and white worms to optimize live feed quality, enriching feed with essential omega-3 fatty acids, and reducing reliance on antibiotics through integration of probiotics and novel oral vaccines administered *via* bioencapsulation. Cryopreservation techniques will be employed to extend shelf life and stabilize feed supplies.

The consortium, comprising research groups (AU, AAU, DTU), live feed producers and biotech companies (FISHLAB, E-NEMA, W42), and aquaculture firms (VENØ, SKAGENSAL, AQUAPRI, ALPHAAQUA, NORDICHALI, LANDAKVA), aims to commercialize these innovations, creating new employment opportunities and driving industry growth.

The DELIFEED project represents a pivotal effort to revolutionize aquaculture through sustainable feed innovations, with far-reaching benefits for industry, animal welfare, and marine biodiversity.

IDENTIFYING COST-EFFICIENT MICROALGAE COMBINATIONS FOR EUROPEAN FLAT OYSTER *Ostrea edulis* SPAT GROWTH

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Although substantial advances have been made in the hatchery production of the European flat oyster *Ostrea edulis* in the past decades, current methods are still insufficient to meet the demands of spats for aquaculture and for restoration projects. One of the factors that can constrain production is nutrition, which account for 30-60% of costs. It has been shown that different ratio of mixed microalgae diet can improve broodstock conditioning and improve growth, survival and settlement rates of larvae. This is why in most hatchery several strains of microalgae are cultivated. Once produced, spat grow-out occurs in micronursery, then nursery or in the wild. In nursery, it is frequent that a single species of diatom such as *skelatonema* sp. is used as food and added to partially or non-treated local seawater to reduce costs. In areas where spat cannot be placed in nursery using local contaminated water, the first grow-out of spat in biosecured area such as a micronursery should be cost-efficient. We evaluated the effect of single or mixed diets on spat in micronursery.

O. edulis spat produced from a single swarming event in spring 2023 were used for two feeding experiments. Spat were placed in 2L tanks with constant aeration in a flow-through system, supplied with 27.5 ppt 1µm filtered and UV treated seawater at 15 ºC. Five microalgae diets, plus a no-feeding control, were tested: Diet 1: 50% *Chaetoceros* sp., 25% *Pavlova gyrans*, 25% *Tisochrysis lutea*; Diet 2: 50% *Rhodomonas salina*, 50% *Chaetoceros* sp.; Diet 3: 50% *Chaetoceros* sp., 50% *T. lutea*; Diet 4: 50 % *P. gyrans*, 50% *T. lutea*; Diet 5: 100% *Chaetoceros* sp. After 7 days acclimatization, spat were fed at a constant concentration of 20 cells μl^{-1} (equivalent *T. lutea* volume) for total experimental time of 35 days. For experiment 1: 210 spat (10-15 mm) were distributed in 6 treatment tanks for measuring clearance rate and oxygen consumption; for experiment 2: 15g of spat (5-10 mm) were randomly allocated to each treatment in triplicates (18 tanks in total). Weight gain was calculated at the end of the experimental period, and their organic mass was stored at -20 ºC for fatty acid analysis.

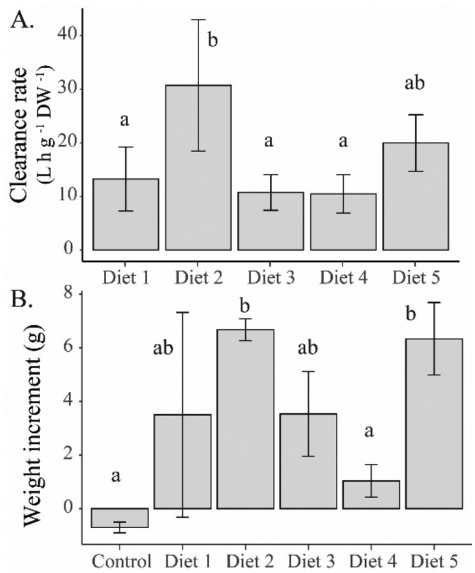

Figure 1: Clearance rate (A) and weight increment (B) of *Ostrea edulis* spat fed 5 microalgae combinations (mean ± SD).

Oxygen consumption was similar for spat fed with each of the 5 diets. Clearance rate was significantly higher for spat fed diet 2, whereas the highest weight increment was observed for spat fed diet 2 and 5 (Fig. 1). These results suggest that *O. edulis* spat show a preference towards *R. salina* similarly to conditioning adults, when compared to diets including microalgae of smaller cell size (*R. salina* volume being ~*678* μm^3, *P. gyrans* and *T. lutea* ~217 and 133 μm^3, respectively). In contrast to the observations on larval stages, the use of *Chaetoceros* sp. as single species diet yielded higher growth compared to mixed diets with *T. lutea*. Conversely, the best results in term of growth were obtained by its combination with *R. salina*. Fatty acid composition analysis is currently being carried out.

Funding: This study was part of the project LTA BOOST supported financially by Innovation Fund Denmark.

TRANSCRIPTOMIC PROFILING TO IDENTIFY BROODSTOCK QUALITY BIOMARKERS IN EUROPEAN EEL

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The development of European eel (*Anguilla anguilla*) aquaculture relies on closing its life cycle in captivity. Assisted reproduction techniques using hormonal treatments to induce sexual maturation and vitellogenesis are currently used to successfully produce embryos and larvae. However, a variation in the number of vital offspring between batches is often observed, which can derive from the physiological status of the female broodstock at the onset of hormonal administration. In this context, the identification of possible broodstock related biomarkers is necessary to improve its management strategies. In fact, molecular markers have been of particular interest as the abundance of some maternal mRNAs has been linked to embryonic survival and hatching success in European eels.

In this work we evaluated the potential use of transcriptomic analysis and gene expression to identify RNA-based biomarkers to identify broodstock quality indicators in batches with high and low survival rate of larvae originating from farm-raised females European eel fed a formulated diet. Broodstock females and males were subjected to a standardized hormonal treatment scheme and strip-spawned for subsequent fertilization in standard conditions. Blood samples were collected immediately after spawning. A sample of embryos derived from each of the female considered was also collected at 4, 24 and 48 hours post-fertilization. Buoyant eggs were moved to conical incubators and reared until hatching. Batches from which larvae reached the first-feeding stage were considered to contain eggs of good quality. Following this criterion, two groups were created: high quality (n = 5) and low quality (n = 5). RNA was extracted from the blood and the embryos and sequenced through RNA sequencing with Illumina platform. Data generated were mapped against the European eel reference genome (NCBI, GCA_013347855.1) with Tophat2. Comparison between the two groups at the four levels (i.e blood, embryos at 4, 24 and 48h) to detect Differentially Expressed genes (DEG) was performed with Deseq2.

The comparison of the blood transcriptome detected 166 upregulated and 38 downregulated genes in females producing high-quality batches, and the PCA plot showed that samples from low- and high-quality groups clustered into two groups (Fig. 1).

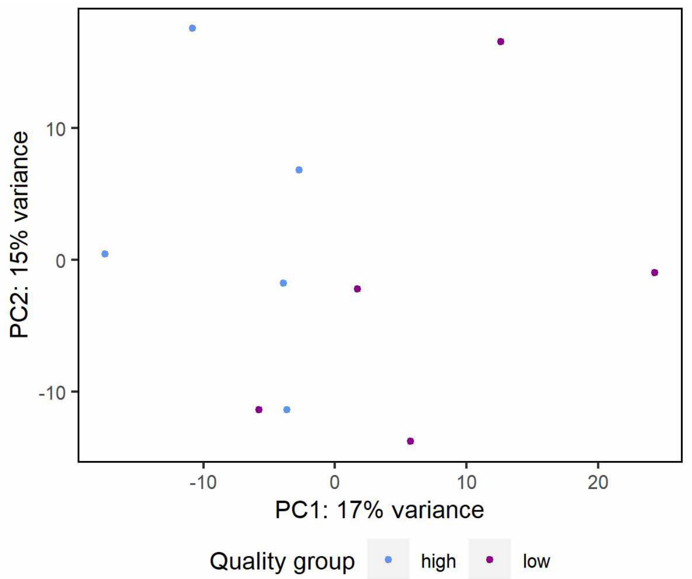

Figure 1: PCA plot of the differential expression analysis for European eel blood from females producing high-quality and low-quality batches.

(Continued on next page)

However, the number of differentially expressed genes in was markedly reduced unfertilized eggs and none were detected in embryos at advanced developmental stages.

This study highlights how markers that capture the difference between good and bad spawning groups can be detected on the mother site rather than after fertilization and highlight the blood as promising tissue for the evaluation of the quality of the female broodstock.

FABRICATION OF NANOLIPOSOMAL CARRIERS CONTAINING POLYPHENOLIC COMPOUNDS

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The main objective of the present study was to encapsulate polyphenols using nanoliposomes in various ratios of lecithin and cholesterol (9:1, 8:2, 7:3 and 6:4 lecithin-cholesterol) to overcome their application challenges in food products, such as low solubility, undesirable sensory attributes, and instability during processing and storage. Then, the effect of lecithin-cholesterol concentrations on particle size, particle size distribution, encapsulation efficiency (EE), physical stability of nano-liposomes and stability of phenolic compounds loaded in nanoliposomes during the storage time were evaluated. The average particle size as Z-Average was in the range of 191.73-553.60 nm. By decreasing the amount of cholesterol in the liposome structure, the particle size also decreased. The particle size distribution was in the acceptable range of 0.3-0.4 (PI≤0.5). Incorporating cholesterol resulted in a shift of the zeta potential from -41.94 to -51.73. Higher zeta potential values indicate a higher and longer-term stability of the particles. The highest efficiency of encapsulation and stability during storage was obtained in the ratio of 1-9 lecithin-cholesterol. Adequate mixing of polyphenol powder and the highest encapsulation efficiency were achieved at low concentrations of lecithin, which is of vital importance in the commercial application of liposomes. All in all, encapsulation can protect the polyphenol from adverse environmental and processing conditions.

DIVING INTO THE DEPTHS: UNVEILING THE MAIN ETIOLOGIES OF PISCINE LACTOCOCCOSIS WITH A NOVEL MULTIPLEX QPCR ASSAY

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Piscine lactococcosis poses a significant threat to a wide range of cultured and wild fish populations on a global scale. The disease routinely manifests as per-acute to acute hemorrhagic septicemia and is often associated with high levels of morbidity and mortality. Until recently, *Lactococcus garvieae* was thought to be the only causative agent of the piscine lactococcosis; however, further research has proven that the disease episodes are attributed not only to *L. garvieae* but also to the other two highly pathogenic *Lactococcus* sp. known as *L. petauri* and *L. formosensis.* These three bacterial species share phenotypic and genetic similarities that often lead to diagnostic challenges due to substantial misidentification using traditional microbiological and molecular methods. Thus, rapid, sensitive, and specific molecular diagnostic tools for correct differential diagnosis of the aetiologies of lactococcosis in fish are warranted.

In the current study, we successfully developed a novel TaqMan species-specific multiplex qPCR for simultaneous identification and quantification of *L. garvieae*, *L. petauri* and *L. formosensis* following pangenome analysis of publicly available genomes of these bacterial species. The newly developed assay showed high sensitivity and specificity when tested with 139 bacterial isolates, representing the three bacterial species, recovered from multiple cultured fish species, originated from various geographical locations, including Mediterranean area (Turkey, Italy, Spain, Greece), Northern Europe (Norway), North America (USA) and South America (Brazil), along with a representative panel of closely related and non-related bacterial species. In addition, a successful detection and identification of the bacterial species was achieved using the novel multiplex assay on 126 field tissue samples. from clinical lactococcosis outbreaks, including tissues preserved in RNAlater, archived formalin fixed paraffin embedded (FFPE) tissues and tissues fixed on FTA cards. The new molecular assay performed better at diagnosing piscine lactococcosis than the gold standard protocols currently in use in laboratories.

Overall, the multiplex qPCR assay developed in the current study provides a reliable, rapid, sensitive, and species-specific molecular method for diagnosis, genomic typing, and differentiation of the closely related etiological agents of piscine lactococcosis. Using this robust technique will contribute to the accurate diagnosis of the infection and will assist in the establishment of effective management strategies against lactococcosis in aquaculture.

EVALUATION OF ENVIRONMENTAL INTERACTIONS FROM FISHPONDS

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Pond aquaculture processes are highly interactive with the surrounding environment, particularly the adjacent reed and marsh vegetation. The design and planning of sustainable pond aquaculture requires a thorough understanding of the biophysical processes behind these interactions within the system and with the surrounding environment. However, it remains a challenge to study the internal interactions between the various interconnected subsystems of the pond ecosystem and also to consider the external environmental interactions on a quantitative basis. Therefore, our work aims to create a unified simulation model of a managed fishpond/reed ecosystem to understand the dynamic balances and casual causal relationships behind environmental interactions.

The methodology involved establishing the links between the sub-models of the pond compartment and reed on land and in the pond. Starting from this structure, the model describes the flows of nutrients between the elements, determined by various processes and functions. For modelling and simulation, the Programmable Process Structures framework has been used to enable the unified creation and execution of the underlying multidisciplinary dynamic models (Varga & Csukás, 2022). A previously developed and validated pond model by Sharma et al. (2024) has been used to describe the processes in the pond. In addition to the literature-based description of the various elementary processes for reed plants, we consciously follow the consideration of the associated stoichiometric atomic balance of carbon, hydrogen, oxygen, nitrogen, and phosphorus. The analysis of atomic balances allows for an explicit evaluation of the underlying system on a conservational basis and also allows to check the completeness and correctness of the model under consideration.

Hypothetical scenarios (with different stocking densities, feeding regimes, reed cover, and reed management practices) are tested to obtain preliminary results for different environmental interactions and features such as carbon sequestration, quality of effluent, and biowaste, the quantity of the sediment in the pond, etc.

Acknowledgments
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References

Sharma, P., Gyalog, G., Berzi-Nagy, L., Tóth, F., Nagy, Z., Halasi-Kovács, B., ... & Varga, M. (2024). Reusability check-based refinement of a biophysical fishpond model. Computers and Electronics in Agriculture, 218, 108664. https://doi.org/10.1016/j.compag.2024.108664

Varga, M., Csukás, B. (2022). Foundations of Programmable Process Structures for the unified modeling and simulation of agricultural and aquacultural systems. Information Processes in Agriculture, in the press, https://doi.org/10.1016/j.inpa.2022.10.001

AQUAFEED GREENWASHING: CERTIFICATION, SUSTAINABLE AND RESPONSIBLE

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The transition of aquafeeds from fishmeal and fish oil to alternatives has been in progress since the early 2000s. A wide variety of products are being investigated including microbial proteins, fish and animal processing waste, insect meal, algae and terrestrial plants. Soy, appears to be a promising alternative due primarily to the quantity and quality of protein. Concurrent with the shift toward alternative feedstuffs, there has been a growing awareness of the potential environmental effects of both aquaculture and agriculture, and a desire to make aquaculture "sustainable." In 1990 9the U.S. government passed a law (U.S. Code, Title 7, Section 3103) that defined sustainable agriculture and aquaculture.

In brief, sustainability is site-specific and over the long-term is required to:

(1) Satisfy human food and fiber needs.
(2) Enhance environmental quality and the natural resource base upon which the agriculture economy depends.
(3) Make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls.
(4) Sustain the economic viability of farm operations.
(5) Enhance the quality of life for farmers and society as a whole.

I will present an examination of soy in aquafeed as it relates to the USA Title 7 language as well as the UN Sustainable Development Goals (SDG) 12, 13, 14, 15. Constance (2010 Sustainability 2 (1) 48-72) wrote "because the concept of sustainability is deeply contested, agribusiness is able to exploit the ambiguity surrounding the definition of sustainable and exercise power in attempts to frame sustainable agriculture in their favor." It will be difficult to claim that intensive aquaculture is sustainable without sustainable feeds. In 2013 soybeans, grown in the USA, were certified by (xxx) as being sustainably produced. My presentation will show that this is not the case.

DIGITAL AQUACULTURE MARKETING AND ITS POTENTIAL FOR AQUACULTURE GROWTH IN NIGERIA

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According to MarTech, a digital marketing provider, the world will spend $4.7 trillion on marketing by 2025. According to the Food and Agriculture Organization of the United Nations (FAO), aquaculture production in Nigeria has been growing at an average annual rate of 10% over the past decade. In 2019, Nigeria produced an estimated 136,000 tonnes of fish from aquaculture, making it one of the largest producers of farmed fish in Africa. The majority of the aquaculture production in Nigeria is based on tilapia and catfish, which are the most commonly cultivated species in the country. Other species farmed in Nigeria include Nile tilapia, Clarias Garie Pinus (African catfish), and Heterotis niloticus (Nile perch). The growth of the aquaculture sector in Nigeria has been supported by the government and development partners, who have provided infrastructure, training, and support to farmers. The government has also implemented several policies and programs aimed at promoting the development of the aquaculture sector, such as the National Aquaculture Development Plan (NADP) and the Agricultural Promotion Policy (APP). Additionally, the Nigerian government has provided support for research and development in the sector, which has led to the introduction of improved technologies and practices.

This paper focuses on the potential of digital aquaculture marketing and its potential in Nigeria.

GENOMICS-BASED R & D OF HIGH-QUALITY ALL-MALE BIGMOUTH BASS

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Bigmouth bass (*Micropterus Salmoides*; Fig. 1a), also known as Californian black bass, has become a fish star with expanding aquaculture volume in the mainland of China. It is a popular resource of raw material for grill fish or sauerkraut fish. However, due to the present status of low-quality germplasm (usually caused by the confused parental pedigree and inbreeding) and poor antipathogenic capacity of most cultured populations, high-quality new varieties are urgently required for rapid expansion of aquaculture scale and economic value.

By integration of whole-genome sequencing and resequencing of male and female fishes, we constructed high-quality chromosome-level genome assemblies (for both genders; Fig. 1b, c) and performed a genome-wide association study (GWAS) to localize a sex-differentiated region (SDR) on the sex chromosome (Chr10; Fig. 1d) and male-specific regions (MSR; Fig. 1e) for designing male-specific markers (Fig. 1f). Subsequently, we screened out XY females among fry after treated with estrogen at an early stage (Fig. 1g), and then generated certain YY supermales for future breeding of all-male fry.

As we know, all-male fry usually own certain advantages in growth, disease resistance, feed utilization efficiency, and uniform specification (i.e., convenience for capturing and processing). The combined outcome of low cost for production of this target all-male variety will improve the overall culture scale of bigmouth mass to a high level in South China.

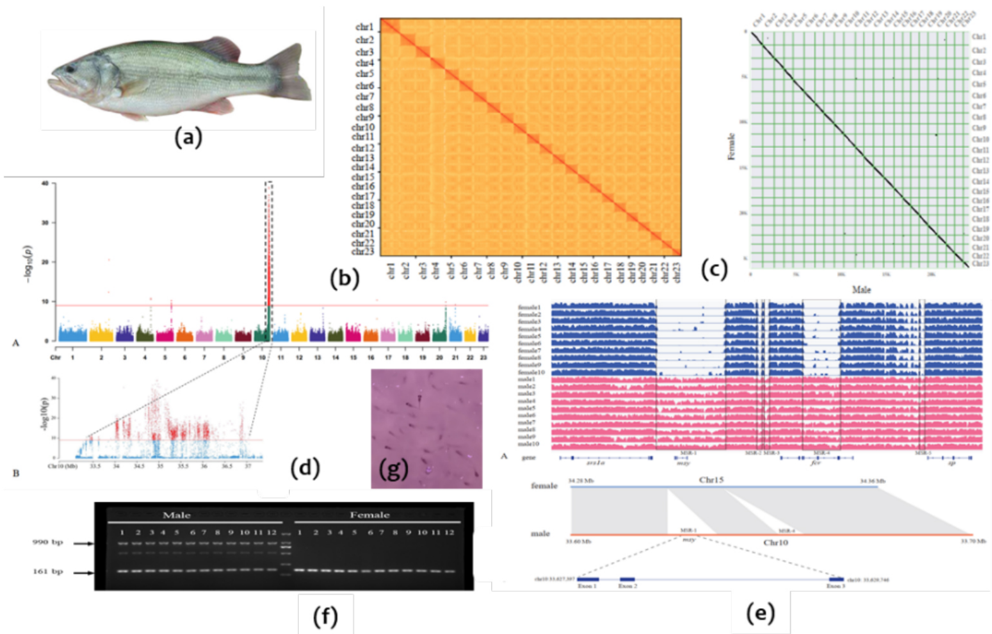

Fig. 1 GWAS identification of molecular markers for generation of all-male bigmouth bass. (**a**) An image of the cultured bigmouth bass. (**b**) Hi-C interactions among the 23 chromosomes in the hyplotypic XY genome. (**c**) A whole-genome synteny analysis between this XY genome and previously reported female (XX) genome. (**d**) Localization of a significant SDR (A) on Chr10 (B). (**e**) Characterization of male-specific insertions on the Y chromosome (A~ B). Only one coding sequence within MSRs of the Y chromosome (*msy*) was predicted (C). (**f**) PCR detection of a sex-specific marker in collected samples.

EFFECTS OF GLUCOSE DURING THE YOLK-SAC LARVAE AND THE PRE-LEPTOCEPHALUS STAGES IN FRESHWATER EEL (*Anguilla japonica*)

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Freshwater eels are commercially important in Northeast Asia, but mass seed production has not been successful, in part because of their very unique life history. These eels typically spend 5-10 years maturing in freshwater and spawn around 200 meters deep near the Mariana Trench. After hatching, the larvae are known to feed on marine snow, which consists of dead plankton carcasses and fecal matter. While it is generally known that carbohydrates are not important for fish larvae because they feed on zooplankton, the marine snow that eel larvae eat are known to contain various carbohydrates in the form of polysaccharides and simple sugars such as glucose and galactose. In addition, previous studies have reported that eel larvae have relatively high expression of glucose transporter, and that growth can be improved by adding glucose in the feed. Therefore, this study aimed to confirm the effects of glucose feeding for eel early larvae.

Experiment 1: Investigating the potential of nutritional enrichment with glucose before the first feeding.

Immediately after hatching, the eel larvae were housed in 300 L cylindrical tanks at approximately 50 individuals/L. The water flow rate was approximately 1.8 L/min. Larvae were maintained in a recirculating filtration system, 50% water changes per day, water temperature 23.2 ± 0.3 °C, DO 7.3 ± 0.1 mg/L, salinity 33.8 ± 0.7 ppt, pH 8.27 ± 0.05. Glucose was added to seawater from 1 days after hatching (DAH) and maintained at concentrations of 0%(Control), 0.5%, 1%, and 2%. We analyzed total length, body depth, hyaluronan content, and expression of genes at 6 DAH.

Experiment 2: Investigating the potential of nutritional enrichment with glucose after first feeding.

At 6 DAH, the eel larvae were housed in 20 L round acrylic resin tank at 500 individuals. The water flow rate was approximately 1.0 L/min. The first feeding was performed at 7 DAH. The slurry feed based on the eggs of spiny dogfish consisted of fish meal, krill meal, dry milk and vitamins. Glucose was added to the feed at concentrations of 0% (Control), 0.1%, 0.3%, and 0.5%. The slurry feed (6 mL) was supplied five times daily (09:00, 11:00, 13:00, 15:00, and 17:00) on the bottom of each tanks. We checked total length, body depth, and survival rate at 15 and 30 DAH.

Experiment 1:

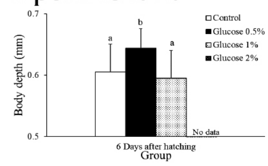

Fig. 1. Growth of *Anguilla japonica* larvae. Body depth. Values are means ± SD. Different letters represent significant differences ($P < 0.05$).

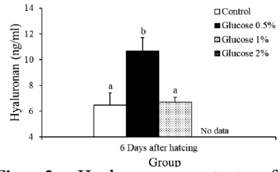

Fig. 2. Hyaluronan content of *Anguilla japonica* larvae. Values are means ± SD. Different letters represent significant differences ($P < 0.05$).

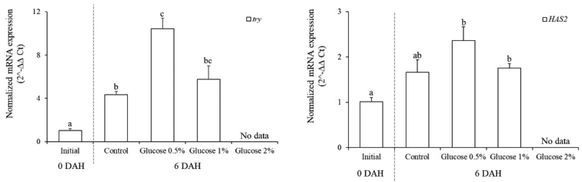

Fig. 3. mRNA levels of eel larvae. Abbreviations: *try*: pancreatic trypsin; *HAS2*: Hyaluronan synthase 2. Values are means ± SEMs. Different letters represent significant differences ($P < 0.05$).

Experiment 2:

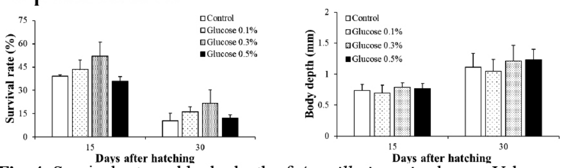

Fig. 4. Survival rate and body depth of *Anguilla japonica* larvae. Values are means ± SD.

At 6 DAH, all larvae perished in glucose-2% added group. No significant difference in total length was observed, but there was a significant increase in body depth, hyaluronan and expression of *try* and *HAS2* at glucose-0.5% added group.

The results indicated a relative improve in growth and survival in glucose-0.3% added group. In conclusion, we suggest that a suitable glucose provision could be effective for eel larvae.

MEASUREMENT OF HEAVY METALS IN MUSCLES OF WILD *Cyprinus carpio* IN CASPIAN SEA COAST; GUILAN PROVINCE, IRAN

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Heavy metals, as one of the main groups of pollutants in aquatic ecosystems, enter aquatic environments as a result of natural activities and also mainly as a result of human activities, and may accumulate in the bodies of aquatic organisms, including fish (esp. food fish), and pose a potential risk for health, ecosystem and living organisms are considered.

In this study, with the aim of investigating the amount of heavy metals lead, zinc, cadmium and mercury in wild *Cyprinus carpio* of Caspian sea coast; Guilan province, Iran province and investigating the effect of their weight and size on the level of pollution with these heavy metals and also investigating the level of contamination with the aforementioned heavy metals in different areas of the Guilan province, Twenty-four samples (pectoral muscles) of common wild carp were completely-random collected from fish retail center in six stations (Astara, Hashtpar, Anzali, Rasht, Kalachai and Kiashahr) of Guilan province.

The results of this study showed that the amount of heavy metals lead, zinc, cadmium and mercury in the studied samples are lower than the permissible limit stated in FAO / WHO, also other results obtained from this study showed that there is no significant difference between the weights, total lengths and standard lengths of the studied common carps between the two sexes, the sampling stations and the average amount of metals obtained in the tests.($P>0.05$).

TABLE. Heavy metals' (zinc, cadmium, lead and mercury) some indices in wild common carp

Index	Zn	Cd	Pb	Hg
No. Samples	24	24	24	24
Mean	24.73	0.003	0.025	0.084
Median	21.79	0.003	0.025	0.0495
Min.	9.46	0.003	0.025	0.027
Max.	59.24	0.004	0.30	0.282

THE USE OF *Ulva* IN INTEGRATED MULTI_TROPHIC AQUACULTURE (IMTA), AN ECOLOGICAL APPROACH FOR SUSTANABLE AQUACULTURE

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The scarcity of freshwater, overfishing and declining ocean biodiversity, marine eutrophication by anthropogenic activities, and the increasing demand for seafood have all required attention from a more comprehensive, global perspective.

Moving from conventional aquaculture toward an ecological approach to developing and managing a sustainable aquaculture that cares for environmental and sociological aspects can bring relief to at least some of these problematic issues.

Nutrient removal using Integrated Multi-Trophic Aquaculture (IMTA) systems is a promising ecological approach for sustainable aquaculture. The rationale behind the IMTA systems is to convert the excretions of the organisms cultured upstream into valuable food for the organisms cultured downstream. In marine IMTA systems, algae and halophyte plants have a high capacity for nutrient uptake per unit of culture area and can be an essential additional valuable product. In addition to nutrient removal by the green seaweed Ulva sp., the IMTA system proved to be a reliable source of sustainable biomass for human consumption, animal feed, and high-value by-products for the food additive industries.

IMTA systems in offshore cage cultures and land-based facilities will improve the food conversion rate (FCR) and diversify the mariculture products, ultimately increasing profit for the farmer. In addition, they will often create additional jobs and, no less importantly, will reduce environmental pollution.

EFFECTS OF THREE DIFFERENT LEVELS OF DISSOLVED OXYGEN ON THE FEED INTAKE, GROWTH AND APPETITE OF FARMED ATLANTIC SALMON

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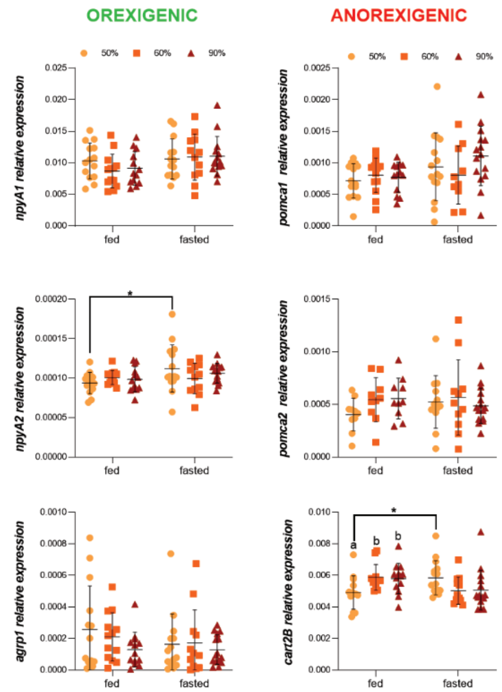
Introduction

Atlantic salmon (*Salmo salar*) can experience low and sometimes large variations in levels of dissolved oxygen (DO) in sea cages. The decreased DO levels can limit aerobic energy metabolisms, reduce feed intake and negatively affect growth performance. The hypothalamus regulates appetite, feed intake and energy balance but how the neuronal cell populations involved in appetite regulation respond to low levels of DO is unknown. These neurons express orexigenic peptides *neuropeptide y* (*npy*) and a*gouti-related protein 1* (*agrp1*), and the anorexigenic peptides *proopiomelanocortin a* (*pomca*) and *cocaine- and amphetamine-regulated transcript* (*cart*). Together these neuropeptides contribute to either stimulate or inhibit feed intake and might be at the base of the changes in appetite under different oxygen levels.

Thus, in this study we aimed to examine the response of long-term mild and moderately low DO on the expression of neuropeptides involved in control of appetite in the hypothalamus and feed intake and growth in Atlantic salmon.



Materials and methods

Post-smolt Atlantic salmon of 1035 ± 13 g were reared in triplicates tanks at either DO levels of 50%, 60% or 90%, at 12°C, 22 ppt salinity and continuous light at the Institute of Marine Research at Matre, Norway. Fish were fed twice a day for 1.5 hour and uneaten feed was collected to assess feed intake. Following two months, 15 fish from each experimental group were collected right after the first meal of the day (fed group) or a 24 h fasting (fasted group). Growth biometry and brain samples for gene expression analysis were collected.

Results

The fish at 90% DO performed best, with a considerably higher body weight, body length, and CF than the 60% and 50% DO groups. The 60% and 50% DO groups consumed much less food but maintained an equal FCR compared to the 90% DO group. Analysis of the hypothalamic region showed a lower mRNA expression of *cart2b* in the 50 % DO group than the 60 and 90 % DO. In the same 50 % DO, fasting the fish for 24 hours induced an increase of *npya2* and *cart2b*. This shows that these neuropeptides are involved in modulating appetite during low oxygen levels although their individual impact remains to be understood.

Funding

This work is part of the NoviFEED project - EEA grants (PT-INNOVATION-0099). A.S. is funded by PON doctoral scholarship, COD. DOT1412034, CUP F85F21005750001.

ASSESSING THE PHYSIOLOGICAL RESPONSE OF GILLS AND INTESTINE THROUGH SMOLTIFICATION IN ATLANTIC SALMON EXPOSED TO TWO DIFFERENT PHOTOPERIODS

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The Atlantic salmon (*Salmo salar*) life cycle involves a transformation called smoltification, where the fish undergo a series of physiological changes that allow them to move from freshwater (FW) to seawater (SW). This process is primarily synchronized by increasing daylength; therefore, applying a 6-week period of reduced light (LD 12:12) to induce smoltification followed by a period of constant day light is a common practice in aquaculture. But knowing the exact time when the salmon have developed smolts characteristics, is still a concern for the aquaculture industry. Gills are considered the main osmoregulatory organ coping with osmotic challenges, but other organs like the intestine also play an important role. The Na^+/K^+-ATPase (NKA) is the main enzyme that allows the cell to cope with the osmotic gradient and the NKA activity in gills is commonly used as the main indicator of smoltification status. However, analyzing the expression of genes related with osmoregulation in the intestine may also help to assess smolt status.

This study aimed to characterize the development of smoltification in Atlantic salmon exposed to two photoperiods: constant light (LL) and a 6-week LD12:12 winter signal (LD). A total of 500 parr (33.23 ± 5.41 g) were randomly distributed into 10 flow-through tanks (FW, 10°C) at the Department of Biological Sciences (Bergen, Norway). After the winter signal, both groups were maintained in constant light. Fish were sampled every other week for a period of 8 weeks. Smoltification was assessed by measuring gill NKA activity, plasma cortisol and glucose, and gene expression of the isoforms $\alpha 1a$, $\alpha 1b$ and $\alpha 1c$ of the NKA, the Na^+-K^+-$2Cl^-$ cotransporter (*nkcc*), the cystic fibrosis transmembrane conductance regulator isoforms *cftr-i* and *cftr-ii*, and the Na^+/HCO_3^- cotransporter (*nbc*) in the gills and in the posterior midgut (PMG).

The fish in the LD group showed a coordinated osmoregulatory change, particularly at the 4th week after the winter signal. This included an increase in gill NKA activity, plasma glucose and cortisol levels, as well as a peak in transcription of gill *cftr-i* and PMG *nka*α*1c*. In contrast, basically no change in these biomarkers were observed for the LL group over time. In conclusion, an increase in daylength induces and synchronizes smoltification in Atlantic salmon. Additionally, analysis of genes (like *nka*α*1c)* in the PMG can be used as complementary biomarkers to the gill NKA activity.

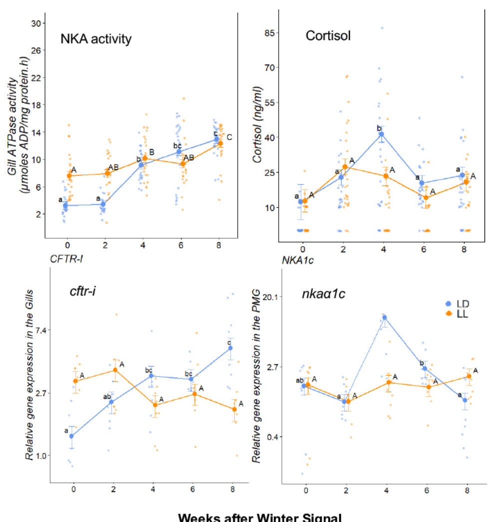

Figure 1. Results of the most relevant biomarkers assessed during smoltification. Figures display mean ± standard error over time. Capital letters show significant differences between samplings in the LL treatment, while lowercase letters display the same in the LD treatment.

A FATTY FATE: HOW DIETARY FATTY ACID PROFILES MODIFIED BY MICROALGAE INCLUSION INFLUENCE FATTY ACID METABOLISM IN RAINBOW TROUT *Oncorhynchus mykiss*

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Essential fatty acids for fish need to be derived by their diets and fish oil is still a main source for EPA and DHA. With increasing inclusion of vegetable oils in fish diets, the n-3 long chain polyunsaturated fatty acid (LC-PUFA) content in fish is decreasing. Microalgae have already been evaluated in several studies for their application as a feed ingredient in aquaculture and due to their high contents of PUFA, they are considered as a valuable lipid source in fish feed. As the fatty acid profiles differ between species, their inclusion in diets should be balanced to the requirements of the fish. Especially microalgae with higher contents of stearidonic acid (SDA) could play an important role in enhancing the n-3 LC-PUFA biosynthesis in fish. In this trial the use of microalgae as n-3 fatty acid source was tested. The influence of EPA and DHA in fish oil free diets with elevated SDA contents was further evaluated, with a focus on the fatty acid biosynthesis in rainbow trout.

The microalgae species *Isochrysis galbana* (14.7 % ALA, 10.1 % SDA, 1.3 % EPA, 9 % DHA of total fatty acids), *Tetraselmis chui* (14.7 % ALA, 6.2 % SDA, 6.8 % EPA) and *Schizochytrium limacinum* (0.3 % ALA, 0.4 % SDA, 0.7 % EPA, 55.8 % DHA) were used to formulate four isonitrogenous, isolipidic and isocaloric diets with varying fatty acid profiles. 180 rainbow trout were held in a recirculating aquaculture system in triplicates of trial diets with 15 fish each. They were fed daily until apparent satiation for 56 days. For digestibility analysis the fish were fed for two more weeks and were manually striped. The fatty acid balance method was used to evaluate the bioconversion of fatty acids in the fish.

The combination of *I. galbana* and *T. chui* resulted in highest growth performance despite lower overall diet digestibility, which was compensated by higher feed intake. There was no dietary influence on the whole body nutrient composition, but the different dietary fatty acid profiles were mirrored in the fish with higher dietary DHA contents resulting in higher DHA contents in the fish. However, DHA appearance showed no product inhibition on fatty acid biosynthesis. Microalgae can be used as an efficient source for fatty acids in fish diets considering their different fatty acid profiles. Dietary DHA contents did apparently not influence the biosynthesis of this fatty acid in rainbow trout but higher EPA contents resulted in increased DHA production.

WEANING OF ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*) WITH COMMERCIAL AVAILABLE DIETS IN EUROPE

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Introduction

The development of artificial diets for rearing Atlantic bluefin tuna (ABT) is crucial for several reasons. Overall, it will support sustainability, economic viability, and reduced environmental impact in tuna aquaculture. This trial aimed to establish a benchmark of artificial ABT feeding with readily available commercial diets on the European market, for future studies and diet improvement, focusing on artificial weaning and feeding of ABT.

Material & Methods

Over 12 weeks, an ABT feeding trial was conducted at the Instituto Oceanográfico de Murcia (IEO). Two Skretting artificial feeds, Gemma Silk (GS) and Magro, were assessed across three ABT rearing stages: Weaning, pre-ongrowing phases 1 and 2. Weaning occurred between 23-41 days post hatch (dph) when ABT larvae were introduced to GS at 23 dph, taking 9 days for full weaning from sea bream (Sparus Aurata L.) yolk sac larvae. In pre-ongrowing phase 1 (41-84 dph), a 50:50 GS and Magro diet was supplied, followed by exclusive Magro feeding in pre-ongrowing phase 2 (84-106 dph).

Results & Discussion

The application of Skretting commercial start feeding diets, resulted in expected survival rates during weaning, ranging from 26% to 37% across replicas, in the range of previous weaning experiments. Pre-ongrowing phase 1 had the highest specific growth rate with 8.1 versus 3.8 in pre-ongrowing phase 2. However, phase 2 had the highest gains in wet weight and length, alongside with lower feed conversion ratio (FCR).

It is important to highlight that the exclusive utilization of Magro led to decreased FCR rates, suggesting its capability to improve subsequent on-growing phases for ABT, as a logical progression, the next step of this trial involves the continuation of the artificial feeding regimen beyond the 200 g weight threshold. Another aspect worth contemplating for future trials involves exploring the potential to decrease or eliminate the use of YSL in the early stages of ABT larvae.

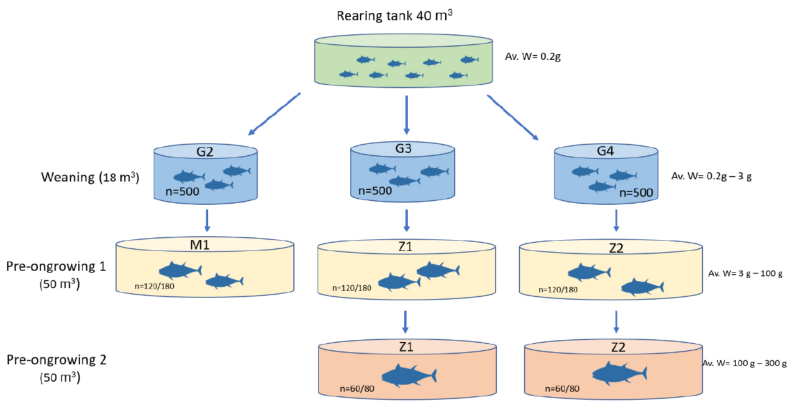

Fig. 1.: Trial design

TRAINING THE NEXT GENERATION OF AQUACULTURE PROFESSIONALS IN EUROPE AND BEYOND

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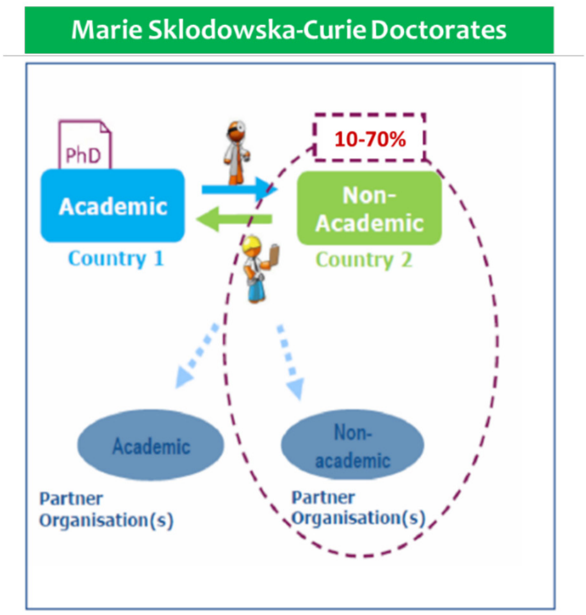
"Give a man a fish, and you feed him for a day. Teach a man how to fish, and you feed him for a lifetime". Although the original reference of this statement is disputed, there are few who dispute its truthfulness. However, we would like to share the experiences of our aquaculture training programmes, because life-long learning does not stop after teaching a man/woman how to fish. For example, teaching everyone how to fish would lead to a collapse of the fish population. Aquaculture has seen a steep increase in the past decades, but further growth to secure future aquaculture production is linked to considerable societal challenges in a complex interrelated manner: (1) environmental impact of aquaculture, (2) climate resilience, (3) resource scarcity, (4) biodiversity conservation, (5) social equity, (6) food security, (7) health and disease, (8) market access, (9) technological adoption and (10) policy and governance.

Although experts in different disciplines are needed to enforce scientific breakthroughs, transdisciplinary visions are crucial for implementation of change. EATFISH, EASYTRAIN and RASOPTA are three Marie Sklodowska Curie doctoral training networks educating early-stage disciplinary researchers in a transdisciplinary manner through a three-year training programme around individual PhD research projects. For PhD students, research and education is not only a means of acquiring knowledge and skills, but also a way of contributing to the advancement of their fields and the society at large. To understand such situations in the complex web of interacting sectors, goals, and interests our approach is founded on bridging the frequently occurring "knowing–doing" gap and shift away from more traditional, discipline-oriented education. Real-life issues as identified by PhD students through visiting and working at aquaculture industries were the point of departure for a student-active learning. These observations have been systematically linked to science, business, governance and societal aspects in the training programme.

At AQUA24 we will share our experiences in transdisciplinary training of young researchers and address the impact on the career of the researchers, aquaculture innovation and society.



CULTIVATION OF TWO MEDICAL PLANTS INTEGRATED WITH COMMON CARP (*Cyprinus carpio* L) IN AQUAPONICS SYSTEM

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Aquaponics can rightly be called a technology of the future as it combines the use of wastewater with the production of high-quality and economically viable products—aquatic organisms and plants. Medicinal plants can be used as a source of biologically active substances that can replace antibiotics, which are responsible for bacterial resistance in the treatment of various diseases in humans and animals. To date, aquaponic technology is innovative, and therefore studies addressing the issue related to the combined cultivation of medicinal plants and fish in this innovative technology are severely limited.

The purpose of this study is to compare the effects of cultivating oregano and thyme in an aquaponic system on the growth indicators of fish and plants and the hydrochemical indicators. The experiment was conducted in an innovative aquaponic system, located at the Agricultural Faculty of Trakia University in Stara Zagora, Bulgaria. The initial weight of the fish was average 245±18.9g. The seedlings of oregano and thyme were transferred to aquaponic pots and placed in deep water culture tanks in the specified system. The duration of the experiment was two months. At the end of the experiment, the weight of the fish was 447.16 ± 15.86 g. The hydrochemical parameters observed during the experiment are presented in Table 1. The growth parameters of oregano and thyme are shown in Figure 1.

Table. 1 Hydrochemical parameters during the experiment

Parameters	Oregano /Origanum vulgare/	Thyme /Thymus vulgaris/
oxygen mg.L-1	7.19±0.03	7.13±0.03
conductivity µS.cm-1	248.46±1.21	256.58±1.38***
pH	7.63±0.07	8.47±0.09***
ammonium	1.05±0.22	1.95±0.42*
nitrite	0.023±0.01	0.021±0.005
nitrate	2.14±0.69	1.41±0.35
phosphate	5.33±1.19	4.78±1.18

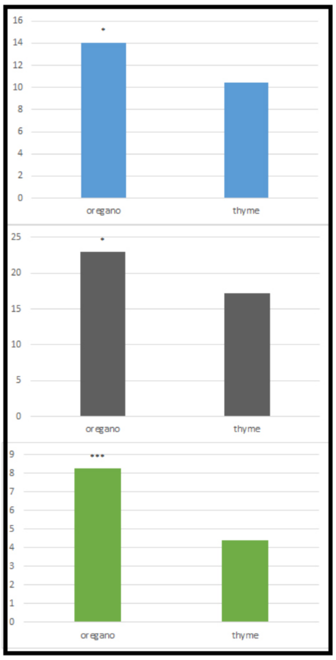

Fig.1 Growth parameters of oregano and thyme during the trial

DEVELOPING NOVEL APPROACHES FOR THE NURSERY CULTURE OF GREENSHELL™ MUSSELS IN NEW ZEALAND

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New Zealand's Greenshell™ mussel (*Perna canaliculus*) industry stands as the cornerstone of the nation's aquaculture sector, boasting an annual production exceeding 100,000 tonnes and contributing over $350 million NZD to the economy. However, the growth and long-term sustainability of this industry is threatened by the highly inefficient use of wild spat that is crucial for seeding its farms. Despite harvesting over 350 billion spat from the wild each year to supply the industry, only 1.5 billion of these are retained on farms from seeding through to harvest, representing losses of over 99%. This presentation will summarize the findings from several studies which were undertaken to begin to develop alternative approaches to nursery culture of Greenshell™ mussels to reduce spat losses and improve production efficiency. This presentation will demonstrate that although further research is required, common approaches to the nursery culture of bivalves used elsewhere and in other species, might also represent a promising approach for the nursery culture of Greenshell™ mussels. This presentation will also outline the next steps required for transforming the Greenshell™ production cycle in New Zealand.

INTRODUCTION OF TORULA YEAST IN RAINBOW TROUT DIET IMPROVED THE EFFICIENCY OF PLANT-BASED DIET AND INFLUENCED IMMUNITY

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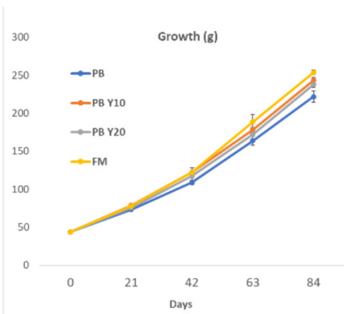
The sustainable development of aquaculture is threatened by climate change, which not only affects the availability of raw materials used in fish feed but also the farming conditions, which are becoming increasingly stressful. Nutrition is one of the levers that can be activated to mitigate the consequences of climate change on farmed fish. Yeast extracts not only contain molecules with immunostimulant properties such as beta-glucans and nucleotides, but they are also rich in proteins, which make them interesting as an alternative to fishmeal and to increase fish robustness. Among the yeasts, torula (Cyberlindnera jadinii, a teleomorph of Candida utilis) is particularly attractive because it can be can be grown on hydrolysates of lignocellulosic materials, a by-product of the forestry industry and thus do not compete with human food.

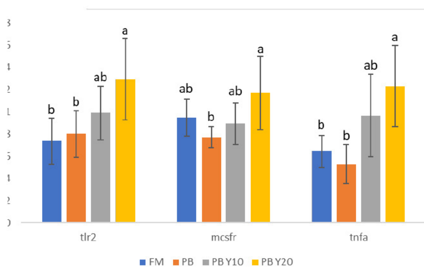
In the present project, we evaluated whether the addition of 10% or 20% torula yeast could improve the efficiency of a plant-based diet devoid of fishmeal and compete with 20% fishmeal diet in rainbow trout. The digestibility of torula yeast was measured, and a growth trial was conducted for 12 weeks, after which blood and intestinal samples were collected to analyze immune parameters and intestinal gene expression.

The results indicated that torula yeast had a protein digestibility for trout (93.8%) very similar to that of fishmeal and plant protein concentrates with similar protein content, which make it suitable for trout feeding. Adding 10 or 20% yeast to a plant-based diet significantly improved growth, which seems to be related to a restoration of feed intake. Moreover, the addition of 10% yeast achieved a level of growth comparable to that observed with a diet containing 20% fishmeal. The introduction of yeast also induces an innate immune response in fish, as evidenced by the upregulation of immune genes encoding *tlr2*, *mcsfr*, and *tnfa* and plasma level of lysozyme in a dose-dependent manner.

In conclusion, torula yeast appears to have the potential to enable plant-based diets to compete with fishmeal diets and, in addition, to improve the robustness of rainbow trout by increasing the fish's ability to detect unknown molecules, eliminate pathogens and prepare the fish for an eventual pathogen or inflammatory challenge.

Acknowledgements: This work was funded by the TNA program of AQUAEXCEL2020 EU project.





THE NEGATIVE IMPACTS AND MONITORING OF INVASIVE TOPMOUTH GUDGEON IN CARP AQUACULTURE PONDS

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Introduction

Pond aquaculture in Central and Eastern Europe has faced many challenges, from energy prices to the biological invasion of non-native species. Invasive species such as topmouth gudgeon (*Pseudorabora parva*) pose a significant threat to the fishpond ecosystems that, through direct and indirect effects, could negatively affect pond ecosystem functioning and, ultimately, fish production. The expansion and distribution of invasive species are rapid and often unpredictable; therefore, early detection is crucial to prevent further ecosystem destruction and distribution of such species. The presented study aimed to (1) assess the impact of topmouth gudgeon on common carp (*Cyprinus carpio*) production and pond functioning and (2) find the most comprehensive sampling methods for population monitoring of this invasive species in ponds.

Material and methods

The study was conducted in South Bohemia (Czechia) in six ponds (approx. depth of 1m; approx. pond area 1 ha). Three ponds were stocked with only common carp and were used as control, and three ponds were stocked with both common carp and topmouth gudgeon. Zoobenthos, zooplankton, environmental parameters, and fish (both common carp and topmouth gudgeon) were sampled monthly from March to September 2020. Alongside these samplings, three methods for population monitoring were tested, electrofishing, fish-trapping, and throw-netting, to detect the invasive topmouth gudgeon.

Results and discussion

Topmouth gudgeon had a strong negative impact on the production of common carp in aquaculture ponds. The growth of common carp was remarkably reduced in ponds with the presence of topmouth gudgeon, with the final body weight of carp averaging 740 ± 128 g compared to 1125 ± 144 g in ponds without topmouth gudgeon. Topmouth gudgeon demonstrated a heavy top-down effect on planktonic crustaceans, especially large *Daphnia* spp., contributing to impaired carp growth and increased zoobenthos consumption. In the second part of the study, all monitoring methods detected the presence of topmouth gudgeon, but fish trapping proved to be the most accurate. The other two methods, throw-netting, and electrofishing, gave biased size distributions and underestimated density. Precise monitoring is the first step to the effective eradication of invasive species.

Acknowledgement

The study was supported by the Czech Science Foundation (project: GA ČR 22-18597S).

BIOSAFETY IN SMOLT PRODUCTION AND TRANSPORT - RISKS IN HATCHERIES AND WELL BOATS

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Introduction

Diseases and parasites are main concerns in Norwegian Atlantic salmon farming. In 2023, over 100 million fish died from various causes throughout the various production stages. The most common cause of death was infection disease which accounted for 38%. This situation is not acceptable, and all parties related to the industry agree that progress is necessary. Biosafety is therefore a central concern in Norwegian salmon farming. World Organization for Animal Health has defined biosafety as "a set of management and physical measures designed to mitigate the risk of introduction of pathogenic agents into, or spread within, or release from, aquatic animal populations.".

Farming of Atlantic salmon can be divided into two main activities; on-land smolt production and sea-based grow-out. This project focuses on smolt production and delivery of smolt to sea-based farms. Reasons for this are both the high number of mortalities in smolt production and the uncertainty related to whether disease and mortality in sea-based grow-out originates from infections carried over from smolt production. Biosafety at this stage is thus essential, and there is need for an overview of risk factors and measures related to infection of salmon in these technical solutions and systems for smolt production and transfer to sea.

Material and methods

This project covers results from a study identifying risk factors related to technical solutions at hatcheries with RAS technology (recirculating aquaculture systems) and well boats, and potential measures to reduce risk. Such risk factors and measures are related to the technical solutions' design, construction, cleaning, maintenance, and how they facilitate operation according to biosafety principles. Both risk factors and potential measures were identified based on literature review of research literature, document study of technical drawings and other documents provided by industry, interviews and observations and discussions during visits at RAS-facilities and vessels.

Results

Risk factors for RAS facilities are divided into four main groups: Introduction to the facility, spread inside the facility, growth inside the facility, and additional risks. Risk factors for well boats are divided into three main groups: Introduction through intake water, spread between fish groups, and infection of external fish. Measures are proposed to mitigate the identified risk factors. The lists are not exhaustive, and risk magnitude is not quantified, so effect of measures is not calculated. Still, the measures' potential for risk reduction and implementation (cost/benefit) are discussed.

In the presentation, we will suggest key measures for biosafety in RAS hatcheries and well boats for smolt transfer to sea-based farms.

SATELLITE-BASED INFORMATION SERVICES SUPPORTING OPERATIONAL ENVIRONMENTAL RISK MITIGATION IN THE SOUTHERN AFRICAN MARINE AQUACULTURE SECTOR

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The marine aquaculture industry of southern Africa faces environmental pressures such as harmful algal blooms (HABs) and marine heatwaves, which can negatively affect production, animal health, and system water quality, potentially resulting in devastating economic losses. Satellite-derived information is of great value to the industry as it offers early warnings and a synoptic scale environmental overview of the location, size and intensity of external threats, facilitating appropriate risk mitigation steps. Under the South African National Oceans and Coastal Information Management System (OCIMS) project satellite-derived indicators and information services have been co-designed between earth observation specialists and the fisheries and aquaculture industry. The result is a fit-for-purpose decision support system that provides the capability of near real time monitoring, assessment, and mitigation for environmental risks. The Tool utilises freely available Copernicus satellite data, knowledge about local marine ecosystems and oceanographic patterns, and localised algorithms to provide maps and regionally relevant indicators (e.g. phytoplankton biomass, HAB warnings, temperature anomalies) through an interactive web application, facilitating operational support for daily decision making. These services have also been regionally expanded into southern Africa and the western Indian Ocean through the GMES&Africa programme's Marine and Coastal Operations for southern Africa and the Indian Ocean (MarCOSIO) project. We will provide specific examples of applied operational risk mitigation during harmful algal bloom events, and how these services support daily decision making in the South African abalone industry.

Acknowledgements
The authors would like to acknowledge the OCIMS project, which received funding from the Department of Fisheries, Forestry and the Environment and the Department of Science and Innovation; as well as the MarCOSIO project, which received funding from the African Union Commission through the GMES&Africa programme.

DEVELOPMENT AND OPTIMIZATION OF HATCHERY *Codium tomentosum* CULTIVATION METHODS FOR OFFSHORE PRODUCTION

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Introduction

European seaweed industry is experiencing a shift towards seaweed aquaculture and one of the species that is showing high potential for cultivation in Portugal, and presents diverse applications is *Codium tomentosum*, however, methods for its offshore cultivation are only now being developed and optimized. On the other hand, established *C. fragile* cultivation in Korea involves the use of seed stock regenerated from isolated utricles and medullary filaments, that are seeded in twine in a nursery and deploying ropes at sea. *C. fragile* is also sometimes produced through the settlement of wild zygotes onto culture twine, however, the regeneration capacity method has shown higher biomass yields compared to zygote seeding (Hwang et al, 2007). This study aims to explore the reproductive mechanisms and attachment processes to twine of the local species *C. tomentosum* for the optimization of offshore cultivation.

Methods

The work is divided into three experiments. Experiment 1 aims to investigate the sexual reproduction mechanisms of *C. tomentosum*, by identifying and inducing gamete release using two different methods and following the development of *C. tomentosum* zygotes and germlings. Experiment 2 aims to study *C. tomentosum* medullary filaments attachment to 5 different types of twine and follow its development. Experiment 3 aims to identify the optimal density and resting time (time without water) for *C. tomentum* attachment and development in the selected twine. All experimental work is carried out in climate chambers under controlled conditions. The germlings/filamentous settlement rate and development are assessed through microscope observations, with image and video capture and analysis.

Preliminary Results

Initial observations show that gamete release, zygotes formation and germling development occurred with both methods tested, however, contamination levels were different between the two methods. An initial trial carried out with three different twine substrates and two different concentrations of *C. tomentosum*, showed that the two concentrations tested only affected settlement rate in one of the tested twines, but the type of twine always did ($p < 0.001$).

Acknowledgments

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References

1. Hwang, E.K., Baek, J.M., Park, C.S. (2007). Cultivation of the green alga, *Codium fragile* (Suringar) Hariot, by artificial seed production in Korea. In: Borowitzka, M.A., Critchley, A.T., Kraan, S., Peters, A., Sjøtun, K., Notoya, M. (eds) Nineteenth International Seaweed Symposium. Developments in Applied Phycology, vol 2. Springer, Dordrecht.

OXYGEN CONSUMPTION IN SENEGALESE SOLE *Solea senegalensis* POST-LARVAE IN FED AND FASTED STATE

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Respirometry analysis was carried out in Senegalese sole post-larvae aiming to: (i) characterize the oxygen consumption in fasted and fed state as function of fish weight; (ii) assess the impact of diet, feeding level, or a combination of both factors, on the oxygen consumption rates. This analysis was conducted in the context of a trial where fish (age at start: 16 days after hatching, DAH) were fed three diets with different protein, fat and energy contents, at two feeding levels (100% and 80%), for 100 days (age at the end: 115 DAH).

Respirometry measurements occurred at 41, 48, 62 and 90 DAH, with four replicates per tank, both in the fed and fasted states. Fish considered to be in fed state were fed up to half an hour before the respirometry test and their gut checked for the presence of feed. Fish considered to be in fasted state were subjected to a 5-hour fasting before the test. Measurements were made with fish placed in an individual chamber containing an oxygen probe, by closed respirometry until reaching an oxygen saturation level of 85-90%. In total, 575 datasets (dissolved oxygen saturation time series) were generated. To ensure data reliability and representativeness, all datasets were cleaned (e.g., removing outliers), transformed (e.g., converting from oxygen saturation to oxygen consumption, based on chamber and fish volume, corrected for blank measurements) and quality assessed (e.g., regression analysis). Datasets that did not meet the criteria defined in the quality assessment stage were not considered in further analysis.

Allometric models, characterizing the average oxygen consumption of Senegalese sole post-larvae as a function of weight, in fed and fasted state, were obtained:

(i) in fed state, oxygen consumption (mgO_2/fish/day) = 10.50 × fish weight (g) $^{0.78}$;
(ii) in fasted state, oxygen consumption (mgO_2/fish/day) = 9.02 × fish weight (g) $^{0.81}$.

The impact of diet and feeding level on oxygen consumption were assessed based on a two-way approach: (a) comparison by sampling point, considering as variable of interest the relative oxygen consumption (mgO_2/g fish/day); (b) comparison of all measurements, considering as variable of interest the normalized relative oxygen consumption (mgO_2/g^{bw_exp} fish/day). Based on the results of this analysis, neither diet nor feeding level appear to have a clear effect on the oxygen consumption of fish in both the fasted and fed state.

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SHIPPING PROTOCOL FOR MARINE FISH YOLK-SAC LARVAE: A MODEL STUDY ON MEAGRE *Argyrosomus regius*

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The transportation of early-life stages of fish, especially fertilized eggs, from hatcheries to aquaculture and research facilities is a commonplace procedure. However, challenges such as low hatching rates and mortality have been observed during egg shipment due to factors such as extended shipping times and water quality degradation. Shipping yolk-sac larvae may offer an alternative solution to address these challenges. This research aimed to develop a protocol for shipping yolk-sac larvae, focusing on critical factors such as stocking density and transport duration.

Three different larval concentrations (4000 larvae L^{-1}, D4; 8000 larvae L^{-1}, D8; 12000 larvae L^{-1}, D12) were tested in replicates at three different simulated shipping times (24h, ST24; 36h, ST36; 48h, ST48). After hatching in fiberglass incubators, larvae were transferred to polyethylene shipping bags and underwent simulated shipping conditions. Temperature, dissolved oxygen levels, ph, NH_3 and NO_2, were measured during the experiment to monitor water quality changes. Larval survival was assessed at the end of the simulation of shipping (unpacking). Surviving larvae were transferred to incubators to evaluate also the survival rate after a 24-hour post-transportation period (incubator).

Results showed that larval survival was not affected at any density tested (D4, D8, D12) within ST24 (Fig.1 A). On the contrary, extending the shipping time decreased significantly larval survival in the higher densities (Fig.1 B and C). Larval survival was found to be negatively correlated with decreased levels of O_2 and pH, and increased NH_3 and NO_2. The developed protocol, may provide guidelines applicable and adaptable to other desired marine species in aquaculture.

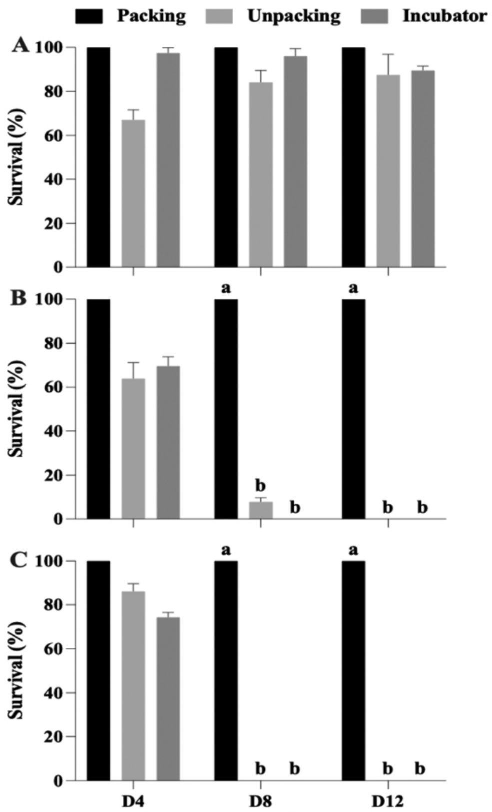

Fig.1 Mean (±SEM) larval survival (%) density during the simulation of shipping of meagre (Argyrosomus regius) yolk-sack larvae obtained from spawning 2. The shipping time imposed on the larvae during the experiment were **A.** 24h (ST24) **B.** 36h (ST36) and, **C.** 48h (ST48). Superscript letters above columns indicate differences between different time points (packing, shipment, incubator) for each given experimental group (4000larvae L^{-1}, D4; 8000 larvae L^{-1}, D8; 12000 larvae L^{-1}; D12) (two-way ANOVA, Tukey HSD, $P < 0.05$).

OFF-FLAVOUR PRODUCING MYXOBACTERIA HAVE AN APPETITE FOR OTHER MICROBIAL MEMBERS OF RAS – IS GEOSMIN PRODUCTION AFFECTED WHEN PREDATOR ENCOUNTERS PREY?

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Tainting of fish due to the off-flavours geosmin and 2-MIB is a long-known problem in Recirculated Aquaculture Systems (RAS)-reared products. While several attempts have been made to physically, chemically and biologically remove these bothersome compounds, none have proved more efficient than depuration of the fish at the end of the growth cycle, although this procedure is both costly and potentially distressing for the fish. Regardless of the attempts of removal of off-flavours, one curious fact still stands: Why are these secondary metabolites at all produced in RAS systems from the bacteria's point of view? Molecular studies point to one of the major bacterial producers of these compounds in RAS, especially geosmin, being the group of myxobacteria (Lukassen et al., 2021). These bacteria are ubiquitous on the planet (accounting for 2% of the total OTUs on Earth Microbiome Project) (Wang et al., 2021) and have a very peculiar lifestyle because they feed cooperatively on other bacteria. Due to this predatory lifestyle, it has been speculated that they are important in shaping microbial communities in soil (Petters et al., 2021). This theory might also hold true for RAS environments where they are commonly found. Transcriptomic studies of myxobacteria show that the geosmin synthase gene *geoA* is upregulated during predation (Pérez et al., 2022). To further investigate whether geosmin might stimulate predatory activity in RAS, we performed a predation assay with two myxobacteria isolated from RAS and using a collection of bacteria also isolated from RAS as prey. The volatiles produced during predation was measured in real time using Proton Transfer Reaction-Mass Spectrometry (PTR-MS).

Preliminary results show that myxobacteria could feed on most of the selected bacteria (21 out of 28 preys), as determined by clearing zones of the prey colonies. Interestingly, the bacteria that avoided predation all displayed a rapid swarming behavior. Production of protective polysaccharides or toxins secreted by the prey bacteria likely make the prey bacteria avoid predation, as previously documented in predation studies with *Pseudomonas* (Akbar & Stevens, 2021). All bacteria without swarming behavior were susceptible to predation, but some highly swarming members of the order Flavobacteriales were also susceptible to predation. Results from the real-time measurements of volatiles produced are currently undergoing analysis but will be presented at the conference.

Two myxobacteria isolated from RAS predated readily on most, but not all, selected RAS bacteria. This trait likely gives them an important role in the RAS microbiome and deserves more investigations from an ecological point of view. Understanding the relationship between predation and geosmin production will provide an ecological frame for optimized RAS management by reducing off-flavour problems directly at the bacterial source.

DEVELOPMET OF A VACCINE AGAINST VIRAL NERVOUS NECROSIS IN GROUPER AQUACULTURE USING VIRUS-LIKE PARTICLES PRODUCED BY PLANT EXPRESSION SYSTEM

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Viral nervous necrosis (VNN) is a major viral disease affecting the central nervous system of over 170 marine fish species, including grouper. It is caused by the nervous necrosis virus (NNV), a non-enveloped, positive-sense single-stranded RNA virus belonging to the Betanodavirus genus. Fish infected with NNV exhibit symptoms such as abnormal swimming behavior and anorexia, with mortality rates reaching up to 100%. Currently, no commercial vaccine is available for grouper VNN disease, presenting an urgent need for an effective solution in the grouper aquaculture industry. In this study, we aimed to develop an effective vaccine against VNN using NNV virus-like particles (VLPs) produced through a plant expression system. The coding sequence of the RGNNV capsid protein was subcloned into an expression vector, fused with a rubisco transit peptide, and expressed in *Nicotiana benthamiana* using agrobacterium transient transfection. The expressed recombinant protein exhibited VLP properties, as confirmed by SDS-PAGE, Western blot, and transmission electron microscopy. To assess the immunogenicity of the produced RGNNV VLPs, immunization trials were conducted on red-spotted groupers (*Epinephelus akaara*) and seven-banded groupers (*Epinephelus septemfasciatus*) with varying antigen dosages and booster vaccinations. The challenge results demonstrated that the RGNNV VLP vaccine provided protection against NNV infection in both grouper species, showing significant reductions in mortality rates compared to the unvaccinated control group. Additionally, antibody titration assays revealed antigen dose-dependent antibody production in vaccinated fish. In conclusion, the RGNNV VLP vaccine developed through the plant expression system poses promise for use in preventing VNN disease outbreaks in grouper aquaculture.

NATURAL SELECTION QUANTIFIED: REVERSING DIRECTIONAL SELECTION IN A DOMESTICATED SPECIES

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Atlantic salmon has been exposed to directional selection for aquaculture-related traits for >12 generations. Consequently, farmed salmon deviate from wild conspecifics in multiple traits, but especially growth rate under aquaculture conditions (increased in farmed) and survival in the wild (reduced in farmed). Not surprisingly, interbreeding between escaped salmon and wild populations has resulted in alterations of fitness-related traits in naturally produced introgressed individuals in the wild, including reduced survival in fresh water which may result in reduced production of wild salmon. However, the long-term consequences of introgression are not fully known and there is a knowledge gap related to what extent natural selection can reconstruct the original characteristics of the wild salmon populations.

In order to elucidate the impact of natural selection on the long-term consequences of introgression we conducted a study where domesticated salmon was planted out as eyed-eggs in a river with a fish trap for smolt recapture. Surviving individuals were recaptured as age 3-5 year old-smolts and thereafter reared under aquaculture conditions until reaching maturation. These individuals were then used as brood stock and crossed with individuals of identical genetic background that had been held for the entire generation under aquaculture conditions and thus not exposed to natural selection in the wild. The offspring of these unique crosses were thereafter compared in a pedigree-controlled common-garden growth study, in order to quantify the reversal of the genetic gain achieved through directional breeding for the main trait targeted: increased growth. Here we document natural-selection induced loss of growth capacity in offspring of farmed salmon, thus documenting the first evidence of de-domestication in fish.

IDENTIFISH: DIGITAL TOOLS FOR PRECISE IDENTIFICATION OF ESCAPED AND WILD SALMON

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Considerable variation in Atlantic salmon morphology creates challenges for developing reliable and agreed criteria for manual identification of farmed escapees. It is therefore desirable to develop a digital tool that can provide a precise and objective classification of salmonids with associated uncertainty. Thus, the IdentiFish project was established to develop a general and precise machine vision to distinguish between escaped and wild salmon.

Based on a unique data set collected through a national monitoring program for escaped farmed salmon in Norwegian water courses and a commercial salmon fishing app Elveguiden, the project group will develop a model for distinguishing escaped farmed salmon from wild salmon using machine vision. While the national monitoring program surveys ~200 rivers annually, Elveguiden collaborates with over 500 management teams and landowners and has over 55,000 registered anglers on the platform.

The machine vision model will be made publicly available on the Institute of Marine Research's website, and at the same time implemented in Elveguiden's app. An app capable of distinguishing between escaped and wild salmon using machine vision can contribute to more precise monitoring of escape events. Immediate classification via app provides opportunities for rapid reporting to management authorities which can reduce the reaction time to implement necessary measures, such as targeted recapture as well as limit the extent of the escape event and thus minimize financial losses.

The project is an example of how artificial intelligence can be used to strengthen monitoring of wild salmonids and contribute towards sustainable aquaculture production. The project is funded by the Norwegian Seafood Research Found (FHF 901937) and is a collaboration between researchers, developers, and entrepreneurs: Norwegian Institute of Marine Research, Elveguiden (https://elveguiden.no/en), Norwegian Veterinary Institute and Norwegian Institute for Nature Research.

Fig 1: Example of a salmonid classified using machine vision. The red area at the head and at the fin-tail region, and the orange area in the middle of the fish (the fish's side) indicate that shape and the dot pattern have probably been important in the determination of this fish.

FROM SCIENCE TO ADVISE: RISK OF GENETIC IMPACT FROM ESCAPED FARMED ATLANTIC SALMON FROM A GIVEN FARMING LOCATION

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Norway is the largest producer of Atlantic salmon, and the government has established a strategy against escapes from aquaculture facilities with a vision that genetic influence on wild salmon populations shall not occur. The strategy has a two-pronged approach; 1) through escape-proof design and operation of aquaculture facilities and risk-based supervision by management, escapes of farmed fish shall be reduced as much as possible, and 2) in the event of escapes, genetic effects on wild populations (genetic introgression) shall be reduced to a minimum.

Since 2019, the Institute of Marine Research has assessed the risk of further genetic change in wild populations due to introgression of aquaculture Atlantic salmon escapees, using Bayesian networks (Figure 1). This method illustrates risk factors that can lead to possible consequences, and the uncertainty associated with these factors. Thus far the 13 production areas along the Norwegian coast have been assessed. While management have conveyed that this methodology provides the opportunity to operationalize risk-based supervision, the current per production area resolution is too low for assessing the consequences of escapes from a given location. Thus, in order to identify farming locations that in the event of escape have increased risk of causing further genetic introgression, the Norwegian Directorate of Fisheries put forward an order to the Institute of Marine Research, a neutral knowledge provider in advisory capacity to the Ministry of Trade, Industry and Fisheries.

The purpose of the order was to investigate the possibility of increasing the resolution of the current risk assessment in such a way that it could be used to assess the consequences of escape from a given location, on the individual wild populations. In the ongoing pilot project, the dispersal of escaped Atlantic salmon the geographical location of aquaculture facilities in connection to nearby water courses, the water courses attractiveness to escaped salmon and the resilience of the wild populations were assessed. The current talk therefore provides an example of "from science to advise" in a risk assessment framework.

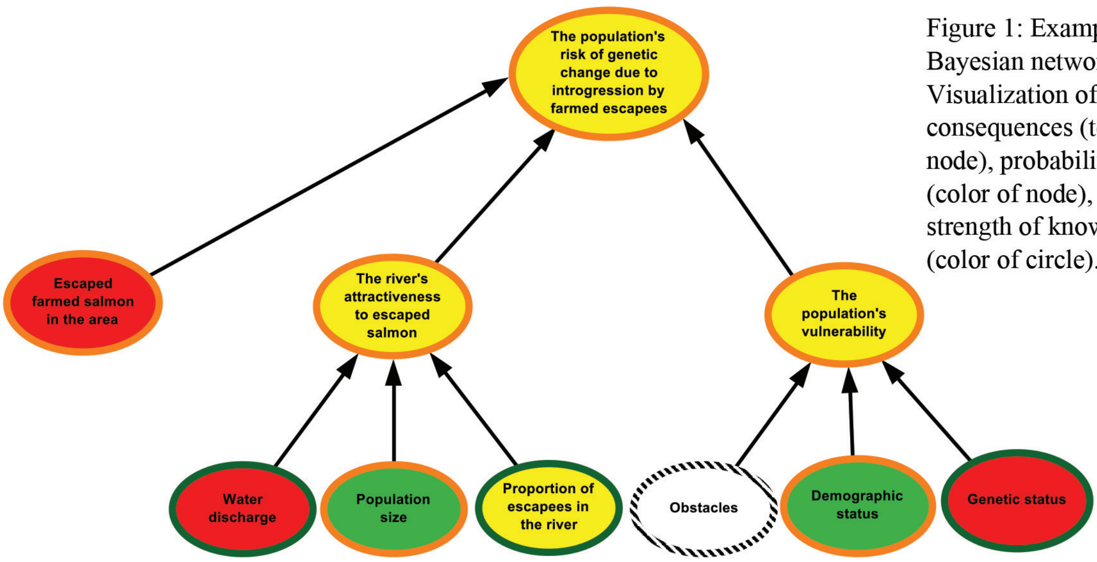

Figure 1: Example of Bayesian network. Visualization of consequences (text in node), probabilities (color of node), strength of knowledge (color of circle).

THERMAL EXPOSURE HISTORY INFLUENCES THE NASAL RESPONSES OF ATLANTIC COD TO BIOLOGICAL STRESSORS

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Climate change is no longer a distant threat to aquaculture production. In recent years, its ramifications have become more evident and prevalent, especially with reports of several episodes of long periods of elevated or erratic temperatures, and low dissolved oxygen levels. In addition, acidified aquatic environments due to carbon dioxide (CO_2) have been identified and included in future climate projection models. These climate stressors have resulted in mass mortality events in several fish farms. Besides these widely known climate stressors, there are several secondary stressors that are less studied but pose significant compound concerns. For example, jellyfish blooms have affected marine farms and their frequent occurrence in recent years has been implicated to prolonged period of elevated temperature. The severity of some diseases has also been altered by the changing environments, especially where the virulence and pathogenicity of the causative agent is temperature dependent.

The olfactory organ of fish serves a crucial role in sensing stimuli, odorants and chemical signals in the environment. Its anatomical position makes it a portal of entry for a number of pathogens. Hence, besides its function in olfaction, the presence of mucosa-associated lymphoid tissue in the olfactory rosette highlights its important role in immunity. Earlier studies have shown that the olfaction of fish is highly vulnerable to climate change, particularly with CO_2 exposure. We have previously documented that elevated temperature affected the olfactory organ of Atlantic cod (*Gadus morhua*), which carried associated implications on the nasal responses to *Francisella noatunensis,* an intracellular bacterium which is the causative agent of francisellosis. It remains to be studied how repeated cycles of prolonged exposure to elevated temperature affect the nasal responses and what are the implications associated when fish are exposed to secondary stressors.

A trial was designed where a group of Atlantic cod were exposed to three temperature scenarios: 1) constant temperature at 12°C, the common production temperature, 2) constant elevated temperature at 16°C, the upper thermal limit, and 3) fluctuating temperatures, 12°C → 16°C → 12°C. Three weeks after, a group of fish were exposed to secondary stressors, i.e., jellyfish bloom and *F. noatunensis*. Preliminary results indicate that nasal morphometries changed following exposure to a jellyfish bloom. Increased number of mucus cells and prevalence of olfactory epithelial hyperplasia were observed. In addition, *lamina propria* degeneration was identified in several individuals. The occurrence of these changes was higher in Groups 2 and 3. In addition, inter-treatment differences in the regulation of olfactory receptors, immune and stress markers were documented among the groups. In the infection trial, th e pathogen was detected in the olfactory organ, though differentiating bacterial load among the groups could not be conclusively established. Interestingly, pathologies related to francisellosis were more severe in Groups 2 and 3. Genome-wide transcriptomic analysis is currently on-going and will be presented at the meeting.

The work was supported by the Norwegian Troms and Finnmark county (TFFK2021-179, SecureCod) and the Norwegian Research Council (No. 194050, Insight; No. 335990, Frantic).

A QUALITATIVE APPROACH TO IDENTIFY RISK FACTORS FOR REDUCED FISH WELFARE AND MORTALITIES IN NORWEGIAN ATLANTIC SALMON SMOLTS

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High mortality and reduced fish welfare has a major influence on the reputation of Norwegian Atlantic salmon farming, and it represents economic losses for producers. To make a shift towards a more sustainable production, welfare need to improve and the mortalities must be reduced. Studies have found high mortalities after sea transfer of salmon smolts, and a connection between such mortality and which freshwater farm the smolt group comes from has been suggested. This indicates that the conditions experienced in the freshwater farm will affect the performance and survival after transfer to seawater. The aim of this study was to identify qualitative and quantitative risk factors in the freshwater farms for mortality after sea transfer for Atlantic salmon smolts, and describe how management and organisational factors affect this outcome.

A qualitative approach using individual interviews was applied to explore the aims. Participants for the interviews were recruited from fish farming and fish health companies. A total of 15 in-depth interviews was conducted online using Teams, 10 with operations managers at freshwater farms, and five with fish health personnel who had experience working with fish health in freshwater farms for Atlantic salmon. The participants represent nine different companies, with three companies providing fish health services, and six companies producing Atlantic salmon. A semi structured interview guide was used, with the following topics: Factors influencing smolt mortalities, factors influencing fish welfare and husbandry practices. The transcribed interviews were analysed by Systematic Text Condensation, a systematic thematic cross-case analysis strategy.

The results showed that the risk factors for mortalities after sea transfer in Atlantic salmon are a combination of non-infectious factors and infectious diseases that are mediated by management practices and organisational factors in the freshwater farms. In addition, risk factors related to the transfer itself were identified. The participants reported that even if the infectious diseases are brought from the freshwater farm and cause mortalities after sea transfer, non-infectious diseases and factors cause the majority of the smolt-related mortality after sea transfer. These risk factors relates to production and management practices including smoltification strategy, large diversity in smolt size within groups, production of large smolts, strategies for destruction and sorting, densities, handling, suboptimal feeding, and water quality management. The intensity of the production was also highlighted by the participants. The organisational factors identified in the interviews included formal structures such as being part of a large company, culture and knowledge such as motivation and competence, interactions such as communication and production planning, and factors related to the physical conditions of the farm.

By using a qualitative approach to investigate potential risk factors for smolt mortalities after sea transfer, this study aimed to identify the perceptions and experiences of relevant stakeholders in the production of smolts. Risk factors identified in this study will be used in a complementary quantitative study.

COST-EFFECTIVE GENOMIC PREDICTION OF CRITICAL ECONOMIC TRAITS IN RUSSIAN STURGEONS *Acipenser gueldenstaedtii* THROUGH LOW-COVERAGE SEQUENCING

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Low-coverage whole-genome sequencing (LCS) offers a cost-effective alternative for sturgeon breeding, especially given the lack of SNP chips and the high costs associated with whole-genome sequencing. In this study, the efficiency of LCS for genotype imputation and genomic prediction was assessed in 643 sequenced Russian sturgeons (~13.68×). The results showed that using BaseVar+STITCH at a sequencing depth of 2× with a sample size larger than 300 resulted in the highest genotyping accuracy. In addition, when the sequencing depth reached 0.5× and SNP density was reduced to 50K through linkage disequilibrium pruning, the prediction accuracy was comparable to that of high-depth sequencing. Furthermore, an incremental feature selection method has the potential to improve prediction accuracy. This study suggests that the combination of LCS and imputation can be a cost-effective strategy, contributing to the genetic improvement of economic traits and promoting genetic gains in aquaculture species.

In this study, SNP panels with different densities were generated by linkage disequilibrium pruning of sequencing data at different depths, and the genomic prediction performance under different densities of SNP was compared. The results showed that a density of 50K for SNP was sufficient to obtain accuracy similar to that of high-density SNP with less bias.

To leverage the advantages of sequencing data, we investigated the potential of incremental feature selection, which involved ranking the SNPs based on the strength of their association with the phenotype as determined by a GWAS, to improve the accuracy of genomic prediction. As shown in Fig. 1, the genomic prediction accuracy increased by 4.9%, 1.1% and 9.2% for caviar yield, caviar color and body weight, respectively.

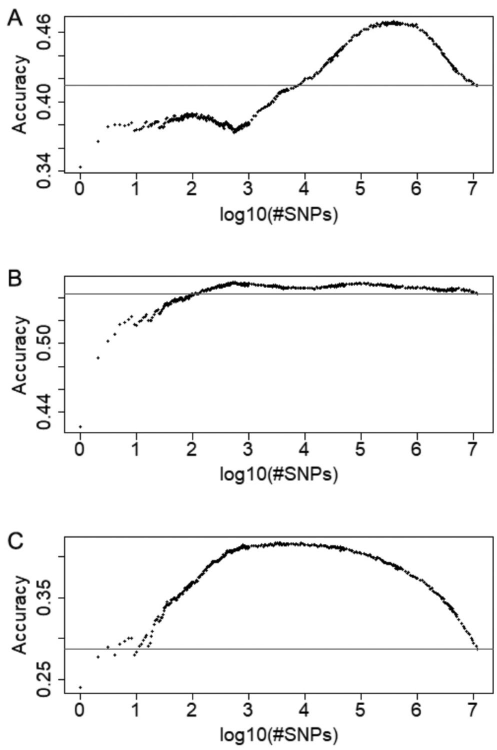

FIGURE 1 Accuracy of genomic prediction of (A) caviar yield, (B) caviar color and (C) body weight.

SEASONAL INFLUENCE AND PARASITE DENSITY IMPACT ON THE NUTRITIONAL AND ORGANOLEPTIC QUALITY OF ECONOMICALLY RELEVANT FISH SPECIES: EUROPEAN HAKE AND POUTING

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The increasing occurrence of parasites, namely Microsporidia and Anisakids, in economically relevant fish species such as Pouting (*Trisopterus luscus*) and European hake (*Merluccius merluccius*) raises concerns regarding the quality and safety of seafood products, presenting a significant public health risk. Therefore, this study aims to understand how Microsporidia and Anisakids, both bearing significant parasitic loads in these fish species, impact the quality and nutritional muscle traits across different seasons.

Samples were collected from European Hake from Southeast Ireland and Gulf of Biscay, and from Pouting captured in Portuguese waters. Pouting and European hake muscle samples from 30 individuals per season were analyzed for proximal composition (ongoing), color, texture, pH, and parasites. Microsporidia, the most prevalent parasite group in Pouting muscle, was categorized into four intensity levels: absence (0), low (1-20), medium (20-50), and high (>50). Anisakids, the most prevalent parasite group in European hake muscle, was categorized into three density levels: low (1-5), medium (5-10), and high (>10).

When looking at how organoleptic properties varied with seasonality, significative differences ($p < 0.05$) were observed in Pouting. Spring and Summer had higher muscle lightness (L*), redness (a*) and yellowness (b*) when compared with Autumn and Winter. In terms of textural properties, Spring showed the lowest Cohesiveness and Chewiness values.

The parasitization level also significantly ($p < 0.05$) impacted pouting muscle color. A decrease in muscle lightness (L*) was observed, particularly with higher microsporidia parasitic levels (>50), which could compromise consumers quality requirements. Overall, muscle samples collected during Spring and Summer exhibited higher quality compared to those collected during Autumn and Winter. Ongoing research includes parasite infection level determination, nutritional assessments, and analyses focusing on European hake.

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UNDERSTANDING THE OCCURRENCE, ALLERGENICITY, AND IMPACT OF ANISAKIS SPP. ON SEAFOOD SAFETY AND QUALITY: AN HOLISTIC AND INTEGRATED APPROACH

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Parasitic species are ubiquitous, existing in equilibrium with aquatic host organisms. Disruption of this delicate balance between host and parasite can lead to outbreaks, identified as a primary factor contributing to the growing occurrence of Anisakis spp. in commercially available wild fish. The European Food Safety Authority (EFSA) recognizes that the occurrence of Anisakis spp. in fishery products for human consumption poses a serious public health hazard, emphasizing the need for comprehensive studies on emerging seafood-related zoonotic diseases such as human anisakiosis. Despite the reported increase in Anisakis spp. in highly consumed fish species in recent years, the current knowledge is far from satisfactory. European Union's measures for minimizing the risk of sending contaminated products to the market can't guarantee safe products with zero risk of contamination. One of the reasons is that Anisakis allergens can persist in the muscle even after removal of dead larvae or exposure to heat/freezing as mitigation measure. This inefficiency in the parasite control methodologies highlights the need to develop more reliable techniques for ensuring safe products.

To tackle these challenges, this study aims to monitor the occurrence of Anisakis spp. in wild European hake (*Merluccius merluccius*) and Pouting (*Trisopterus luscus*) from NE Atlantic Ocean across various seasons, to understand its potential impact on fish nutritional and organoleptic quality, and safety, By adopting a holistic and integrated approach, we aim to unravel the complexities associated with emerging parasitic threats, contributing valuable insights to safeguard public health. The information generated by this study will allow policymakers, food industry stakeholders and consumers to make informed decisions and create safer products.

Acknowledgments:
This work was funded by Innovation Pact, Project No. C644915664-00000026, known as the "Blue Bioeconomy Pact" and by FCT in the framework of the Programme Portugal2020 to CIIMAR (UIDB/Multi/04423/2021 and UIDB/Multi/04423/2022), and the PhD fellow to Carla Sousa (2023.00570.BDANA).

SHELF-LIFE EXTENSION OF GILTHEAD SEABREAM (*Sparus aurata*) FILLETS BY COMBINED APPLICATION OF IN-PACKAGE COLD ATMOSPHERIC PLASMA AND ACTIVE PACKAGING

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Cold atmospheric plasma (CAP) is considered as an effective antimicrobial element which consists of several reactive chemical species obtained from electrical discharges in atmospheric gases. The generation of cold plasma inside sealed packages (in-package processing) allows a local reaction, while preventing any post-process contamination, and thus forming an improved preservation method of perishable fresh foods, such as fish. Polylactic acid (PLA) is a linear, aliphatic, thermoplastic and bioplastic polymer, obtained from renewable resources, which presents similar properties to several convensional synthetic polymers and is approved by The Food and Drug Administration (FDA) of the United States for direct contact with foods. Natural additives like plant essential oils (EOs) present antimicrobial and antioxidant properties and are categorized as GRAS, while rosemary (*Rosmarinus officinalis* L.) EO has been attributed to the higher antioxidant, antimicrobial, fungicidal, and anticancer activity. The aim of the study was the evaluation of the effect of in-package cold atmospheric plasma processing on the quality and shelf-life of gilthead seabream (*Sparus aurata*) fillets during refrigerated storage. PLA films containing 0 and 20% rosemary EO used for fish packaging combined with in-package CAP processing were evaluated for their preservative effect on fish fillets, based on microbial and physicochemical quality parameters.

Two types of PLA pellets (neat PLA and PLA with 20% rosemary EO) were used for the production of films to be used as packaging materials for the fish fillets. The film production was based on a solvent-casting method, using chloroform as a solvent, while the films were characterized in terms of their water vapor permeability, mechanical properties and optical properties. In-package CAP processing was applied on fresh gilthead seabream (*Sparus aurata*) fillets, which were pre-packed in PLA pouches (0 and 20% REO) and fish shelf-life was evaluated during isothermal storage at 2℃. Untreated fillets sealed in similar PLA pouches and stored at 2℃ were tested as Control samples. Quality evaluation of fish fillets was based on microbial enumeration (Total Aerobic Mesophilic, Total Aerobic Psychrotrophic and *Pseudomonas* spp.), pH measurement, color parameters determination, lipid oxidation and total volatile basic nitrogen (TVBN). CAP treatment resulted in lower populations of all the microbial counts in fish fillets, compared to the Control fillets, during refrigerated storage, while rosemary EO active packaging limited the lipid oxidation induced by the CAP treatment.

ULVA: TOMORROW'S "WHEAT OF THE SEA", A MODEL FOR AN INNOVATIVE MARICULTURE

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The global struggle to secure enough food for the growing human population has met a rising interest in developing coastal areas.

Marine seaweeds are a promising alternative for supplying novel food and valuable bioproducts for human and animal consumption.

Due to the unique characteristics of *Ulva* species, we have identified these green algae as the most suitable organisms for an innovative kind of European mariculture.

Species of *Ulva* have been extensively analysed for their value as food, feed, food ingredients, and chemical components.

In mariculture, *Ulva* can be cultured in land- and sea-based facilities. An *Ulva* farm can produce a yield several times higher than terrestrial plants.

As an excellent ecological biofilter, *Ulva* also provides ecosystem services, thus reducing environmental impact and enhancing the sustainability of the growing land- and sea-based fish farming industry.

The European Cooperation in Science and Technology (COST) operates an EU-funded instrument that enables researchers and innovators to create a collaborative research network across a wide range of scientific disciplines.

The main goal of our "SeaWheat" COST Action project is to create a comprehensive step change in the knowledge of the entire *Ulva* genus.

The "SeaWheat" project consists of 210 experts and specialists from 36 countries.
The "SeaWheat" project combines interdisciplinary approaches to sustainable marine resource use, covering all facets involving *Ulva* spp.: biology, ecology, aquaculture, engineering, economics and social sciences.

"SeaWheat" will bridge the scientific, regulatory, and social practical gaps in *Ulva,* paving the way to commercial production in the blue-biotech industries.

This advanced knowledge promoted by SeaWheat Action will create businesses and job opportunities in maritime and coastal economies, resulting in a significantly positive impact on societal welfare.

Funded by the European Union

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SeaWheat
COST Action CA20106

SIDE STREAMS FROM THE GREEN SECTOR AS FEED INGREDIENT FOR GAMMARIDS (AMPHIPODA: GAMMARIDEA)

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To meet the growing demand for feed ingredients in aquaculture, there is a need to explore alternative and sustainable feed sources. Feathers and grass are low value products from the green sector that are available in many European countries. Both raw materials, however, have poor a digestibility and cannot be directly digested by fish. Many lower trophic organisms on the other hand, such as many gammarid shrimp species that typically feed on dead organic material with poor nutritional value, are known to thrive on low digestible feeds. Gammarids also have suitable protein and fatty acid profiles for fish feed and comprise a natural part of the diet of a wide selection of fish species in nature.

To investigate the feasibility of turning side streams from the green sector (grass pulp and feathers) into raw materials for aquafeeds by secondary bioproduction of low trophic organisms, multiple feeding experiments have been conducted in the project BlueGreenFeed. In one small-scale feeding experiment, juvenile gammarids were fed with four different feeds (feathers, grass pulp, a more digestible mixture of feathers and grass pulp, and sugar kelp as a positive control) for 22 days. For each treatment, the experiment was run in triplicates, with 30 individuals per beaker. Data on growth and survival was recorded, and the chemical composition of both the gammarids and the feeds will be analysed.

Initial results from the feeding experiment showed no significant differences in survival rate between treatments. In terms of the specific growth rate (% SGR), the gammarids fed with kelp and the more digestible mixture of grass pulp and feathers showed a significantly higher growth rate than those fed exclusively with feathers. These results suggest that there is a higher potential for gammarids to grow on and bio-convert pre-processed feathers and grass with both increased digestibility and bioavailability, compared to the same feed stock when untreated. Data on the effect of the feed on the biochemical composition of the gammarids is in progress and will be presented at the conference.

a

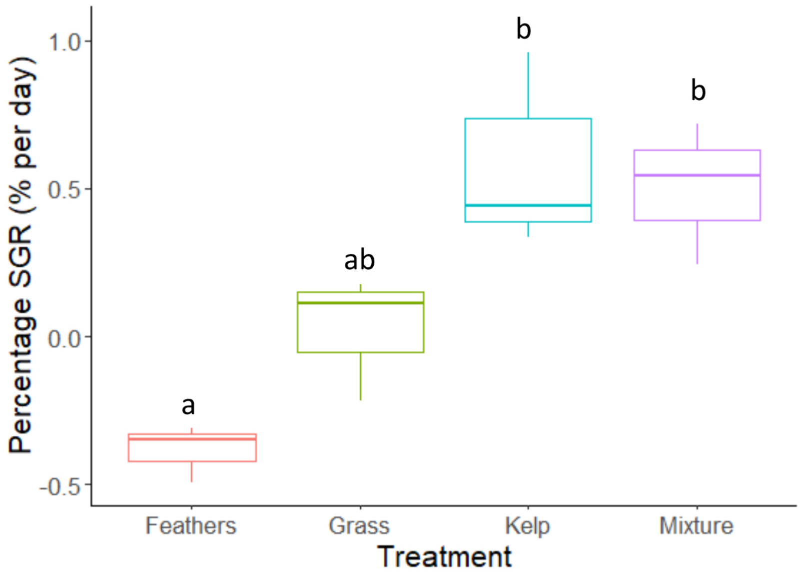

Figure 1: The specific growth rate for the total biomass for gammarids fed on feathers, grass pulp, kelp and a more digestible mixture of feathers and grass. Different letters indicate significant differences between the treatments.

SIZE MATTERS FOR *Octopus tetricus* PARALARVAE

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Octopuses have been intensively researched for more than 50 years because of their excellent potential for aquaculture due to their high demand for human consumption. Different octopus species produce either large embryos that hatch directly into benthic juveniles or small embryos that hatch into a planktonic stage called paralarvae before transitioning through a settlement phase and becoming fully benthic. High mortalities are commonly experienced in cultured paralarvae due to the absence of suitable diets. The few successful attempts at rearing paralarvae have used zoeae larvae of various crab species as the sole or as a complimentary diet, however, zoeae cultures are labour intensive and costly. Initial feeding behaviour studies with *Octopus tetricus* paralarvae suggest that they rapidly accept artificial pellets, with the proportion of paralarvae capturing artificial pellets being equal to those capturing live prey.

Despite the apparent potential for artificial pellets to provide a viable alternative to provisioning live feed for the culture of paralarvae, minimal research in this area has been reported. The aim of this study was to evaluate whether artificial pellet size and shape affects the capture success of *O. tetricus* paralarvae. For this, the capture success of six different artificial pellets was tested in a crossed experiment for size (i.e., small, medium, or large) and shape (i.e., spherical, or rectangular). A total of 250 paralarvae aged 2 days post-hatch were individually observed for up to 5 min with size, shape and handling time of captured particles being recorded.

A total of 42 % (105/250) paralarvae captured one of the artificial pellets. The number of paralarvae capturing artificial pellets was not affected by the shape of the pellets, but there was a strong preference for smaller artificial pellets with 57 out of 105 paralarvae that captured any size of pellet selecting the small pellets (Fig. 1). The handling times ranged from 1 to 485 sec across all shapes and sizes of artificial pellets. The mean handling time was affected by the artificial pellet shape with shorter mean handling times of 13 ± 41 sec for rectangular pellets compared to 31 ± 97 sec for spherical pellets, regardless of their size.

Developing artificial pellets has the potential to greatly improve effectiveness and reduce associated costs of octopus paralarvae cultures, however, fundamental knowledge on their feeding behaviour is needed. The results from this study suggest that smaller pellets can lead to more captures and that size can affect the handling times. The next step will be to increase the palatability of the artificial pellets to increase handling times and thus consumption.

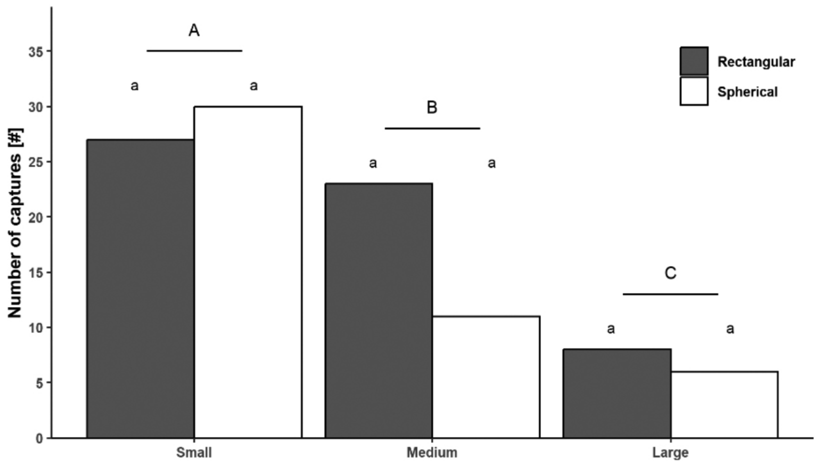

Fig. 1. Total number of captures by 2 dph old *O. tetricus* paralarvae for each of the six artificial pellets consisting of three sizes, each in two shapes (n = 105). Different upper-case letters indicate significant differences amongst different sized pellets regardless of their shape ($P < 0.05$). Lower case letters indicate that there were no significant differences amongst different shapes within the same size ($P > 0.05$).

ENHANCING THE POTENTIAL OF *Calanus finmarchicus* AS RAW MATERIAL FOR SUSTAINABLE AQUACULTURE FEED INGREDIENTS

Alternative ingredients & raw material processing

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One of the challenges the aquaculture sector faces is access to sustainable, nutritional and cost-effective feed ingredients. The zooplankton *Calanus finmarchicus* (*C.finmarchicus*), widely available in subarctic waters of the North Atlantic, has a beneficial chemical composition for use in fish feed. Only recently a commercial quota on *C. finmarchicus* has been set, opening possibilities to harvest and utilize this nearly untapped resource, with an estimated new production per year of 290 million tonnes in the Norwegian Sea alone.

Calanus is a natural feed for fish– and it has a composition that makes it relevant both for feed and human consumption. *C. finmarchicus* biomass consists of astaxanthin rich lipids – with high n-3 PUFA content, proteins – with a well balanced amino acids composition, and free amino acids - that are known to induce strong feeding responses. Currently oil is sold as high value marine oil for human consumption, while market for the protein fraction is at an early stage commercially.

In the CalaFeed project focus has been on increased knowledge to enhance the potential of using *C. finmarchicus* as a feed ingredient for aquaculture. Researchers have studied how to treat the catch after harvesting to keep the nutrients up to finished products. In addition, different protein ingredients (fish protein hydrolysate (FPH) and fish protein concentrate (FPC) are tested in feeding trials with salmon.

The high innate enzymatic activity is a challenge for preserving lipids and proteins. Today, *C.finmarchicus*, is frozen on board, but freezing is an energy demanding process and not so relevant for feed ingredients- nor for preserving large biomasses on-board. In addition, even during frozen storage enzymatic degradation of lipids takes place. Ensiling technologies with the reduction of pH is a commonly used preservation method for feed raw materials. Both enzymatic degradation and oxidation may be influenced by storage pH. In the current study – the effect of different pH´s were investigated- to evaluate how pH can be used to control the unwanted biochemical changes during the silage process – and if ensilage can be an energy -efficient alternative to freezing the biomass on-board. Calanus has also high content of small molecules, such as free amino acids, that may act as attractants to fish. The current study focuses on finding suitable on-board handling – and processing methods for producing nutritional ingredients from *C.finmarchicus*. The results from feeding trials show that both Calanus FPH and FPC stimulated feed intake, growth, and health of salmon in the first challenging period after seawater transfer. To our knowledge, this is the first study that evaluates if ensiling technologies can be a relevant method for preserving *C.finmarchicus* biomass for feed purposes.

THE IMPACT ON HEALTH USING THE CORRECT NUCLEOTIDES

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The long-held idea that free nucleotides (NT) are non-essential nutrients for aquatic animals and dietary supplementation is not necessary is beginning to change. Most scientists working in this field would say instead that NT are conditionally essential nutrients. First, we know that an animal's capacity to endogenously synthesize Nucleosides (NS) and NT might not be able to meet an increased metabolic demand, such as might occur when an animal is rapidly producing energy, requiring large amounts of AMP and ATP as energy intermediates. Secondly, rapid cell division and tissue synthesis during early life, or perhaps during an urgent immune response, significantly increases anabolic requirements for NS and NT in their roles as building blocks of DNA, and as components of RNA, NT mediate the rate of DNA transcription and protein synthesis. While animals do have an NT recovery and recycling pathway which takes about three months to become active, the lifespan of many farmed aquatic animals to harvest is short, and they do not benefit much from recycled NT.
Boosting growth rates of rapidly growing juveniles by enhancing gene expression and cell proliferation

Increasing enzyme secretion in the gut.
- Improving an animal's primary stress response.
- Supporting robust innate and adaptive immune responses.
- Improving reproductive performance of both males and females.
- Enhancing an animal's response to vaccination, by making an antibody response stronger and the duration of improved immunity longer.

Another benefit of dietary NT is that the external sensory organs of all aquatic animals are sensitized to the presence of dissolved NT in water as an indicator of nearby food .

Certainly, there are numerous whole yeast products containing significant amounts of NT available for use in feed formulations but feeding yeast to carnivorous and omnivorous fish lacking enzymes capable of digesting the complex polysaccharide cell wall consisting of mannans and glucans often limits the benefit. To ensure that the quantities of NS and NT are adequate to meet increased conditional demand, supplementation of feeds with purified or semi-purified nucleotide supplements is recommended.

The number of published papers focusing specifically on feeding semi-purified or purified NT to aquatic animals is currently quite limited. The research reports that will be summarized herein are essentially the entirety of what is currently available. Nevertheless, these reports show there are many benefits provided by supplementing feeds with NT feed additives. Benefits to growth, improved stress resistance and gut health, increased immunity and disease resistance have been shown for a few different species of fish and crustaceans important for aquaculture.

APPLICATION OF SURROGATE BROODSTOCK TECHNOLOGY IN EUROPEAN PERCIDS

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The technique of forming and development of fish gametes of one species in a fish individual of another species, is known as surrogate broodstock technology (Yoshizaki et al. 2011; Marinovi□ et al. 2021). This protocol considers the transplantation of primordial germ cells or germline stem cells from a donor into a recipient of a different strain or species. Through the application of this technology, from GSCs of one male individual, we can obtain several (or many) recipients of both sexes who are able to produce donor-derived gametes. Development of this technique could provide benefits such as accelerated production of endangered species in one generation; rapid spreading of rare desirable features; overcoming reproduction problems in difficult-to-spawn species and, combined with the cryopreservation of donor cells, enabling biodiversity preservation on a completely new level.

Protocols for spermatogonia transplantation have already been described for several fish families. This research aimed to adapt a protocol for interspecific transplantation of spermatogonia developed for other species to percid species. Specifically, we transplanted SSCs from juvenile Volga pikeperch (*Sander volgensis*) to pikeperch (*Sander lucioperca*) surrogates. To prevent the formation recipient's endogenous gametes, triploidization of recipients was carried out with the application of hydrostatic pressure following the protocol of Káldy et al. (2021). A 10-min pressure of 8000 PSA was applied to fertilized eggs from the 5th-minute post-fertilization at 14 °C. Donor gonads were dissected and dissociated through an enzymatic dissociation procedure. The obtained cell suspensions were stained with a PKH-26 fluorescent linked dye that would enable tracking of cells after transplantation. Approximately 2500 PKH-labelled cells were transplanted into the abdominal cavity of recipient larvae at days 9 and 16 post-hatch. Injected larvae, together with controls that received no manipulation were further kept in the RAS system, following the larviculture protocol of Ljubobratovi□ (2019). Using fluorescent microscopy, the transplantation success was evaluated 50 days after the treatment. Autofluorescence of recipient specimens which has been occasionally noticed in other species is excluded. Fluorescence coming from the applied stain was detected in dissected fish of both groups, however, it was outside of the expected place in the genital ridge and was originating from the peritoneum. The mortality rate in control exceeded both treatment groups. The main outcomes of this trial can be specified as follows: 1. Triploid pikeperch larvae can survive total anesthesia and microinjection even before the swim bladder inflation. 2. Designed protocol of dissociation and staining of cells from juvenile male gonad of Volga pikeperch can be considered effective. 3. The current attempt of transplantation cannot be defined as successful due to the lack of incorporation of cells into the genital ridge.

Káldy, J., Patakiné Várkonyi, E., Fazekas, G. L., Nagy, Z., Sándor, Z. J., Bogár, K., ... & Ljubobratović, U. (2021). Effects of hydrostatic pressure treatment of newly fertilized eggs on the ploidy level and karyotype of pikeperch Sander lucioperca (Linnaeus, 1758). Life, 11(12), 1296. DOI: https://10.3390/life11121296

Ljubobratović, U., Péter, G., Alvestad, R., Horváth, Z., & Rónyai, A. (2019). Alcalase enzyme treatment affects egg incubation and larval quality in pikeperch (Sander lucioperca). Aquaculture International, 27, 917-929. DOI: https://10.1007/s10499-018-0323-6

Marinović, Z., Lujić, J., Li, Q., Iwasaki, Y., Urbányi, B., Yoshizaki, G., & Horváth, Á. (2021). Cryopreservation and transplantation of spermatogonial stem cells. Germline Development in the Zebrafish: Methods and Protocols, 37-47. DOI: https://10.1007/978-1-0716-0970-5_4

Yoshizaki, G., Fujinuma, K., Iwasaki, Y., Okutsu, T., Shikina, S., Yazawa, R., & Takeuchi, Y. (2011). Spermatogonial transplantation in fish: a novel method for the preservation of genetic resources. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 6(1), 55-61. DOI: https://10.1016/j.cbd.2010.05.003

THE RANK OF INTENSIFICATION FACTORS STRENGTH IN INTENSIVE POND PRODUCTION OF COMMON CARP (*Cyprinus carpio L.*)

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Intensive common carp (*Cyprinus carpio L.*) pond production offers an innovative alternative to the traditional extensive and semi-intensive farming methods typically applied in Europe. With the advent of global warming, water resources are becoming increasingly scarce, necessitating enhanced productivity per unit of water used in aquaculture systems. To address these challenges, a trial was conducted focussing on the intensive rearing of two-summer-old Common carp (mean individual weight 0.5 ± 0.1 kg).

The study utilized a fractional factorial design (2^{4-1}, resolution IV) to explore the impacts of four distinct factors on production performance. The factors considered were: (1) Feeding Rate; compared *ad libitum* feeding (4% of body weight) against restricted feeding (2% of body weight). (2) Stocking Density; assessed low density (1.1 t/ha) versus high density (2.2 t/ha). (3) Aeration Rate; examined low aeration (11 kW/ha) versus high aeration (25 kW/ha). (4) Co-culture with Pikeperch; evaluated the effects of monoculture against polyculture involving pikeperch (*Sander lucioperca* L.) reared in cages.

The results showed that feeding rate was the most significant factor influencing the growth, meat quality, water quality, and stress parameters of the common carp. Higher stocking densities, while potentially economically advantageous in the short term, posed risks to fish welfare in the long run. Increased artificial aeration negatively affected meat and water quality; however, its cooling effect during extreme temperatures could be beneficial in acute instances. The trial also demonstrated that cage production of pikeperch is viable even within an intensive pond production system.

Based on the findings, several recommendations can be made for the feasible intensification of common carp pond aquaculture. Feeding regimes should prioritize efficient feed utilization rather than merely maximizing growth. Stocking densities should not exceed the welfare limits of the fish, with a gross yield maintained below 11 t/ha. Optimized paddle wheel aeration should be employed to favor algae development, preferably at rates below 11 kW/ha. Additionally, incorporating species and technology diversification, such as the inclusion of pikeperch in polyculture systems, can enhance overall productivity and economic sustainability.

Acknowledgment
This trial conduction was financially helped by the European Union's Horizon 2020 research and innovation program, grant agreement No. 652831 (AQUAEXCEL2020), and European Regional Development Fund, and the Hungarian Government, the project GINOP-2.3.2-15-2016-00025.

CAN ENVIRONMENTAL ENRICHMENT BOOST LUMPFISH (*Cyclopterus lumpus*) WELL-BEING AND LICE REMOVAL WHEN USED AS CLEANER FISH IN SALMON (*Salmo salar*) AQUACULTURE?

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The integration of cleaner fish, such as the lumpfish (*Cyclopterus lumpus*), in salmon farming has become increasingly vital for controlling infestations of crustacean ectoparasites (*Lepeophtheirus salmonis* and *Caligus elongatus*). Cleaning behaviour is a mix of inherited traits and life experience. Previous laboratory scale experiments on habituation of lumpfish have highlighted the potential of environmental enrichments (EE) to reduce stress during first salmon encounter and potentially promote lice consumption after arrival at commercial salmon farms. Here, we report findings on the effect of EE on lice consumption based on four commercial scale studies conducted from 2020 to 2022. Juvenile lumpfish were reared in tanks and exposed to life-like salmon models for 7 days at the production facility prior to stocking in salmon cages. Visual predatory cues are considered a strong effector on inherited stress responses, and we hypothesized that lumpfish preconditioned to these cues became habituated prior to deployment, and upon first interspecific interaction initiated cleaning behaviour earlier compared to lumpfish without preconditioning. Two deployments were arranged as randomized duplicates with EE lumpfish and control lumpfish in separate net pens, while the other two were arranged as mixed "common garden" groups, with control and enriched lumpfish in the same net pens. Stomach contents from lumpfish (523 in total) were collected on day 2 and day 7 after deployment at sea with some variation in sample size (n = 8 to 20). Using a GLM model, data on the number of lumpfish having consumed, or not consumed, lice revealed a significant increase in lice consumption on day 2 with EE lumpfish. This difference was not observed on day 7. EE also had an effect on neurotransmitter levels in the telencephalon, where serotonin levels were significantly lower, presumably as a result of interacting with the EE models. Plasma cortisol levels below the detection limit, suggested that the models did not initiate a strong HPI-axis stress response. We conclude that EE improves cleaning efficacy of salmon after deployment and has the potential to impact brain regulation associated with stress and/or social interaction. In conclusion, this research demonstrates effects at commercial scale of easily implementable EE protocols, and highlights the importance of EE as a tool for optimizing the efficacy of lumpfish in commercial salmon aquaculture.

EFFECT OF REPEATED STRESS ON E. SEABASS WELFARE MONITORED VIA ESTABLISHED AND NOVEL TECHNOLOGIES

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Monitoring fish welfare is of paramount importance in aquaculture from an ethical, legal, and economic perspective. The development of new technologies such as cameras, implant tags, and various types of loggers could allow efficient and timely monitoring in real time, ensuring the welfare of the farmed animals. In this study, we experimentally tested the effect of repeated stress on established welfare indicators while also including the application of a previously developed camera-based welfare monitoring technology. Specifically, we induced change in welfare as crowding-induced stress and then recorded and analyzed fish behavior. Physiological and growth markers as well as visual cues were used to determine the levels of stress and access shifts in welfare while the repetition of the stress protocol was done to assess the habituation of the fish.

A trial was performed in a Recirculating Aquaculture System where juvenile European seabass of approximately 250 g were subjected to a protocol of repeated stressing, induced via crowding, at intervals of 15 days over a two-month period. Over this period, growth performance was monitored (weigh measurements, FCR) along with visual inspection for external abnormalities (ulcers, scales, fins, and tail issues). At sampling points, blood was collected, and cortisol measurements were used to quantify stress levels. For the duration of the trial, the behavior of the fish was recorded via ip cameras and behavioral indicators such as the speed were analyzed via methodology previously developed[1].

There were no significant differences between the control and the stressed groups in terms of growth with both groups increasing in weight by 150 g by the end of the trial. Similarly, baseline cortisol levels did not differ between the groups, exhibiting low stress values within the typical seasonal range at approximately 200 mg/ml. However, there were slight differences in conversion efficiency, with the stressed group exhibiting higher FCR values overall. Moreover, the prevalence of external abnormalities differed between stressed and non-stressed groups, with the former exhibiting higher, and increasing over time, frequency of visual cues compared to the control group. Regarding behavior, preliminary results suggest that there are no significant differences in the speed caused from the stressor. The stressed group appeared to have a lower swimming speed than the control group, but this difference was apparent throughout the whole experimental period, even before any stressor was applied. After the stressor applied, the difference between the control and the stressed group increased slightly. This could possibly indicate a weak effect of the stressor on the behavior of the treated group. These results highlight the importance of incorporating multiple indicators in the overall welfare assessment of farmed fish.

Georgopoulou, D.G., Vouidaskis, C., Papandroulakis, N., 2024. Swimming behavior as a potential metric to detect satiation levels of European seabass in marine cages. Front. Mar. Sci. 11:1350385. doi: 10.3389/fmars.2024.1350385

DEVELOPMENT OF SMALLER DOSE VOLUME VACCINES FOR FARMED FISH

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Fish vaccines have been and are evolving parallel to the growing fish farming industries globally. In the 1990s a relatively high dose of 0.2 ml oil-based vaccine could result in severe adverse reactions in the abdominal cavity of injection vaccinated salmon. New vaccines were developed with half that dose, 0.1ml in the early 2000s, with an improved welfare and safety profile. This also included vaccines for other species than salmonids. Up to six bacterial and viral components needed to be added in these vaccines, requiring more research and technology development in order to ensure acceptable efficacy and safety. Based on further RnD, refinement of both downstream and formulation processes involved, PHARMAQ launched the micro dose injection concept in 2008; a 0.05 ml dose volume containing the same antigens. This was the first product of its kind, and the volume enabled vaccination of even smaller fish, while still maintaining efficacy and further improvement of safety. This concept was proven successful and has since been the foundation for most of the Pharmaq vaccines developed across species; Salmon, trout, Atlantic cod, cleaner fish, seabass and bream, tilapia and pangasius. Since 2013 mono- and di-valent vaccines have also been developed with the further reduced dose volume; 0.025ml. These volumes are injected alone or in combination with the micro dose vaccines while still maintaining as low injected volumes as possible. Safe dosing and administration are still fully possible with modern and calibrated injection systems, automated or manual. The development and experiences with the smaller dose volume vaccine concepts across species will be presented and discussed.

DIETARY METHANE-UTILIZED BACTERIA (*Methylococcus capsulatus*) PROTEIN AS A REPLACEMENT FOR FISHMEAL IN DIETS FOR SIBERIAN STURGEON (*Acipenser baerii*)

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Introduction

Global Acipenseridae production has doubled since 2010, now exceeding 100,000 tonnes annually (EUMOFA). A current trend to ensure the sustainability of the aquaculture sector is the increasing use of alternative ingredients for fishmeal in aquafeed (Cottrell et al., 2020). Despite their recent popularity as aquafeed alternatives, terrestrial plants face expansion challenges due to unfavorable nutritional components and the environmental impact of increased demand for arable land. Single cell proteins (SCPs) from high-protein microorganisms are promising as an aquaculture nutrient supplement because of their rich amino acid profile and high protein content. Methanotroph bacterial protein meal (MCP), produced from the fermentation of *Methylococcus capsulatus* grown on natural gas, contains approximately 90% dry matter, over 73% crude protein, and more than 5% crude lipids, making it comparable to or superior to fish meal. Therefore, this study was designed to assess the impact of partially replacing fishmeal with MCP in 25%, 50 and 75% on the performance and somatic indexes of Siberian sturgeon.

Materials and methods

Four isoproteic (52 %), isolipidic (10%), and isoenergic (18.4 Mj/kg) diets were formulated and extruded in a 3 mm diameter. All the diets were extruded by a commercial aquafeed manufacturer (Exot Hobby s.r.o, Černá v Pošumaví, Czech Republic) using a twin-screw extruder. Five hundred and forty Siberian sturgeon (23.15±0.35g) were randomly assigned in 300 L-tanks (45fish/tank) connected to a recirculating system (total volume 11,400 L) and fed four diets (three replicates per diet) for 70 days. The fish were hand-fed twice daily, at 9:00 and 15:00, with an excessive amount of feed. Any uneaten feed was removed twenty minutes after each feeding and dried to measure the feed intake.

Table 1. Production performance, feed efficiency and somatic indexes of Siberian sturgeon (*Acipenser baerii*) fed graded levels of methanotroph bacterial protein meal for 70 days.

Growth indices	Experimental diets				SEM	ANOVA	Regression	
	MCP0	*MCP25*	*MCP50*	*MCP75*		*p-value*	*p-value*	*ARS*
IBW (g)	22.88	23.33	23.3	23.1	0.1			
FBW (g)	98.69	94.83	96.14	98.02	1.68	0.883	0.966	0.099
DWG (g/fish/day)	1.08	1.02	1.04	1.07	0.02	0.847	0.936	0.099
SGR (%/day)	2.09	2	2.02	2.06	0.03	0.706	0.792	0.092
FI (g/fish/day)	0.74	0.72	0.73	0.74	0.004	0.259	0.69	0.081
FCR	0.82	0.85	0.93	0.97	0.02	0.084	0.007	0.48
VSI (%)	6.29	6.38	6.38	6.74	0.12	0.623	0.223	0.056
HSI (%)	1.63	1.71	1.72	1.95	0.05	0.08	0.016	0.399
GSI (%)	4.02	3.83	3.79	4.03	0.08	0.678	0.985	0.099

(Continued on next page)

Results

The inclusion of MCP did not have a significant effect (P < 0.05) on final body weight, daily weight gain, specific growth rate, feed intake, feed conversion ratio, visceral somatic index, hepatic somatic index, or gastrointestinal somatic index (Table 1). Additionally, feeding sturgeons with dietary MCP did not impact survival rates after the 70-day trial. However, a positive correlation between MCP levels and hepatic somatic index was observed.

IBW: initial body weight, FBW: final body weight, DWG: daily weight gain, SGR: specific growth rate, FI: feed intake, FCR: feed conversion ratio, VSI: viseral somatic index, HSI: hepatic somatic index, GSI: gastrointestinal somatic index, SEM: standard error of means, Regression:between MCP and tested index, ARS: adjusted R-squared

Conclusion

Our research demonstrates that fishmeal can be effectively substituted with MCP at levels up to 75% without negative eefects on fish performance or feed utilization. It is crucial, however, to closely monitor the hepatic index, as it is positively correlated with increased MCP levels.

References

Cottrell, R.S., Blanchard, J.L., Halpern, B.S., Metian, M., Froehlich, H.E., 2020. Global adoption of novel aquaculture feeds could substantially reduce forage fish demand by 2030. Nature Food 1, 301–308.

EUMOFA (European Market Observatory for Fisheries and Aquaculture Products). The Caviar Market. Production, Trade and Consumption in and Outside the EU; EUMOFA (Europe, Directorate-General for Maritime Affairs and Fisheries): Brussels, Belgium, 2019.

APPROACHES FOR CARBON DIOXIDE DEGASSING UNIT AUTOMATION

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Introduction

The development of energy- efficient recirculating aquaculture systems (RAS) for Atlantic salmon is one of the main future goals of aquacultural engineering. RAS facilities are on its way to have more and more automated technologies. However, CO_2 degassing units normally are not regulated or automatised. They just running on full power even if they have strip out very little CO_2 from the production water. Energy prices increased a lot over the last years and an automation of CO_2 Degassing units could help to significantly reduce energy costs in fish production. Degassing performance can be measured through sensors and displayed on the operating computer of the control panel of the RAS unit. This could be used to regulate the pump, and blower performance to transport the high CO_2 air more economically (save energy) out of the production facility. With machine learning and reliable robust sensors an automated degassing unit could be developed and used to safe energy and reduce the environmental footprint of close containment systems.

Material and methods

Two single RAS units (1 m^3) in Sunndalsøra (Landing MiniRAS) were used (without fish, CO_2 was gassed artificially) with different water salinities (0 and 12 ppt), alkalinities (50, 200 mg/L $CaCO_3$), dissolved CO_2 concentration (5, 10, 20 mg/L dissolved CO_2), water flow rates through the degasser (hydraulic loading rates (HLR) 10, 20, 30 l/m^2s (Water flow rate equal 1000 (21.50 hz), 2000 (22.70 hz) and 3000 (24.30 hz) l/h) and 3 different degassing fan speeds were adjusted to compare the degassing performance (water and air phase) and estimated energy usage under these conditions, each.

Results

There was only a tendency that the degassing efficiency was lower (-10-20%) under the high alkalinity treatment (200 mg/L) in combination with lower water flow rates (1000 and 2000 L/h). At 3000 L/h the 12 ppt salinity and 200 mg/L alkalinity test showed the lowest CO_2 stripping efficiency. There was no significant difference measurable between the 2 salinities used. A combined approach of Gas : liquid ratio (Fan speed vs. water flow rate) combined with energy logger measurement gives really easy the most efficient CO_2 removal in g/h x kWh. 2000 and 3000 l/h remove significant more g CO_2 / kWh than 1000 l/h.

Discussion

The different water quality adjustments were done at one day for a specific CO_2 concentration. We tested quite a wide range of variables. We could not find differences in degassing efficiency under different salinities. It can be that the accuracy and pression of the used setup was not enough to verify what was needed to find the effect of salinity. We propose how to use a set of robust sensors that process the incoming data (machine learning) and regulates the Gas to liquid (Blower to Pump operation) ratio of the degassing set based on the energy consumption and target CO_2 concentration (Figure 1).

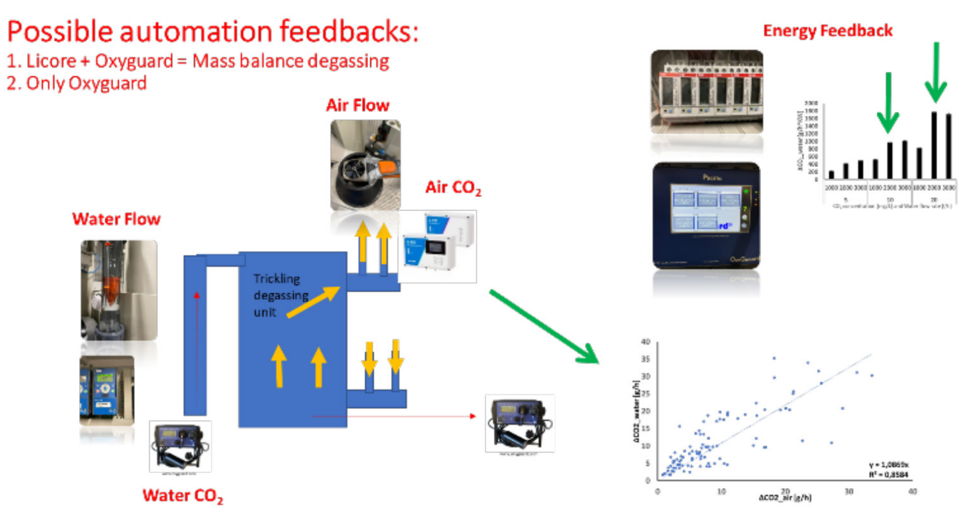

Figure 1: Proposal for automated mass balanced CO_2 degassing, containing submerged and air CO_2 sensors, energy loggers and air/water flow meters.

UNMASKING THE CULPRITS BEHIND NEW ZEALAND'S GREENSHELL™ MUSSEL *Perna canaliculus* FARM FEAST

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Mussel farming in known to enhance coastal habitats by attracting a higher diversity of fish by providing both shelter and feeding opportunities. However, when the feeding opportunities are the cultured mussels themselves, it results in substantial production inefficiencies. In the Firth of Thames region of New Zealand, mussel farmers report predation by fish contributes to mussel losses of up to 100%. However, the species responsible for these losses and the production stage at which they occur remain anecdotal. In this study, remote underwater video (RUV) was used over a 6-month period to observe fish activity at four different stages of production in mussel farms in the Firth of Thames, New Zealand. The RUV enabled the identification of the fish species inhabiting mussel farms, including the two most commonly observed species that were also found to be responsible for consuming cultured mussels, confirming the effectiveness of RUV methods for this research application. Four fish species were identified as frequent inhabitants of the mussel farms, with Australasian snapper (*Chrysophrys auratus*) and parore (*Girella tricuspidata*) the most abundant at three out of the four mussel farm sites investigated (Figure 1). Australasian snapper was the most common predator, taking 2880 bites of the mussel dropper lines in a single 11 min 47 sec video recording. Snapper formed feeding aggregations targeting dropper lines holding juvenile mussels (> 20 mm SL) in particular, indicating their potential to remove mussels from nursery farms in a short amount of time. Observations of the feeding behaviour of parore indicated their potential to contribute to the losses of mussels on recently seeded spat farms with these fish recorded tearing apart seeded lines, possibly targeting the macroalgae that is seeded out with the mussel spat. This knowledge can be used to begin to develop mitigation strategies aimed at reducing crop losses in mussel aquaculture.

Figure 1: Snapshots from RUV deployed at four different mussel farm sites in the Firth of Thames, New Zealand. Aggregations of Australasian snapper (top) and parore (bottom) feeding on dropper lines at nursery mussel farm sites.

NEW TECHNOLOGY, NEW WORKPLACES, NEW CONFLICTING OBJECTIVES FOR PERSONELL IN NORWEGIAN FISH FARMING

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Introduction and research gap
Norwegian fish farming is undergoing major concept changes, yet continues to be Norway's second most hazardous occupation. The industry is going from mostly open netpens along the coast, to also semi-closed and submerged units offshore and units on land. Along with the technology, the workplaces have transformed. It is need for knowledge about the operational conditions in the different workplaces in the current and emerging salmon farming concepts, and consequences for working conditions.

A qualitative study
The empirical foundation of this study is personnels' descriptions of operational conditions at their workplaces. The data is gathered through semi-structured research interviews of 14 persons representing different concepts in Norwegian fish farming, as well as three workshops with national and international industry representatives, researchers, and other stakeholders. This is an activity in the project "OHS in aquaculture – risk management in different production forms", financed by the Norwegian Seafood Research Fund (project number 901801).

Results
The study indicates differences between the well-established workplaces, and the workplaces that are newly established or under planning.

Operational conditions at the well-established workplaces, either in traditional or new concepts, have similarities with what is found in earlier research. Our empirical studies the last decades have revealed constraining operational conditions in Norwegian fish farming. Limited time and personnel is still common, giving stress, long hours, lack of rest, inadequate working positions. For larger operations, more organizations and vessels need to cooperate, which leads to complex collaboration, sometimes among personnel without experience or training. Conflicting objectives usually emerge, sometimes leading the personnel in the operations to prioritize production and fish welfare over their own protection. As in other industries, quality and safety management has led to more systematic measures. In addition, it has led to a discussion on whether management tasks may conflict with each other and with safety, fish welfare, etc.

At the newly established or planned workplaces in new fish farm concepts, the risks may be higher, but the operational planning is more thorough. On the one hand, these new production forms include larger units of fish, and operations that are unfamiliar for the personnel. Some workplaces have harsher weather conditions, and all involve personnel from more organizations, heavier equipment, and more energy involved in the operations. On the other hand, the larger concepts give stable working platforms, and the increased risks lead to more awareness on mitigating measures. The personnel report about well-functioning safety management measures, collaboration about risk assessments and resources to perform operations according to plan. Personnel here have rarely experienced goal conflicts, but these organizations have had few operations.

Further studies should investigate how the operational conditions are experienced when the new workplaces have become well-established, and the concepts are not seen as new anymore.

GOVERNMENTAL MISSION ON SUSTAINABLE FEED FOR FARMED FISH AND LIVESTOCK – POTENTIAL FOR NORWEGIAN LOW TROPHIC AQUACULTURE?

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The Norwegian government has launched missions under their long-term policy priorities where research-based knowledge and skills are regarded prerequisite for achieving the concrete goals. The mission on sustainable feed sets the objective of ensuring that all feed for farmed fish and livestock shall come from renewable sources, thus reducing greenhouse gas emissions from food systems. Today, less than 10% of the feed for fish farming is from Norwegian sources. The mission also suggests increasing security of supply by develop a stronger feed ingredients industry in Norway. The aquaculture industry conveys that the prospected future production growth depends on increase in novel feed raw materials. Various assessments of potential sources of feed raw material hold benthic filter feeders (bivalves, blue mussels, tunicates) as primary candidates, with their nutrient qualities, production potential and relatively low environmental footprint. We will address the potential for production, some prospects for industry development and the main challenges for realizing the scale of production needed for contributing as a feed ingredient.

MATERIAL, ENERGY, AND INFRASTRUCTURE REQUIREMENTS OF NEW PRODUCTION SYSTEMS FOR SALMON AQUACULTURE

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Introduction. Most of the production of grow-out salmon in Norway, takes place in traditional open net cages. However, with this production system, there has been several challenges, such as spread of sea lice and escaped farmed salmon, that have led to negative environmental impacts. To battle these challenges, the Norwegian government incentivised the development of new production systems, such as offshore and (semi-)closed systems. Several producers are also producing post-smolt or grow-out fish in land-based farms. These production systems play a key role in sustainable growth in the industry.

Traditional cages have a quite standardized design, with floating collars in plastic with nylon nets. It is estimated that around a third of the plastic used is recirculated. Even though there are many different technical solution and concepts within each production system, it is expected that the material and energy requirements will be larger. This may lead to a burden shift, with higher environmental impacts in other countries, linked to the production of materials, mainly plastics, steel, and concrete. It is important to investigate how the material and infrastructure demands changes, and the opportunities for circular economy for material use. Several of the new production systems have a higher energy demand than production in traditional cages, which may lead to a development of the national electricity grid. This research aims to map supply chains of novel production systems with a focus on types and amounts of materials, energy, and infrastructure requirements.

Methodological approach. This work is based on reviews of existing and planned production systems, peer reviewed articles on aquaculture production in (semi-)closed, land-based and offshore systems from different countries, and company reports and newspaper articles from Norwegian industry media.

Results and discussion. New production systems show promising potential in reducing sea lice levels and escapees, as well as utilizing area unsuitable for traditional aquaculture. Employing new production systems can change the aquaculture supply chains and will in most cases require more materials such as steel, plastic and concrete, and more energy per kg produced salmon than traditional systems do. This contributes to the overall sustainability of salmon and it’s important to investigate the implications of this shift to assess the overall environmental, social, and economic impacts of salmon production in novel production systems.

This research is part of the COMPAREIT project, and is funded by the Research Council of Norway (grant number # 319647)

EVALUATION OF GLYCEROL AS AN ALTERNATIVE CARBON SOURCE IN A LAND-BASED SALMON RECIRCULATING AQUACULTURE SYSTEM IN NORWAY

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Aim:
We evaluated glycerol as an alternative carbon source to methanol and acetic acid in a Denitrification. Objectives included assessing denitrification performance, incomplete denitrification, user-friendliness, substrate efficiency, costs, and health and safety.

Materials and Methods:
Research was conducted in a commercial salmon RAS in Norway. Biofilters were supplemented with glycerol vs. acetic acid. Performance metrics included total dissolved nitrogen (TDN) removal, pH stability, and chemical oxygen demand (COD) consumption. Economic analysis compared costs per kilogram of TDN removed. Health and safety were assessed across hazard categories. Total gas pressure residual (TGP res) was evaluated as an indicator of denitrification performance.

Results:
Glycerol demonstrated effective denitrification, with similar or higher TDN removal. Glycerol maintained stable pH, enhancing bioreactor stability and allowing higher dosing without compromising user-friendliness. Complete glycerol consumption indicated efficient utilization. Glycerol was 1.5 times more cost-effective than acetic acid and received the highest health and safety scores. TDN removal correlated well with TGP res.

Discussion and Conclusions:
Glycerol is a suitable and advantageous carbon source for denitrification in land-based salmon RAS. Benefits include stable pH levels, complete COD consumption, superior cost efficiency, and reliable performance indication through TGP res. Glycerol's high health and safety scores make it a preferred choice. Recommendations: 1. Use glycerol at concentrations below 80% to maintain low viscosity and a lower freezing point. 2. Monitor nitrite production and consider higher COD levels and extended hydraulic retention times if nitrite production persists. 3. Install sensors for detecting total dissolved gas pressure and dissolved oxygen levels. 4. Conduct periodic analysis of dissolved nitrogen species to refine performance calibration. This study highlights glycerol's potential as a sustainable and efficient carbon source, encouraging its broader adoption in aquaculture systems.

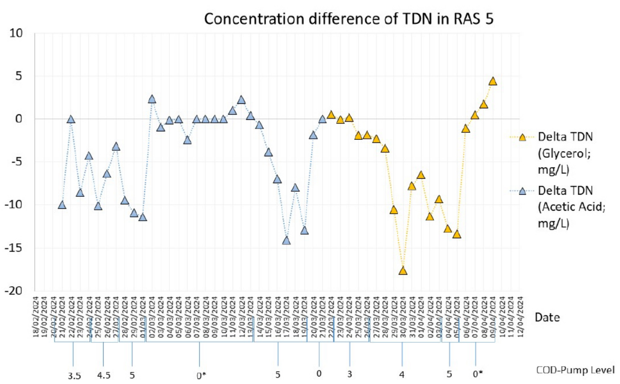

Figure 1: TDN-removal after denitrification, using Acetic Acid vs. Glycerol

ASSESSMENT AND EVALUATION OF CALCIUM HYDROXIDE AS AN ALTERNATIVE TO IRON CHLORIDE FOR PHOSPHATE REMOVAL IN A COMMERCIAL LAND-BASED SALMON RECIRCULATING AQUACULTURE SYSTEM

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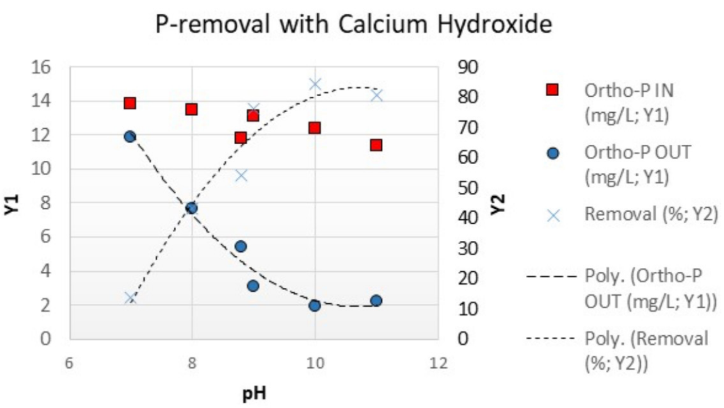
Aim:
This study evaluates the effectiveness of calcium hydroxide (Ca(OH)□) for phosphate removal in a Zero Water Concept Phosphate Removal System used in a commercial land-based salmon Recirculating Aquaculture Systems (RAS).

Materials and Methods:
A phosphate removal sub-system was integrated into the RAS, featuring tanks for pH regulation (Ca(OH)□), iron(III) chloride dosing, mixing, and sedimentation. Phosphate removal was tested by alternately using Ca(OH)□ and iron(III) chloride. Water samples were collected daily and analyzed for phosphate and iron concentrations using Hach Lange methods.

Results:
Calcium hydroxide removed over 80% of phosphate at a pH of 9-11, integrating well with existing pH regulation and providing economic benefits by supplying alkalinity for the nitrification process. Iron(III) chloride was less effective due to high system alkalinity and iron efflux.

Discussion and Conclusions:
Calcium hydroxide is highly effective for phosphate removal in RAS, offering practicality and cost-efficiency. The predominance of calcium phosphates in sludge enhances reuse potential in agriculture or industry. Regular sludge removal is crucial for maintaining system efficiency, i.e., pH-balance. Calcium hydroxide is recommended for phosphate removal due to its efficiency, safety, and economic advantages. Effective sludge management is essential to ensure optimal operation and support sustainable reuse.



THE IMPORTANCE OF QUALITY IN NORWEGIAN SALMON EXPORTS

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A large and growing literature on international trade focuses on differences in product quality and its implications for trade dynamics. However, this is an issue that has received limited attention in relation to the seafood trade despite the large quantities of seafood being traded and the significant heterogeneity in qualities. Using Norwegian firm-level customs data this paper investigates the heterogeneous effects of varying quality on the export of different product forms of salmon. It is widely accepted that increased product quality increases trade values, but a major challenge has been to estimate quality at the product level and quantify the effect on trade values. We estimate market specific elasticities of substitution, and follows the empirical strategy suggested by Khandelwal (2010) to extract a measure of quality from the residual export variation. Our results suggest a large and significant effect from quality on export value of both fresh whole salmon and fresh fillets of salmon. We control for traditional gravity variables and find that the negative distance effect on export value is reduced as quality increases. Our results adds to a growing literature exploring the micro fundaments of the aquaculture supply chain which are particularly important in obtaining insights with respect to the pricing strategies of different seafood exporters in different markets.

EFFECTS OF INSECTICIDES USED IN CONVENTIONAL AND ORGANIC FARMING ON THE NATURAL FOOD BASE OF FISH

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Introduction

Pest management is a pivotal aspect of agriculture, conventional and also organic, and in the contemporary context, the utilization of pesticides assumes a significant role in securing a sustainable global food provision. The application of these agents serves to diminish agricultural losses while concurrently enhancing both the accessibility and quality of food resources (Hedlund et al., 2019; Umetsu and Shirai, 2020; Tudi et al., 2021). Agriculture stands as an indispensable facet of human existence. Presently, the advocacy for the advancement of organic production holds a prominent position in ongoing discussions. Sustainable food methodologies form an integral component of the European Green Deal initiative. The European Commission has established a goal whereby, by the year 2030, no less than 25% of agricultural acreage within the European Union (EU) is targeted to be devoted to organic farming practices (European Commission, 2021). Among the primary objectives of organic farming lie the preservation of biodiversity, the safeguarding of regional ecological equilibrium, and the maintenance of water quality (European Commission, 2023).

The primary objective of this study was to elucidate and to compare the potential toxicity of acetamiprid, flupyradifurone, both conventional insecticides and spinosad, which is approved for organic farming, and also potential toxicity of insecticides formulated with mentioned substances, toward aquatic organisms. We were particularly interested in investigating the impact on invertebrate species that hold significance as natural prey for fish, such as Daphniidae, Naididae, and Chironomidae larvae. The aforementioned organisms belonging to the macrozoobenthos form the food base of fish, especially in European carp (*Cyprinus carpio*) ponds (Kajgrova et al., 2021).

Material and methods

The flupyradifurone-based insecticide Sivanto Prime (registered by Bayer S.A.S), which contains 17.1% flupyradifurone, and acetamiprid-based insecticide Mospilan 20 SP (registered by Nisso Chemical Europe GmbH), which contains 20% acetamiprid, and spinosad-based insecticide Spintor (registered by Dow AgroSciences s.r.o.), which contains 22.75% spinosad, were chosen to assess the potential effects on *Daphnia magna*, *Tubifex tubifex* and *Chironomus riparius*. Toxicity tests were carried out according to OECD Methodology No 202 (*Daphnia magna*) and No 235 (*Chironomus riparius*). The test for *Tubifex tubifex* was modelled based on the methodology described by Maestre et al. (2009).

Results

Based on the toxicity tests performed, the 48hEC50 for Daphnia magna 140 mg/l, 489 mg/l and 0,00406 mg/l were calculated for Mospilan 20 SP, Sivanto Prime and Spintor, respectively. For Chironomus riparius, 48LC50 values were calculated as follows: 0,0879 mg/l, 0,107 mg/l and 0,0526 mg/l. For Tubifex tubifex, 96hLC50 values of 0,033 mg/l, 2 mg/l and >4 mg/l were calculated.

Conclusion

Citlivost jednotlivých bezobratlých necílových organismů vůči insekticidům se výrazně liší. Z výše uvedených výsledků se dá usuzovat, že organismy, které tvoří potravní základnu ryb, jako například Daphnia magna, Chironomus riparius či Tubifex tubifex jsou velmi citlivé nejen vůči konvenčním pesticidům, ale v případě Daphnia m. a Chironomus r. take vůči pesticidům, které jsou schváleny po použití v organickém zemědělství. Schvalování nových pesticidních přípravků a jejich aplikace vyžaduje velmi citlivý a rozumný přístup tak, aby v co nějnižší míře ovlivňovaly vodní ekosystémy. Studie ukazuje, že přípravky schválené pro použití v organickém zemědělství mohou být pro některé organismy mnohem toxičtější a ovlivňovat je vice, než konvenční pesticidy. Při posuzování použití pesticidních látek je nutné zvažovat nejen účinky čisté účinné látky, ale take pesticidního přípravku jako takového.

(Continued on next page)

References

Hedlund et al., 2019; Umetsu and Shirai, 2020; Tudi et al., 2021, European Commission, 2021, European Commission, 2023, Kajgrova et al., 2021, OECD 202, OECD 235, Maeste et al. 2019

Hedlund, J., Longo, S., York, R. (2020). Agriculture, Pesticide Use, and Economic Development: A Global Examination (1990–2014). *Rural Sociology, 85*(2), 519–544.

Tudi, M., Ruan, H.D., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., Phung, D.T. (2021). Agriculture Development, Pesticide Application and Its Impact on the Environment. *International Journal of Environmental Research and Public Health, 18*(3), 1112.

Umetsu, N., Shirai, Y. (2020). Development of novel pesticides in the 21st century. *Journal of Pesticide Science, 45*(2), 54–74.

European Commission. (2021). *Communication from the Commission to The European Parliament, The Council, The European Economic and Social Committee and The Committee of the Regions Empty on an Action Plan for the Development of Organic Production* [online]. European Union. [visited on 29-09-2023]. Available on: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52021DC0141R%2801%29

European Commission. (2023). *Organics at a glance* [online]. European Union. [visited on 29-09-2023]. Available on: https://agriculture.ec.europa.eu/farming/organic-farming/organics-glance_en

OECD. (2004). *Test No. 202: Daphnia sp. Acute Immobilisation Test*. OECD Guidelines for the Testing of Chemicals, OECD Publishing.

OECD. (2011). *Test No. 231: Chironomus sp., Acute Immobilisation Test*. OECD Guidelines for the Testing of Chemicals, OECD Publishing.

Maestre, Z., Martinez-Madrid, M., & Rodriguez, P. (2009). Monitoring the sensitivity of the oligochaete Tubifex tubifex in laboratory cultures using three toxicants. *Ecotoxicology and Environmental Safety, 72*(8), 2083–2089.

Kajgrova, L., Adamek, Z., Regenda, J., Bauer, C., Stejskal, V., Pecha, O., Hlavac, D. (2021). Macrozoobenthos assemblage patterns in European carp (Cyprinus carpio) ponds – the importance of emersed macrophyte beds. *Knowl. Manag. Aquat. Ecosyst., 422(9).*

EFFECTS OF "GREEN WATER" TECHNIQUE, DIFFERENT STOCKING DENSITIES, FEEDING PROTOCOLS, AND PHOTO REGIME ON PIKEPERCH *Sander lucioperca* LARVAL REARING PERFORMANCES UNDER COMMERCIAL CONDITIONS

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In the cultivation of different marine fish larvae, several protocols have been developed to tackle existing bottlenecks. This resulted in a stable production for species such as sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). However, existing protocols are species-specific and for other fish can only be used to a limited extent, especially for freshwater fish species, such as pikeperch (*Sander lucioperca*). Intensive pikeperch larviculture presents a challenging task that requires fine-tuned rearing conditions to reach a reasonable outcome. Low survival rates can be caused by improper swimbladder inflation, deformities, and especially cannibalism. To improve the production efficiency in larval fish culture, it is crucial to consider multitude of biotic and abiotic factors and their interactions.

Beside the effect of the "greenwater" technique compared to standard clear water protocols, two different photo regimes (12/L:12/D vs 24/L:0/D) were examined in two identical recirculated systems. Simultaneously, we compared the influence of stocking density levels (50 larvae/L vs 150 larvae/L), while a fourth factor was a post-weaning feed, considering the reduction of production costs (Expensive vs Low price). The trial was conducted in a full design (model: 2^4; 16 combinations. During 47 days, multiple variables were followed: growth parameters - body weight (g) and length (mm) and their coefficients of variations, condition factor and specific growth rate. In addition, the occurrence of jaw and spine deformities, survival and swim bladder inflation rate were assessed. Main effects and interactions were analyzed with SPSS 29.0 (IBM Co., Armonk, NY, USA) in the General Linear Model, Univariate ANOVA. Afterwards, Principal Component Analysis and Pearson correlation (n-1) was applied, to compare the strength of factors. (XLSTAT, Long Island: New York, NY, USA, 2023).

Our findings revealed that a shorter photoperiod had significant ($p < 0.05$) positive effect on swim bladder inflation. Furthermore, a prolonged light period under greenwater conditions improved homogeneity in growth of each cohort, which was shown by lower coefficient of variation. In larvae reared under greenwater conditions, significant higher survival rate ($p < 0.05$) was detected. These results indicate that a supply of algae during the cultivation of pikeperch larvae could improve the rearing success. To reach a reliable production further investigations are required to determine the most efficient conditions with all crucial parameters e.g. a practical photo regime and compare the best combinations in different rearing systems.

FERMENTED PROTEIN AS A SUSTAINABLE, CLEAN AND SCALABLE PROTEIN SOURCE IN ANIMAL NUTRITION

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Mankind produces more than 1.1 billion tonnes of animal feed annually for the nutrition of livestock and pets. The feed industry is dependent on >75% of soy and >65% of the total fish harvested globally as protein to cater to this demand. With increasing population and growing demand for protein-rich food, sustainable and scalable solutions are needed to meet global needs.

String has enabled advanced biomanufacturing of high-quality fermented ingredients for the animal nutrition sector that directly address these gaps. These ingredients are manufactured using a sustainable manufacturing process that enables clean, traceable, safe and consistent output. Our products deliver significant benefits to the animal, feed manufacturer, farmer, and the end customer, ensuring all parts of the supply chain stand to gain by its use.

Our first product to market, PRODG® with >70% crude protein, is a high-quality protein ingredient that addresses the $70 billion protein feed ingredient market. Validation studies in specific aquatic and poultry species demonstrated excellent performance even at high levels of inclusion in feed. Use of PRODG has been validated in different aquatic animals such as the pacific white shrimp, *Litopenaeus vannamei*, rainbow trout, *Oncorhynchus mykiss*, yellowtail kingfish, *Seriola lalandi* and Barramundi, *Lates calcarifer*. Sustainable feeds formulated with PRO-DG result in healthy body weight gain of animals, good digestibility and optimal feed conversion ratio (FCR). In certain species, use of PRODG has demonstrated beneficial effects on the gut health of animals. A pilot study in canine diets demonstrated PRODG to be palatable with positive effect on the gut microbiome.

Our solutions also tackle the challenges of limited fishmeal availability and volatile pricing, fostering greater industry resilience. Its production protects aquatic ecosystems by reducing reliance on wild-caught fish, safeguarding biodiversity and promoting responsible resource management.

String's protein ingredients have been validated in multiple geographies, varied farm management practices and by leading global feed manufacturers. Our vision is to enable the transition of the feed industry to sustainable, clean and, traceable value chains by leveraging the power of biology.

UNRAVELING SPERMATOGENESIS IN SPOTTED WOLFFISH: INSIGHTS FROM JUVENILE MALE TESTES ULTRASTRUCTURE TO BROODSTOCK SPERM CRYOPRESERVATION

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This study aimed to deepen our understanding of male spotted wolffish (*Anarhichas minor*) reproductive biology using two distinct experimental designs involving juvenile and mature broodstock fish.

The first experiemental design involved a detailed histological examination of testes to identify the onset of gonadal maturation and characterize spermatogenic stages in two- and three-year-old juvenile specimens. Light microscopy analysis revealed clear differences between the age groups. Two-year-old fish displayed well-defined interstitial tissue, Sertoli cells, and cysts housing spermatogonia stem cells where meiosis had not yet been initiated. In contrast, three-year-old fish exhibited cysts containing spermatocytes, spermatids, and abundant spermatozoa, indicating the initiation of the spermatogenic cycle, albeit with asynchronous puberty. Immunostaining revealed a significant presence of smooth myoid cells in the interstitial tissue of sexually mature fish, while electron microscopy further revealed synaptonemal complexes with chromatin differentiation and the emergence of centriolar structures.

The second experimental design focused on optimizing semen freezing and cryopreservation procedures in mature broodstock individuals aged over 10 years. Seven freezing extenders (KT, TS-2, OP, MT, MH, HBSS, or SR), with seawater (SW) as a control, were assessed alongside two cryoprotectants dimethylsulfoxide (DMSO) or methanol to evaluate their impact on pre- and post-thaw semen quality.

Results highlighted MT and HBSS extenders as superior in total sperm kinetics at a 1:3 dilution, with DMSO showing optimal results in sperm motility and velocity variants. Moreover, the MT and HBSS groups demonstrated consistent sperm viability after cryopreservation with values similar to fresh samples. Based on viability results from the SYBR-green-14/PI assay comparing fresh and cryopreserved sperm using MT and HBSS, the MT extender emerged as the most effective freezing medium for spotted wolffish broodstock sperm cryopreservation.

In conclusion, this study provides a comprehensive understanding of male spotted wolffish reproductive dynamics, offering valuable insights for both scientific research and aquaculture management.

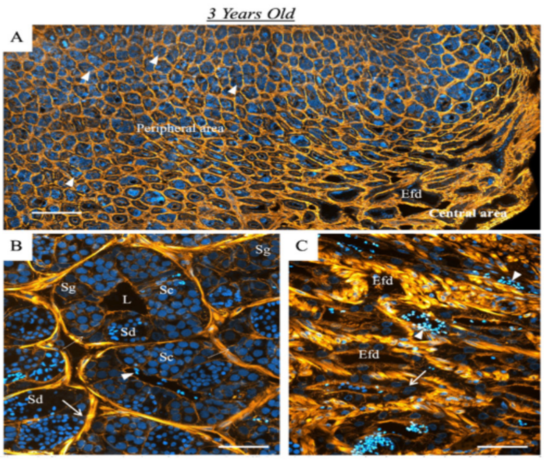

Figure 1. Deconvoluted confocal images, 3-year-old juvenile spotted wolffish testis containing spermatozoa (indicated by arrowheads) as the furthest developed germ cell type, present in large numbers in all tubules. (**A**) Lower magnification of a testis at middle spermatogenesis. (**B**) The peripheral area, showing the germinal compartment different cysts of spermatogonia (Sg), spermatocytes (Sc) and spermatids (Sd). Sertoli cells (arrows) are observed within the germinal compartment. (**C**) Peritubular myoid cells in the interstitial tissue. 30µm. Phalloidin (yellow) and DAPI (blue).

DYNAMIC PROFILING OF WOLFFISH: RNA-SEQ INSIGHTS INTO IMMUNOREPRODUCTION, FATTY ACIDS, LIPIDS, ANTIOXIDANT ENZYMES, AND HORMONAL VARIATIONS ACROSS AN ANNUAL CYCLE

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For the spotted wolffish (*Anarhichas minor*) the study of the general sexual regulatory mechanisms in the male broodstock is crucial for its overall biological understanding and the establishment of a sustainable culture industry. The aim of this study was to establish a comprehensive immune and reproductive physiological seasonal profile of spotted wolffish broodstock by examining, for the first time, the plasma fatty acids composition, sex steroids, antioxidant enzyme activity, and their correlation with cortisol and acetylcholinesterase levels over an annual cycle. Additionally, RNA-Seq technology was utilized to assemble a *de novo* transcriptome and characterize gene expression in the blood of male broodstock fish throughout the annual gamete production process. Blood samples were collected monthly from May 2021 to April 2022. Plasma fatty acids, sex steroids, cortisol, acetylcholinesterase, and antioxidant enzyme activities were analyzed. Total RNA was extracted from blood samples for transcriptome sequencing, and differential gene expression analysis was conducted using RNA-Seq. Our results identified three reproductive stages: resting (April to July), maturation (August to November), and spawning (December to March). Fatty acid composition showed significant seasonal variation, peaking during the resting phase. Testosterone and 11-ketotestosterone levels increased during maturation, while estradiol and maturation-inducing hormone (MIH) remained stable. Cortisol levels showed no significant seasonal variation, but acetylcholinesterase activity was higher during maturation. Oxidative stress markers, such as catalase and glutathione peroxidase, peaked during maturation. RNA-Seq analysis identified over 295,000 contigs, with 78,107 annotated, revealing significant gene downregulation during maturation. Principal component analysis demonstrated distinct clustering based on reproductive stages. Enrichment analyses highlighted the roles of lipid metabolism, oxidative stress management, and immune response in seasonal changes. Key differentially expressed genes involved in the physiological processes described included ELOVL fatty acid elongase 4 (*elov4*), and Apolipoprotein C-I (*apoc1*) for lipid metabolism; Zona pellucida glycoprotein 3 (*zp3*) and Estrogen-related receptor gamma (*esrrg*) for reproduction; 5'-Aminolevulinate synthase 2 (*alas2*) and Nuclear receptor corepressor 1 (*ncor1*) for oxidative enzymes; Prostaglandin E synthase 3 (*ptges3*), and FK506 binding protein 5 (*fkbp5*) for immunity, among others. These genes can serve as potential indicators of the immune reproductive stage and are susceptible to molecular manipulation through advanced gene editing techniques. This profiling provides insights into the physiological and molecular mechanisms of seasonal reproductive changes in male spotted wolffish, with implications for broodstock management and aquaculture practices.

THE EFFECT OF SINGLE AND MULTI-INDIGENOUS PROBIOTICS DIETS ON IMMUNITY, GENE EXPRESSION, HISTOPATHOLOGY, AND PROTECTION AGAINST *Vibrio Parahaemolyticus* Vp5

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The *Vibrio parahaemolyticus* Vp5 carrying pirA and pirB toxin genes cause hepatopancreatic necrosis disease (AHPND) in Pacific white shrimp culture, significantly decreasing shrimp production. The use of probiotic strain based on host-associated probiotic approach is a promising strategy to prevent vibriosis in shrimp culture. Under the laboratory trial, we evaluated the effect of indigenous probiotics *Shewanella algae* A3 and *Serratia marcescens* Van80 UB3 on immunity, histopathology, gene expression, and its protection against pathogen *V. parahaemolyticus* Vp5 of Pacific white shrimp (2 ± 0.5 g) by a 30-day feeding experiment. Five treatment groups using 150 shrimp of post larvae-10 (PL-10) including 10^6 cfu/g single indigenous probiotic A (*Shewanella algae* A3), 10^6 cfu/g single indigenous probiotic B (*Serratia marcescens* Van80 UB3), 10^6 cfu/g multi indigenous probiotic C (mix equal portion of *Shewanella algae* A3 and *Serratia marcescens* Van80 UB3), common feeding without infection (positive control), and common feeding (negative control) were designed. After 30 days of feeding trial, the shrimp were challenged with *V. parahaemolyticus* Vp5 and survival percentage was recorded for 14 days. As a result, Pacific white shrimp showed a significant ($P < 0.05$) higher survival rate in juvenile treated with the multi indigenous probiotics 95.83 ± 1.44% compared to control negative 72.22 ± 1.57%. Gene expression-related immunity in the intestine, muscle, hemolymph, and hepatopancreas including proPO and serine protease, and cathepsine L in multi indigenous probiotic C was increased significantly ($P < 0.05$) compared with A, B, and control group. Gene expression of cathepsine L- related muscle growth was significantly lowest in multi indigenous probiotic C group ($P<0.05$). Immune response parameters after 14-day challenge was higher in multi indigenous probiotic C group compared to the other groups. Histological analysis of the intestine and hepatopancreas indicated that probiotic supplement treatment has better conditions of normal tissue than the negative control. Finally, the results strongly recommended enriched Pacific white shrimp diets with 10^6 living bacteria in mix equal to the portion of *Shewanella algae* A3 and *Serratia marcescens* Van80 UB3 per gram of diet.

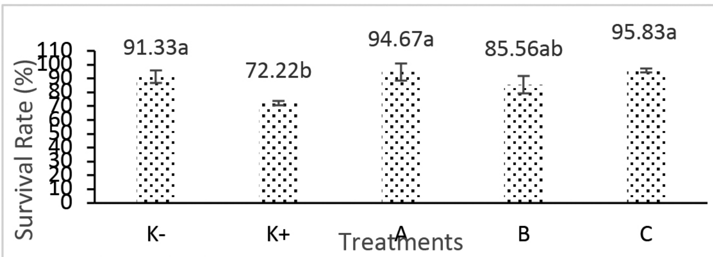

Figure 1. Survival rate of pacific white shrimp after 14-day challenge with 20 µl of *V. parahaemolyticus* Vp5 concentration at 10^5 CFU/mL

ENHANCING SEAFOOD QUALITY AND SUSTAINABILITY BY IMPROVING ANIMAL WELFARE IN WILD CAPTURE FISHERIES

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In this study we use an interdisciplinary approach to identify opportunities and challenges by improving animal welfare in wild capture fishery to enhance seafood quality and sustainability. Using literary exploration, laboratory experiments with live animals, consumer preference assessments and economic modelling, we studied the various steps involved in turning wild fish into food, starting from capture and processing, leading to distribution, and ending up in consumption.

Improper handling and processing of fish can result in stress-induced reductions in meat quality, which carry economic implications such as lower market value and potential rejection of products by consumers. Various fishing gears are associated with different challenges regarding animal welfare, with some renowned for yielding lower-quality catches unsuitable for specific markets. However, proper practices can improve the quality of the catch and consequently reduce food waste. Measures such as lower crowding density and time in the gear are directly related to improved animal welfare as well as meat quality. As climate change intensifies, its impact on marine ecosystems further complicates these dynamics. Through laboratory experiments we found that lower pH and increased temperatures affect fish metabolism, immune system, skin barrier functions and mineralization of scales. These physiological changes undermine fish welfare, which might lower the positive societal outlook towards capture fisheries. These findings highlight the urgency of integrating animal welfare considerations into wild capture fisheries, a necessity strengthened by consumer preferences for ethically sourced products. Seafood products harvested with more humane capture practices could create an increased market demand and product appreciation, reducing the likelihood of waste due to rejected, unsold or underappreciated products. We found that people acknowledge the importance of animal welfare, but only 40 % were willing to pay extra for fish products from producers who priorities animal welfare. This suggests that relying on price-based incentives for improved animal welfare may not be adequate. Economic models further supported this notion, indicating that regulatory measures promoting and enforcing improved capture and processing standards will play a crucial role in motivating industry compliance and encouraging sustainable practices.

In conclusion, this interdisciplinary study combines capture practices, regulatory frameworks, and consumer awareness to address the relationship between animal welfare in wild capture fisheries, seafood quality, and food waste. The study highlights the significance of ethical and sustainable practices in wild capture fisheries for improving seafood quality, lowering food waste, and matching with consumer expectations, global sustainability goals and their advanced interactions in food systems.

A COMPARISON OF BATH, CO-HABITANT AND IN-VITRO MODELS GIVES INSIGHT INTO INFECTION DYNAMICS OF WINTER ULCER DISEASE IN ATLANTIC SALMON

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The winter ulcer disease caused by the Gram-negative bacteria *Moritella viscosa*, is currently one of the major threats to the health and survival of farmed Atlantic salmon. Given the escalating prevalence of *M. viscosa*, we sought to compare three experimental models, the commonly used bath immersion with *M. viscosa*, a co-habitation model with shedder and contact fish, and an *in-vitro* system of fish scale explants. Fish and cell cultures were pre-exposed to commensal Atlantic salmon skin seawater bacteria prior to bacteria challenge.

Mortality was higher (6%) in the bath immersion trial compared to the co-habitation model (2.6%) (Fig. 1 A). At the end of the bath trial the fish were mainly without clinical symptoms. In the co-habitation trial ulcer development was more pronounced, with 25% of the fish having superficial lesions, and 15% had moderate to severe wound development, accompanied by reduced weight. Fish exposed to *M. viscosa* displayed less scale loss as compared to controls (Fig. 1 B), but with no effect of treatment with commensal bacteria prior to challenge. Further, the commensal Atlantic skin bacteria induced morphological changed to skin cells (Fig. 1 C) and induced unspecific immune responses (Fig. 1 D) in the *in-vitro* scale cultures, with large difference towards dead and live bacteria.

Overall, the co-habitation model represented progression of winter ulcer disease as typically observed in the field. In addition, *In-vitro* scale explants represented an excellent model to study transcriptional and morphological responses in the skin towards bacteria.

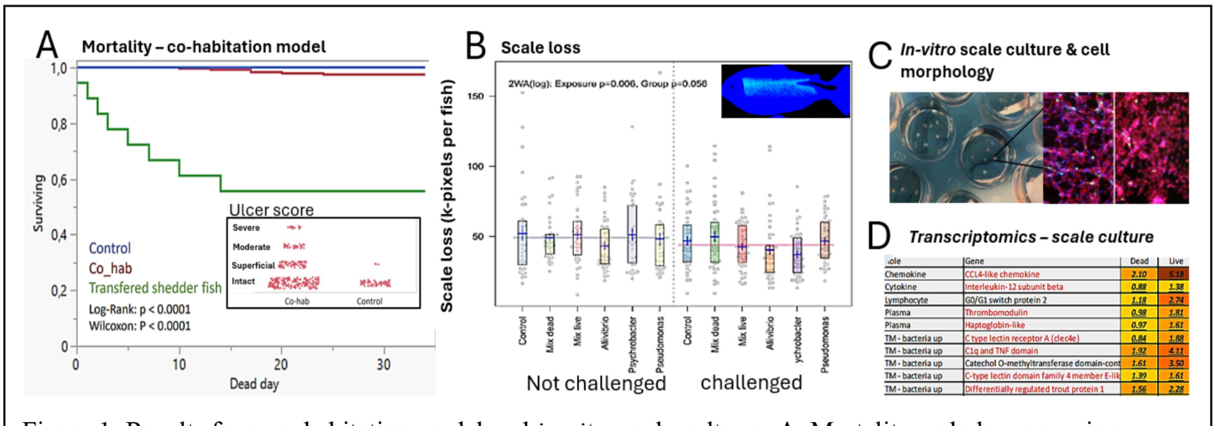

Figure 1: Results from co-habitation model and *in-vitro* scale cultures. A. Mortality and ulcer score in co-habitation model B. Digital assessment of scale loss after pre-exposure to commensal bacteria and infection with *M. Viscosa*. C. *In-vitro* scale culture and cell morphology after adding live and dead bacteria D. Transcriptomic response in scale culture cells to live and dead bacteria.

DENITRIFICATION IN RECIRCULATING AQUACULTURE SYSTEMS USING INTERNAL CARBON SOURCES IS DRIVEN BY DIFFERENT MICROBIOMES DEPENDING ON THE DIET

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Denitrification is a biofiltration process commonly applied in recirculating aquaculture systems (RAS) for converting nitrate-nitrogen (NO_3-N) to nitrogen gas. To meet the heterotrophic conditions required by denitrifing bacteria, fish faeces can be used as an internal carbon source. However, denitrification efficiency depends on the bioavailability of faecal carbon, which varies with faecal composition and thus diet. In this respect, the present study aimed to explore the effect of diet on the denitrification potential of a marine RAS using internal faecal carbon sources.

Four identical RAS (473L) equipped with an up-flow sludge blanket denitrification reactor (DR), were stocked with juvenile European seabass. Fish were fed over a six-week period with two diets in duplo, which consisted of a basal mixture diluted at 15% with a test ingredient: insect meal (IM) and dried distillers grain with solubles (DDGS). 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. The total internal carbon supply was estimated by faecal collection via settling in control systems operating without a DR, with the collected faeces being thereafter analyzed for their content in chemical oxygen demand (COD) and total Kjeldahl nitrogen (TKN). The aforementioned control systems were also used to estimate total NO_3-N supply to the DRs while nitrogen from branchial and urinary losses (BUN) was estimated based on nitrogen balances calculated for the systems with a DR. Denitrification efficiency was quantified as NO_3-N removal based on measurements at the inlet and outlet of the DR over a 24-h period at the end of the trial. Finally, DR sludge was analyzed for its bacterial composition with Illumina MiSeq sequencing targeting the V4 region of the 16S rRNA gene.

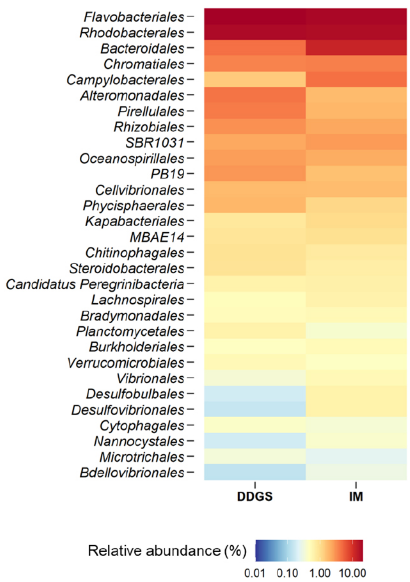

Fig1. Relative abundance of bacteria in the DR sludge for the two diets.

Results showed that DRs operated under similar COD:NO_3-N ratios regardless of the diet (DDGS: 3.1±0.5; IM: 2.8±0.1, $p>0.05$) and were equally efficient in removing NO_3-N per kg dry matter feed (DDGS: 3.1±0.5; IM: 2.8±0.1, $p>0.05$). Despite the similar denitrification performance, diet exhibited a tendency to affect bacterial composition (Fig1; $p=0.075$). A higher abundance of Bacteriodales ($p<0.001$) and Desulfovibrionales ($p<0.05$) was observed for IM. The presence of sulphate-reducing bacteria (*Sulforovum* and *Desulfomicrobium*) was negatively correlated with the total nitrogen (Total nitrogen=BUN+TKN) supply to the DRs ($p<0.05$, $r=-0.95$ and $r=-0.96$, respectively). Considering that these bacteria produce hydrogen sulphide, which can compromise fish health, it is concluded that faeces originating from IM may be less suitable as an internal carbon for denitrification in RAS.

EXPLORING THE PHYSIOLOGICAL PLASTICITY OF OYSTERS (*Crassostrea virginica*) TO EXTENDED HYPOXIA ACROSS DIFFERENT LIFE STAGES

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Hypoxia, defined as a dissolved oxygen concentration (DO) below 2 mg L^{-1}, can be a significant stressor to aquatic organisms, particularly those that are sessile like marine bivalves, who cannot avoid such conditions. Estuarine and coastal environments are more susceptible to hypoxia as anthropogenic nutrient loading fosters algal blooms that deplete oxygen upon decay, putting bivalves at a greater exposure risk of hypoxia. Furthermore, ocean warming exacerbates the challenges of hypoxia for ectothermic bivalves as their metabolic demand rises with temperature, yet meeting this demand becomes increasingly difficult under low oxygen conditions. The plasticity of bivalves to alter their physiology in response to environmental change plays a significant role in understanding their resilience and survival to hypoxia. Furthermore, environmental stress may affect various life stages differently, thus exploring the plasticity and tolerance of different life stages is imperative. The aim of this study is to explore the plasticity of juvenile and adult eastern oysters (*Crassostrea virginica*) to extended hypoxia under warm water temperatures. Juveniles and adults were held under either normoxic conditions or hypoxic conditions for a three-week period at temperatures reflective of summer water conditions in Atlantic Canada (~24ºC). The metabolic rate, anaerobic capacity, mitochondrial activity, and feeding rate of oysters were measured before, during, and after the experiment to understand the efficiency and rate of plasticity at multiple levels of biological organization. Preliminary results suggest that both juvenile and adult oysters exhibit physiological plasticity to low oxygen conditions, though, over an extended period, adults are more resilient than juveniles. This study highlights the potential for oysters across different life stages to acclimate and survive under extended periods of low oxygen, useful information for aquaculture management practices.

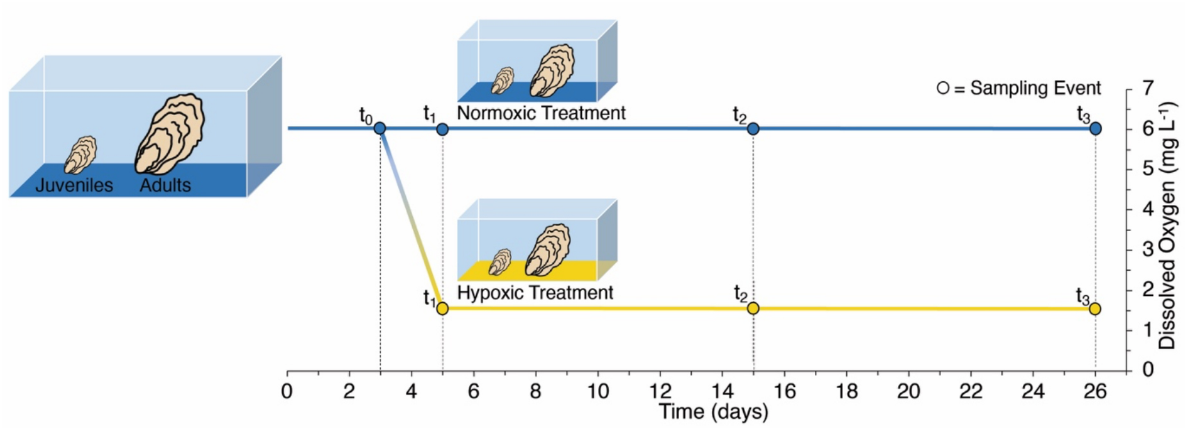

Figure 1. Schematic overview of the experiment. The adult and juvenile oysters were acclimated under normoxic conditions for three days. Oysters were subsequently separated into different tanks with either normoxic (DO > 5 mg L^{-1}), or hypoxic (DO < 2 mg L^{-1}) conditions, both kept at a constant temperature of ~24ºC. Metabolic rate was measured several times a day while anaerobic capacity, mitochondrial activity, and feeding rate measurements occurred at 4 time points throughout the experiment (t_0, t_1, t_2, t_3).

ESSENTIAL OIL COMPONENTS FOR POSTLARVAL CRUSTACEAN HEALTH MANAGEMENT

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Global farming of whiteleg shrimp *Penaeus vannamei* through aquaculture has created huge economic benefits. However, over the past decades, misuse of antibiotics to combat and prevent aquatic disease outbreaks such as vibriosis has led to different levels of antibacterial resistance in various countries. Essential oils (EOs) extracted from plants, and their purified components (EOCs) already proved to possess effective antimicrobial abilities towards aquatic microbial pathogens (e.g. *Vibrio* spp.) in laboratory conditions. Moreover, the benefits of EOCs were extensively studied before by the ARC lab, focusing on the crustacean model *Artemia franciscana* (Zheng et al., 2023). The current study further validated the antibacterial activity and protective ability of EOCs in post-larvae (PLs) of *P. vannamei*.

Three EOCs (citral, R-(+)-limonene and trans-cinnamaldehyde) were applied through a broth microdilution assay at concentrations ranging from 2% to 0.0020%, with dimethyl sulfoxide at 1% as solvent, to determine the minimum inhibitory concentration (MIC) against *Vibrio campbellii* and *V. parahaemolyticus* (AHPND strain) at 5 x 10^5 CFU. The cell density was monitored hourly for 24 hours at OD 600nm. Potential synergistic effects of selected EOCs were analyzed *in vitro*. Finally, an *in vivo* assay involving toxicity assessment was performed, to investigate the LD50 of PLs exposed to different EOCs. Challenge tests were conducted to verify the protective effect of the selected EOCs on the shrimp PLs.

Initial results confirm that (0.5%, v/v) citral has a strong inhibitory effect on *V. campbellii* BB120 after 24 hours exposure (Figure 1). Trans-cinnamaldehyde significantly inhibits the growth of both *Vibrio* strains. The potential benefits of EOCs for mitigating and prophylaxis of vibriosis in shrimp farming practices is highlighted, and demonstrate a valuable and sustainable alternative for combating *Vibrio* during shrimp cultivation. By further exploring their protective efficacy, EOCs offer hope for ecological and sustainable health management in global aquaculture practice.

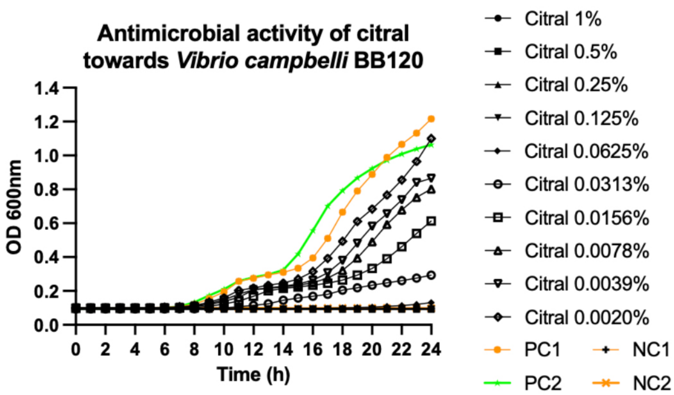

Figure 1: Antibacterial activity (OD at 600nm, Y-axis) of citral (1% - 0.0020% v/v) towards *Vibrio campbellii* BB120 over 24h (X-axis); PC : positive control; NC : negative control

DIETARY SUBSTITUTION OF SOY WITH A NOVEL MIX OF INSECTS AFFECTS OSMOREGULATORY AND STRESS COPING CAPACITIES IN FARMED ATLANTIC SALMON

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The expansion of salmon aquaculture requires diversification of feed ingredients. Insects are advantageous in containing a high protein content and suitable amino acid composition and can be produced using side-stream agro-industrial activities. Also, the diversity of insect species provides a plural of candidate nutrient profiles that can be optimized into a blend ("mix" of species) to match the requirements of farmed fish. While studies confirm the replacement using a single insect species can fulfill necessities to maintain growth, and digestibility, less attention has been given to other important physiological parameters necessary for good fish health and welfare, e.g., assuring shifts in feed compositions do not interfere with the resilience of fish during freshwater (FW), seawater (SW) or their challenging transition is key from a farming perspective. In this study, we evaluated how Atlantic salmon (*Salmo salar*) stress responses and osmoregulatory capacities are affected by substituting soy protein concentrate (SPC) with different inclusions (3%, 25%, and 50%) of a novel mix of insect meal (IM) [black soldier fly larvae (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor*)]. The feed trial included 8 weeks in FW and transfer to SW for 6 weeks. At the end of both FW and SW phases, fish were subject to an acute challenge test (ACT), by high density, to monitor capacities to mount a stress response. Plasma levels of cortisol, ions, and other stress metabolites were quantified and complemented to the expression of genes related to gill osmoregulation and brain plasticity. Our results show the inclusion of insect mix did not affect fish growth, however, resulted in altered responses on stress and osmoregulatory functions (Fig. 1), and revealing salmon handle larger insect substitutions in FW (up to 50 %) compared to SW (up to 25 %). This points to different lifecycle specific requirements in Atlantic salmon.

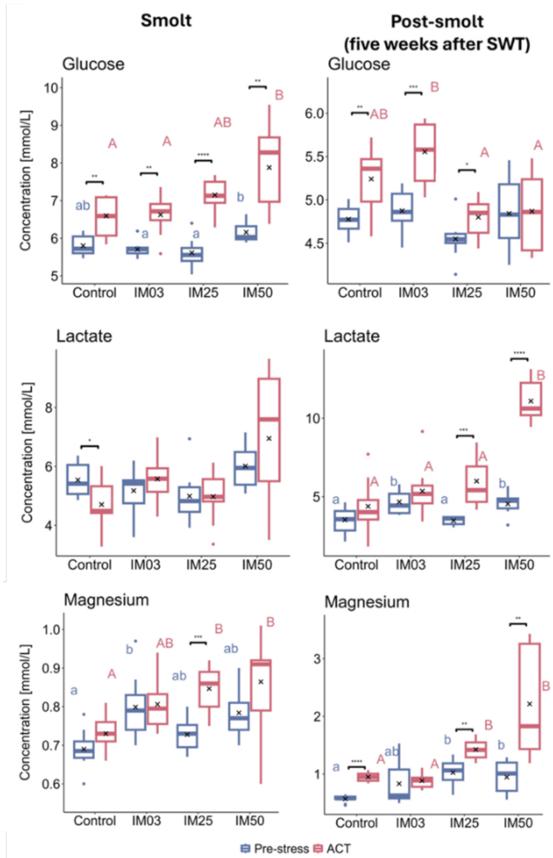

Fig. 1. Plasma glucose, lactate, and magnesium in salmon smolt and post-smolt five weeks after seawater transfer (SWT), with different rates of IM, at baseline (pre-stress) and post-stress (ACT). N = 9 per diet/stress state. Differences among stress states at each diet are marked with (*). Differences between diets at pre-stress and ACT are indicated with lower- and uppercase letters, respectively ($p < 0.05$).

Acknowledgments: Funded by EEA InFishMix-PT-INNOVATION-0094. Analysis performed in FBA-NORCE high throughput state of the art lab (SacLab). Fish trial performed in LetSea R&D center.

A HOLISTIC APPROACH TO EVALUATE THE POTENTIAL USE OF BACTERIAL SINGLE-CELL PROTEIN AS AN ALTERNATIVE PROTEIN SOURCE FOR ATLANTIC SALMON *Salmo Salar* L

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A protein-dense bacterial single-cell protein [SCP:76-87% crude protein (CP) on dry matter (DM) basis], produced from an environmentally friendly fermentation process, using a non-GMO *Methylovorus menthalis*, was examined as an alternative protein source in salmon feeds over a series of four *in vivo* studies. In a freshwater digestibility trial, triplicate groups of juvenile Atlantic salmon (average weight: 23.7 ± 1.0 g), reared at a water temperature of 14 ± 0.2 °C, were fed two digestibility diets: one with a 100:00 blend (reference) and another with an 80:20 blend of reference to the test ingredient (i.e., SCP). The study lasted 25 days, and fecal material was collected using the passive settling column method. Results demonstrated that nutrients in SCP were highly digestible, with apparent digestibility coefficients of DM, CP, energy, and essential amino acids of 85.3%. 92.4%, 87.9% and 85.5-100.0%, respectively.

In a freshwater growth study, SCP was tested in four diets with graded inclusion levels of SCP (0: control, 10, 20 and 30%) by replacing a mixture of commonly used animal and plant protein ingredients. Each diet was fed to triplicate groups of 35 juvenile fish (27.5 ± 0.7 g) for 84 days at a water temperature of 14.2 ± 0.3 °C. Fish fed diets with up to 20% SCP showed feed intake and weight gain comparable to the control group. At the 30% inclusion level of SCP, FI and WG significantly decreased compared to the control, but FCR remained consistent across all treatments, ranging from 0.9 to 1.0.

In a 132-day saltwater growth study, post-smolt fish (423.7 ± 3.8 g), reared at a water temperature of 13.8 ± 0.4 °C, were fed one of four experimental diets containing 0, 10, 20 and 30% inclusion levels of SCP. The feed formulation aimed to primarily replace soy protein concentrate (soy PC) from 24-0%. The findings revealed no significant differences in FI and WG among fish fed 0-20% SCP. The 30% SCP-fed group exhibited inferior feed consumption and growth performance compared to the other groups, but all groups achieved FCR values of 0.9-1.0.

In a 168-day saltwater growth study, four experimental diets with 0, 8, 16 and 24% inclusion levels of SCP, replacing soy PC, were fed to triplicate groups of post-smolt fish weighing 1005.9 ± 22.7 g. Fish were reared at a water temperature of 13.7 ± 0.5 °C. Findings showed no significant difference in FI, WG and FCR between treatments, whereas fish fed SCP from 8-24% had notably higher fillet redness than fish fed the control.

In saltwater trials, whole-body proximate, amino acid, and fillet fatty acid profiles were similar across all dietary treatments. Blood biochemistry and gut histopathology showed no abnormal health signs or enteritis symptoms. The studies concluded that SCP is highly digestible for Atlantic salmon and can be safely included in their diets up to at least 20%.

EVALUATION OF LASERPUNCTURE ON TESTICULAR MATURATION AND SEMEN QUALITY OF THE CATFISH *Sorubim cuspicaudus*

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The catfish *Sorubim cuspicaudus* is a species with the potential to diversify Colombian fish farming. Despite advances in its reproduction in captivity, problems of asynchrony in maturation and low semen volume in males persist. Laserpuncture is an alternative technology to accelerate gonadal maturation and improve reproduction in captivity by applying a low-power laser beam to reproductive acupoints that stimulate the production of hormones and enzymes that control the reproductive cycle. The study aimed to evaluate testicular maturation and semen quality of *S. cuspicaudus* subjected to laserpuncture.

The immature males (n=15) were treated with a Helium-Neon low-laser (17 Mw) at different exposure times: 10, 20 and 25 sec. The laser generated different powers: 0.85, 1.7 and 2.1 Joules (J). The laser was applied to the reproductive acupoints at 2/3 of the ventral region once a week for one month. In addition, a group of immature males without laserpuncture (negative control, n=5) and naturally mature males in earthen ponds (positive control, n=3) were evaluated. Finally, all males (treated and controls) and a group of females in final maturation (n=3) were induced with Ovaprim® (0.5 ml/kg of weight body) to evaluate the seminal quality and fertilizing capacity of the semen. Semen quality was assessed using the computer-assisted semen analysis system (SCA®, Microptic, Spain) and fertilizing capacity was evaluated using fertilization and hatching rates. Testicular maturity was also analyzed using histological sections, and the gonadosomatic index (GSI) was estimated.

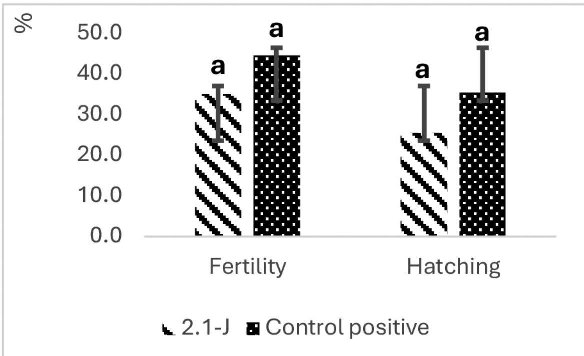
The results indicate that males treated with 2.1 J presented mature testicles. Males treated with less potency (0.85 and 1.7 J) were found to be maturing and did not release semen, as well as the negative control. Males treated with 2.1 J (0.8±0.1) and the positive control (0.9±0.6) registered similar GSI (p>0.05). Total motility ranged between 90.6±5.8% (2.1 J) and 90.9±8.2% (positive control) (p>0.05) (Table 1). The semen obtained by induction with Ovaprim®, both from males matured with laserpuncture (2.1 J) and from the positive control, did not show statistical differences (p>0.05) in fertilizing capacity (Figure 1).

Table 1. Semen quality of *S. cuspicaudus* matured by laserpuncture (2.1 J=17mW*25 sec) and naturally in earthen ponds (positive control). VCL, curvilinear velocity; VSL, straight-linear velocity.

Parameters	Treatments	
	Control positive	2.1 J
Volume (mL)	$0.8±0.0^a$	$1.15±0.3^a$
Motility (%)	$90.9±2.2^a$	$90.6±5.8^a$
Progressivity (%)	$54.4±2.7^a$	$45.8±16.3^a$
Duration of motility (sec)	$44.7±0.0^a$	$39.4±3.2^a$
Concentration ($x10^9$ szp/mL)	$24.8±21.1^a$	$31.1±7.7^a$
Rapid (%)	$3.4±3.1^a$	$5.9±7.8^a$
Medium (%)	$66.0±1.3^a$	$56.1±15.2^a$
Slow (%)	$21.3±1.1^a$	$28.5±16.9^a$
Immotile (%)	$9.0±2.2^a$	$9.3±5.8^a$
VCL (μm/s)	$62.9±4.1^a$	$59.6±15.5^a$
VSL (μm/s)	$46.2±2.0^a$	$40.8±12.7^a$

Different letters indicate significant difference (p<0.05).

Figure 1. Rates of fertility and hatching of *S. cuspicaudus* obtained with males matured with laserpuncture (2.1 J=17mW*25 sec) and naturally in earth ponds. In both cases, the males were induced with Ovaprim® (0.5 ml/kg of weight body).



Different letters indicate significant difference (p<0.05).

The results suggest that laserpuncture accelerates testicular maturity and produces good-quality semen with fertilizing capacity when stimulated with 2.1 J (17 mW for 25 sec) once a week for one month.

COMPARATIVE ANALYSIS OF GENETIC DIVERSITY IN NATURAL POPULATION OF *Labeo rohita* AND *Cirrhinus marigala* BY USING SPECIES SPECIFIC MOLECULAR MARKERS

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Labeo rohita, commonly known as rohu and *Cirrhinus marigala*, popularly known as morakkha are economically most important and extensively cultured fish species in the entire Indian subcontinent. Genetic evaluation of these species is necessary for their proper supervision conservation and profitable production. Various types of molecular markers are available but SSR are most important one due to their equal distribution, abundance in the genome, codominance, polymorphic, low cost detection and multi-allelic nature. In this study ten samples of each species were collected from Head Punjnad, Head Muhammad Wala and Head Trimmu region of river Chenab, Pakistan. After the extraction of DNA, amplification of five simple sequence repeat markers, their resolution on PAGE and allelic scoring for the genotypic data was used for the analysis of different genetic diversity indices with the help of POPGENE version 1.32, FSTAT version 2.9.3.2, Jaccard and Dice similarity coefficient, simple matching analysis and SIMQUAL program of NTSYS-PC package. In case of *Labeo rohita*, total 26 alleles with 260 loci were detected out of which 189 were found polymorphic. Polymorphism for the five markers varies from 43.33%-96% with mean value of 72.69%. Allelic frequency ranges from 0.2000-0.9000 with 0.4600 mean value, allele numbers varies from 4.000-9.000 with average value of 5.2000, gene diversity varies from 0.1800-8800 with mean value of 0.6360 and PIC value ranges from 0.1638-0.8680 with 0.6012 mean value. Pair wise genetic difference among ten collected samples varies from 0.400-0.900 while pair wise genetic similarity ranges from 0.100-1.000. Cluster analysis based on UPGMA divided ten samples of *Labeo rohita* into three clusters and three sub clusters. Similarly, five SSR markers of *Cirrhinus marigala* show 30 alleles with 300 loci across ten samples with 240 polymorphic loci. Polymorphism ranges from 70%-96% with average value of 80%. Allelic frequency varies from 0.4000-0.9000 with average value of 0.6600. Allele numbers varies from 4.000-9.000 with 6.000 as mean value. Gene diversity varies from 0.1800-0.7800 with mean value of 0.4680. PIC value ranges from 0.1638-0.7578 with mean value of 0.4422. Pair wise genetic difference among ten samples of *Cirrhinus marigala*ranges from 0.2000-0.800 and pair wise similarity ranges from 0.200-1.000. Cluster analysis based on UPGMA divided all the samples of *Cirrhinus marigala* into three clusters and three sub clusters. This study reveals that Government of Pakistan and interested agencies should take serious steps for the improvement of genetic structure ofthese species especially *Cirrhinus marigala*.

BIOHYBRID SYSTEMS AS SENSORS IS AQUACULTURE

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Biohybrid systems offer an innovative approach to building sensory systems for aquatic environments by combining living organisms with technological components. These systems are designed to detect environmental changes more rapidly and to recognize a broader range of potential stressors simultaneously. Biohybrid systems enable continuous water quality monitoring, identifying not only traditional parameters such as temperature and acidity (pH) but also harmful substances, invasive species, the presence of pests, or other stressors.

The primary aim of this approach is to develop a more sustainable and efficient method for gathering environmental information about natural habitats and industrially used areas, such as aquaculture. This addresses the industry's increasing demand for environmentally friendly and autonomous monitoring solutions.

At the core of biohybrid sensory systems is the "life form in the loop" principle, a methodology that fosters a mutually beneficial relationship between biological and technological elements. This strategy allows for the autonomous detection of environmental changes, enabling users to respond accordingly, thereby supporting aquatic ecosystems and maintaining operations, including coastal aquaculture.

The methodology is characterised by its use of non-invasive techniques, ensuring that these biohybrid entities do not harm the aquatic environments in which they are placed. Additionally, their autonomous nature significantly reduces the need for human intervention, making the monitoring of environmental parameters more efficient and less labour-intensive.

By merging the capabilities of biology and technology, projects developing biohybrid systems, such as the EU project ROBOCOENOSIS, aim to demonstrate innovative practices in aquaculture. These initiatives aim to not only enhance the sustainability and productivity of aquatic farming but also to encourage a deeper connection between technology and nature.

Figure 1: Prototype of a biohybrid entity measuring several environmental parameters, and, in parallel, biological processes.

UNDERSTANDING MOLECULAR MECHANISMS OF DISEASE RESISTANCE IN RAINBOW TROUT ISOGENIC LINES WITH CONTRASTED SUSCEPTIBILITY TO VHSV AND *Flavobacterium psychrophilum*

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Rainbow trout is one of the most commonly farmed salmonid species across the world. Infectious diseases represent a significant threat to the sustainable development of fish farming. Understanding the basis of fish natural resistance to pathogens is important for genetic selection. This project was developed in the frame of "Advancing European Aquaculture by Genome Functional Annotation" (AQUAFAANG). The objective was to achieve a detailed functional annotation analysis of two rainbow trout isogenic lines chosen based on their resistance/susceptibility status to two pathogens, a bacterium (*Flavobacterium psychrophilum*, Fp) and a virus (Viral Hemorrhagic Septicemia Virus, VHSv). The first isogenic line is resistant to Fp infection by injection and susceptible to VHSv infection by immersion; the second is resistant to VHSv and susceptible to Fp.

Experimental disease challenges were performed by intramuscular injection for standardisation of the stimulation. Two days post-infection, the head kidneys of 32 fish (2 lines; 4 experimental conditions: control Fp, injected Fp, control VHSv and injected VHSv; 4 fish per line and per condition) were sampled for RNAseq and ATACseq libraries construction. The 32 RNAseq and 32 ATACseq libraries were mapped on the Ensembl rainbow trout genome assembly (strain Arlee), and count tables were generated using bioinformatics pipeline nf-core/RNAseq (3.14) and nf-core/atacseq (2.12), respectively. To reduce the false discovery rate during read count, due to the high level of duplication in salmonids and multiple transcript isoforms, we retained uniquely mapped reads and only kept the longest transcript isoform for each gene in the reference database for annotation. Differential gene expression analysis and differential chromatin accessibility analysis were performed with DESeq2 for each disease separately. The integration of RNAseq and ATACseq data was performed by Regularized Generalized Canonical Correlation Analysis (RGCCA), Multi-omic Factor Analysis (MOFA), Weighted Gene Co-expression Network Analysis (WGCNA) and Similarity Network Fusion (SNF), retaining only intersection of the mechanisms detected by the different methods.

Divergent transcriptional responses between the resistant and susceptible lines were revealed at the basal level and following infection. In particular, after VHSv infection, higher induction rates of interferon stimulatory genes were detected in the resistant line. Also, markers of inflammatory response were up regulated in the two lines following Fp infection. In addition, differences in chromatin accessibility were observed between infected and control fish for VHSv but not for Fp challenges. The integration between RNAseq and ATACseq data will lead to a better understanding of molecular mechanisms involved in the contrasted response of the two isogenic lines to both pathogens.

INVESTIGATING AMOEBIC GILL DISEASE (AGD) RESISTANCE IN ATLANTIC SALMON THROUGH A HOLOGENOMIC LENS

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Amoebic gill disease (AGD), caused by *Neoparamoeba perurans,* is one of the major threats to gill health of marine salmonids and hence of major importance in salmon aquaculture. AGD prevention and treatment options are scant, therefore other options such as selective breeding or genetic engineering to increase resilience of Atlantic salmon stock to AGD may present an alternative prevention strategy. A number of studies have addressed AGD resistance in Atlantic salmon using transcriptomic and quantitative genetic approaches. However, another, often overlooked layer can be added to the narrative – namely that of the gill microbiota.

Here we utilised a combined approach of metabarcoding and quantitative genetics to address AGD resistance, by considering the microbiota as an extended resistance phenotype of the salmon. We characterised the gill microbiota of Atlantic salmon subject to an AGD disease challenge, and investigated microbiota composition in relation to common AGD phenotype scoring methods (gill score and amoebic load). We then applied a GWAS approach to investigate SNPs associated with the microbiota and the AGD phenotypic scoring methods.

We found that the overall microbiota composition of gill samples was relatively simple as over 60% of the overall relative abundance of microbial taxa was attributable to two families namely, *Simkaniaceae* and *Arcobacteraceae*. Notably, bacteria of the family *Simkaniaceae* are known to cause epitheliocystis and respiratory distress in Atlantic salmon and may be endosymbionts of the disease causing amoeba. Furthermore, we found significant differences in weighted alpha diversity estimates among samples grouped by amoebic load and significant difference in beta diversity among samples grouped by severity of gill damage. No genome-wide significant QTLs were identified, however four suggestive QTLs were found in relation to gill score, microbiota diversity, and relative abundance of individual bacterial families.

This study highlights the utility of integrating metagenomic and genomic approaches to address the interplay between host genetics, microbiota and disease resistance.

PREDICTION OF MORTALITY IN RECIRCULATION AQUACULTURE SYSTEMS

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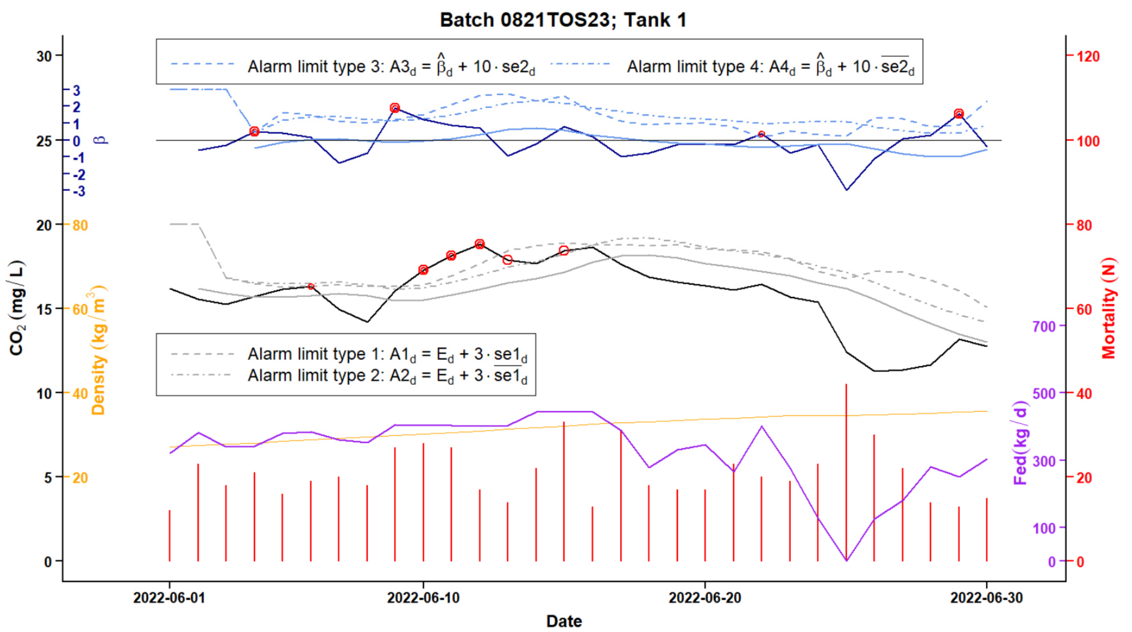
Recirculation aquaculture systems (RAS) provides a way to produce more fish from a restricted resource; water, in addition to enabling control of water entering from and returning to the environment. “Intelligent farming and health control in land-based recirculation aquaculture systems” (2021-2024) is a NordForsk supported project with partners in Norway, Denmark, and Finland. The overall objective is to support farmers producing salmonids in RAS to transition from experience-based to knowledge-based decision making, i.e. Precision Fish Farming (PFF). The PFF concept was introduced recently to facilitate a more sustainable production regarding production margins, environmental impact, health, and welfare in intensive aquaculture. A key ingredient of PFF is a reliable prediction model.

Here we focus on monitoring and predicting mortality and consider management methods that may enable early, preventive changes in feeding. The methods considered are quality control charts of daily mortality counts, forecasting of CO_2 and dissolved oxygen (DO) based on sensor data time series, and use of generalised dynamic linear models. The main data originates from a Scandinavian land-based freshwater rainbow trout farm with 12 tanks, hereof 2 with DO, CO_2, and temperature measurements. The figure below shows one month of observed daily mortality, average CO_2, fish density, and fed feed, as well as predicted mortality from 4 prediction models for one batch of fish. Preliminary analyses showed CO_2 to be in moderate, positive correlation with feed fed but to be uncorrelated with mortality. Mortality showed positive, moderate correlation with temperature. Furthermore, a control chart model showed promising results with respect to predicting mortality.



OCCUPATIONAL HEALTH AND SAFTETY (OHS) IN NORWEGIAN SALMON FARMING – AN EMPLOYEE SURVEY

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Introduction

Employees at Norwegian salmon fish farms and vessels are exposed to several workplace hazards and must handle many risks to ensure personal safety, safeguard fish health and welfare as well prevent escape of fish. This presentation provides findings from an OHS survey about employees' perceptions of health, working environment and safety (1). The survey is part of the project "OHS in aquaculture – risk management in different production forms", financed by the Norwegian Seafood Research Fund (project 901801).

Material and methods

A digital survey was distributed in May-August 2023. It was an updated version of a previous survey (2) based on input from industry, trade organizations and regulatory representatives. 1283 respondents including fish farm workers, vessel crews and operative workers in the supplier industry participated.

Results

Analyses show that most participants consider their health to be good and enjoy their work. Still, many worry that their work may affect their health negatively now or in the future. Furthermore, 62 % have experienced near misses in the last two years, and 17 % have had work-related sickness absence due to illness or injury in the last year. The main causes for both worry and sickness absence are strain/musculoskeletal injuries and injuries.

Most agree that safety has priority when they do their job (86 %). Still, there are challenges related to resources and organization of work. For instance, several of the respondents agree that considerations to production is prioritized at the expense of safety (29 %), that they feel pressured to continue working although safety can be compromised (22 %), that considerations regarding fish welfare and escape mean that safety routines cannot always be followed (22 %), and that inadequate maintenance has reduced the safety level (28 %). Moreover, 31 % agree that it is uncomfortable to point out lack of compliance to safety rules and procedures.

Conclusion

The survey provides information that should be utilized by companies in their risk management. Engaging with the employees who are exposed to hazards, to ensure that their experiences and evaluations are included when measures are developed and prioritized is crucial.

Literature

1. Thorvaldsen T, Kongsvik T, Holmen IM, Størkersen K, Salomonsen C, Sandsund M. 2020. Occupational health, safety and work environments in Norwegian fish farming - employee perspective. Aquaculture.
2. Thorvaldsen T, Kongsvik T, Holmen IM, Størkersen K, Salomonsen C, Holen S. 2023 HMS-undersøkelsen i havbruk 2023. SINTEF-report 2023:0136

CLOSING THE CYCLE OF THE EUROPEAN EEL *Anguilla anguilla*

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Closing the European eel life cycle in captivity for a self-sustained and sustainable eel aquaculture requires efficient hatchery technology and techniques. With this goal in mind, EU-supported research initiatives set out in the beginning of this century to develop procedures for captive reproduction and larval culture aiming at glass eel production. These efforts have led to two decades of successful achievements through dedicated international and national efforts. This targeted research ranges from development of broodstock feeds and assisted reproduction protocols applying hormone therapy, thereby enabling production of high-quality gametes and viable offspring (Tomkiewicz et al., 2019, Jéhannet et al., 2024) to the establishment of culture technology and techniques as well as formulation of feeds for ongrowing larvae sustaining their development into the leptocephalus stage (Politis et al., 2021; Benini et al., 2023; Bandara et al., 2024).

While new knowledge, products and methods have incrementally extended longevity, variable reproduction success, offspring quality and high mortality in the early larval stages challenge upscaling of larval culture and the completion of the larval phase. These challenges have urged researchers to revisit procedures with the aim to enhance quality and survival through different stages. This presentation will overview progress in European eel offspring production and larval culture and discuss bottlenecks and impediments that need attention spanning from reproduction through the larval culture to reach metamorphosis and the glass eel stage in sufficient numbers. Among other, we look at the still limited insights in larval demands to ambient culture conditions and stage specific nutritional requirements and preferences, but also the efficiency required for an efficient hatchery production and commercial closed-cycle production of European eel.

Bandara KA, Politis SN, Sørensen SR, Benini E, Tomkiewicz J, Vadstein O, 2024. Effect of Food Amounts on Larval Performance, Bacteriome and Molecular Immunologic Development during First-Feeding Culture of European Eel. Microorganisms, 12(2), 355.

Benini E, Bandara KA, Politis SN, Engrola S, Nielsen A, Conceiçao LEC, Santos A, Sørensen SR, Tomkiewicz J, 2023. Exploring first-feeding diets for European eel larval culture: insights at morphological, nutritional, and molecular levels. PLoS One 18, e0283680.

Jéhannet P, Heinsbroek LTN, Swinkels W, Palstra AP, 2024 Recent insights into egg quality and larval vitality of the European eel (*Anguilla anguilla*). General and Comparative Endocrinology, 354, 114531.

Politis SN, Syropoulou E, Benini E, Bertolini F, Sørensen SR, Miest JJ, Butts IAE, Tomkiewicz J, 2021. Performance thresholds of hatchery produced European eel larvae reared at different salinity regimes. Aquaculture 539, 736651.

Tomkiewicz J, Politis S, Sørensen S, Butts I, Kottmann J, 2019. European eel – an integrated approach to establish eel hatchery technology in Denmark. In: Eels Biology, Monitoring, Management, Culture and Exploitation: Proceedings of the First International Eel Science Symposium, 2019. 5m Publishing, Sheffield, UK.

PRELIMINARY STUDY: ANTIMICROBIAL RESISTANCE GENES IN BACTERIOPHAGES FROM A NATURALLY PRESERVED WETLAND IN THE MEXICAN ALTIPLANO

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Introduction

Antimicrobial resistance is a worldwide concern for the medical, human or veterinary sector, due to the fact that pathogens such as bacteria, viruses, fungi and parasites have acquired new resistance mechanisms and consequently compromising our ability to treat infections. Bacterial resistance has spread globally at an alarming rate, in 2021 the World Health Organization (WHO) estimated that more than 700,000 deaths annually are attributed to antimicrobial resistant bacteria. The mechanisms by which these bacteria acquire resistance genes include natural and acquired resistance. Natural resistance is inherent to bacterial evolution and exists before antibiotic use, while acquired resistance occurs when bacteria acquire these genes from other bacteria (horizontal transmission), a selection pressure that exists when bacteria are constantly exposed to antibiotics or bacteriophages that integrate their genome into the bacterial genome (Jebri et al., 2020).

Metagenomic studies in aquatic environments have shown that the bacteriophage fraction can comprise more than 60% of the viral total (Breitbart et al., 2004; Rosario et al., 2009), being these bacteriophages an important biological entity in the acquisition of antimicrobial resistance genes in bacteria. In order to know the possible participation of these bacteriophages in a naturally preserved environment, the presence of antimicrobial resistance genes in bacteriophages belonging to a naturally preserved wetland in the Mexican highlands was investigated.

Methodology

The study site was a wetland located in the municipality of Almoloya del Rio, State of Mexico (19°09'15.9 "N 99°29'41.7 "W), where the Lerma River begins. This wetland is part of the Ciénegas de Lerma and is the best preserved to date. Six water samples (50 ml) were taken in sterile tubes and transported under refrigerated conditions to the Faculty of Veterinary Medicine and Zootechnics, UNAM.

To obtain viral particles, each sample was centrifuged at 15,000 xg for 15 min. The supernatant was filtered through 0.45 µm and 0.22 µm membranes. The filtered supernatant with viral particles was concentrated to 1 µl using 100-kDa Corning Spin-X UF Concentrator (Corning, Cat. No. 431491) at 3,000 x g for 15 min (Malki et al., 2021). Each sample received a TURBO DNAase (Thermo Fisher, Invitrogen, Cat. No. AM2238) and RNase Cocktail (Thermo Fisher, Invitrogen, Product Code.10036284) treatment, the reaction was carried out at 37°C for 1 hr and subsequently stopping the reaction at -20°C.

Table 1. Number of antimicrobial resistance genes detected in DNA samples from polyphyletic bacteriophages.

ID	# genes	Drug	Mechanism	Identity	E value
1	10	Fluoroquinolone	antibiotic target protection	42-56%	<9.54 e^{-06}
2	12	Fluoroquinolone	antibiotic target protection	44-48%	<9.8 e^{-06}
3	4	Fluoroquinolone	antibiotic target protection	50-54%	<9.01e^{-06}

(Cdontinued on next page)

Three samples were taken at random for DNA extraction using the commercial AllPrep PowerViral DNA/RNA kit (50) (Catalog Code 28000-50), while the remaining three samples were processed to obtain RNA with the Invitrogen™ TRIzol™ Plus RNA Purification Kit and Phasemaker™ Tubes Complete System (Cat. No. A33254). Library preparation and nucleotide sequencing was performed on the Ilumina miseq platform of the Unidad Universitaria de Secuenciación Masiva (UUSM), Instituto de Biotecnología (IBT) UNAM. DNA libraries were constructed with the Illumina DNA preparation kit following the manufacturer's protocol, and RNA libraries were constructed with the Illumina TruSeq Stranded mRNA sample preparation kit following the manufacturer's protocol. Both libraries were sequenced with the Illumina NextSeq 500/550 High Output Kit v2.5. Sequencing was performed in a 2x75 configuration.

The raw sequences were paired using the FLASh (Fast Length Adjustment of Short reads) program, then edited and filtered by Phred Q>30 quality with Trimmomatic V0.40. The sequences belonging to bacteria and archaea were detected by Kraken2 and subsequently removed. While the identification of bacteriophages was carried out by BLASTn with a value of $e=1x10^{-5}$ and the NCBI viral database. Bacteriophage sequences were taken for analysis using BLASTx and the Comprehensive Antibiotic Resistance Database.

Results and discussion

Sequences similar to antimicrobial resistance genes were detected only in DNA samples; likewise, all the genes detected were against antibiotics of the Fluoroquinolone class (Table 1). These genes were detected in polyphyletic bacteriophages that due to the interaction with the host genome have a great genetic diversity; furthermore, this interaction with the host genome could explain the presence of these antimicrobial resistance genes.

The partial detection of these genes in a naturally preserved wetland could suggest the presence of fluoroquinolone-resistant bacteria. Further studies are needed to test for the presence of these resistant bacteria and how they reached the wetland to determine the impact on public, animal and environmental health.

References

Breitbart, M., Felts, B., Kelley, S., Mahaffy, J. M., Nulton, J., Salamon, P., & Rohwer, F. (2004). Diversity and population structure of a near-shore marine-sediment viral community. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1539), 565. https://doi.org/10.1098/RSPB.2003.2628

Jebri, S., Rahmani, F., & Hmaied, F. (2020). Bacteriophages as antibiotic resistance genes carriers in agro-food systems. *Journal of Applied Microbiology*, *130*, 688–698. https://doi.org/10.1111/jam.14851

Malki, K., Sawaya, N. A., Tisza, M. J., Coutinho, F. H., Rosario, K., Székely, A. J., & Breitbart, M. (2021). Spatial and Temporal Dynamics of Prokaryotic and Viral Community Assemblages in a Lotic System (Manatee Springs, Florida). *Applied and Environmental Microbiology*, *87*(18), 1–18. https://doi.org/10.1128/AEM.00646-21

Rosario, K., Nilsson, C., Lim, Y. W., Ruan, Y., & Breitbart, M. (2009). Metagenomic analysis of viruses in reclaimed water. *Environmental Microbiology*, *11*(11), 2806–2820. https://doi.org/10.1111/J.1462-2920.2009.01964.X

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ESSENTIAL OIL THYMOL EXTRACT MITIGATES THE MUCOSAL STRESS-IMMUNE RESPONSE FOLLOWING INTRAPERITONEAL IMMUNIZATION IN SEA BASS (*Dicentrarchus labrax*)

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Vaccination is an efficent prophylactic method to induce protection in fish against pathogens. However, protection conferred by vaccines is affected by the final producs of the HPI (hypothalamus-pituitary-interrenal) and BSC (brain-sympatho-chromaffin) axis, as vaccination can provoke stress responses at systemic and local levels.

The present study investigates the mitigation of the stress response induced by a bivalent vaccine against vibriosis and pasteurellosis after thymol-carvacrol oil extract treatments known as FishEase-C and FishEase-L in sea bass. Plasmatic and, skin mucus cortisol and mucosal transcript stress-immune response were measured at 1 and 24 hours post-vaccination (hpv).

Our results indicate that FishEase-L (FEL), but not FishEase-C, (FEC) was capable of reducing plasmatic cortisol secretion at 1 hpv and recovering the basal level at 24 hpv, in contrast to vaccinated and vaccinated and treated with FEC groups. A similar pattern was noted in the skin mucus though lesser secretion of cortisol was induced by the FEC.

The expression of stress-immune related gene transcripts (*gr1*, *gr2*, *crh*, *hsp70*, *cox2*, *il1*β, *il6*, *il8*, *il10*, *tgfβ1*, *lysozyme*, *igm*, *igt* and *igd/igt*) revealed that stress-immune gene regulation was not homogenous among mucosa.

Gills were more responsive to the stress effects induced by FEC, as shown by their dramatic expression of *crh*, *hsp70* and *cox2*. In addition, a potent inflammatory response, as evidenced by *il1β* and *il6* expression, was detected in the gills, suggesting higher absorption of this product via the gills. The gills also appeared to be more resilient to the stressful effect of the vaccine, while higher regulation was observed in the skin and intestine.

In summary, administration of FEL was able to negate the regulation of the stress-immune genes in the skin and intestine, mainly at 24 hours post vaccination, and hence mitigate the stress response. Furthermore, our findings show that the sedative effects of essential oils (EOs) is greatly influenced when they are combined or by secondary constituents, trace components and/or concentration utilized to sedate the animal.

INFLUENCE OF VIRAL RE-INFECTION ON TRANSCRIPTOMES OF DISEASE RESISTANT AND SUSCEPTIBLE EUROPEAN SEA BASS *Dicentrarchus labrax, L.* CHALLENGED WITH NERVOUS NECROSIS VIRUS

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Fish viral infections have great environmental and economic implications in aquaculture. As fish health and welfare is a prerequisite for sustainable and profitable production in the Mediterranean area, combating diseases is highlighted as priority for the development and improvement of aquaculture sector. The most significant disease in terms of severity, economic impact and spread, is viral nervous necrosis (VNN). VNN is a devastating disease, which induces cell necrosis accompanied by vacuolation in fish retina and brain. The disease is caused by nervous necrosis virus (NNV), which is a pathogen affecting more than 120 different species worldwide, causing high mortality and morbidity. The first step to move forward on the battle against the NNV disease is to fully understand its progression and its effect on the host.

Aim of the present work is to study how NNV re-infection affects the European sea bass (*Dicentrarchus labrax, L.*) transcriptome, in one disease resistant and one disease susceptible sea bass family. To determine how each family responds to re-infection, we performed RNA-sequencing analysis to assess differential gene expression in brain and head-kidney tissues of experimentally NNV-infected *D. labrax*. Fish were infected by the virus in a long-term study, and one month after the last recorded death, all surviving fish were re-infected by the same NNV strain. Fish tissues were sampled 7 days upon re-infection. A wide range of genes were up- or down-regulated in both *D. labrax* head-kidney and brain.

Genes de-regulation due to infection was more intense for the resistant family than the susceptible family. For both families, the enriched gene ontology (GO) categories included signaling and immune processes revealing their critical role in virus defense. However, only the resistant family utilized vesicle-implicating genes to be protected, as shown by GO enrichment analysis. Pathway analysis revealed a more intense pathways enrichment in resistant family in most KEGG categories. Only a few pathways were commonly enriched in the two families further indicating that the resistant and susceptible families utilize completely different mechanisms to fight the NNV infection.

Concluding, NNV resistant and sensitive sea bass transcriptomes were analyzed following NNV survivors' viral re-infection offering a glimpse on how host attempts to control the infection depending on its genetic background in relation with virus resistance.

Funding: The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: 03990)

FISHMEAL SUBSTITUTION BY FERMENTED SOYBEAN MEAL MODULATION OF IMMUNE AND PHYSIOLOGICAL RESPONSES OF EUROPEAN SEABASS

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Soybean meal is widely used as an alternative to fishmeal in aquafeeds due to its high protein content, availability, and competitive price. Fermentation technology of soybean meal is considered an effective method to reduce anti-nutrient factors and enhance nutrient digestibility, palatability, and immune function. The present study aimed to explore the impact of partial replacement of fishmeal by fermented soybean meal (FSBM) focusing on the underlying mechanisms involved on the immune responses of European seabass, an economically important fish species in European and Mediterranean aquaculture.

Five experimental diets containing increasing levels of fishmeal substitution by fermented soybean (FSM15, FSM30, FSM50, FSM70 and FSM80) and a control diet (CD) were produced by extrusion. European seabass juveniles were assigned to experimental tanks and each diet was allocated in triplicate groups. The feeding trial lasted for 93 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 aseptically and stored at – 80 °C. All procedures related to animal handling and sampling were carried out in compliance with the regulations and laws of both Greece and the European Union. Serum samples were used for biochemical and immunological parameters assessment. Total protein content, glucose, hemoglobin, nitrite ions (NO_2^-), lysozyme, myeloperoxidase, proteases and proteases inhibitors were studied. Total RNA was extracted from fish immune organs and real-time PCR assays were carried out to analyze the expression pattern of different immune-relevant genes (interferon pathway genes, cytokines, immunoglobulin, and T-cell markers).

Replacing fishmeal with fermented soybean meal in aquafeed had no significant effect on the immune system of European seabass when the replacement level was lower than 70 %. There was a moderate effect on some of the parameters studied when the degree of substitution was increased by more than 70%. Overall, the fishmeal substitution with increased levels of fermented soybean meal did not induce statistically important immune responses that would indicate adverse effects on the fish physiology.

The study was funded by the U.S. Soybean Export Council.

CLIMATE CHANGE RELATED STRESSORS IN AQUACULTURE: MODULATION OF GILL MICROBIOTA AND TRANSCRIPTOME IN ATLANTIC SALMON

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Climate change represents a threat to the development of the aquaculture sector. As a consequence, heatwaves are becoming more frequent and have been implicated in the increased occurrence of jellyfish blooms. In particular, *Aurelia aurita,* a common jellyfish found in Norwegian waters, is sensitive to temperature changes. Blooms have been linked with high mortality events, and this is likely related to their capability of causing gill and skin damage, thereby compromising barrier functions. Fish gill mucosal microbiome interacts with the environment, being the primary barrier of defense against external agents, making them useful for monitoring mucosal health. In addition, fish microbiota plays critical roles in many host functions such as nutrient metabolism, stress response, immunity, and protection against pathogens. To date, a limited number of studies are exploring the different interactions between fish mucosal microbial communities and scyphozoans within a climate change context. Thus, this study aimed to explore how multiple climate change-related environmental stressors, such as increasing temperature and limited oxygen availability, interact with salmon gill mucosal microbiota and gill gene expression after jellyfish (*Aurelia aurita*) exposure.

Smolts were reared under three different conditions for three months: Control (C) group was maintained at 12°C, Heatwave (HW) group was maintained at 12°C, gradually increasing up to 17°C and then progressively lowered again to 12°C. Heatwave limited oxygen (HWDO) group was maintained as the HW group, lowering the percentage of dissolved oxygen to 70% at 17°C. Next, fish were tagged and exposed to macerated jellyfish in a common-garden trial for 72 hours. Gill samples were collected, and the microbiota and host transcriptome were sequenced.

Gill microbiota showed a significant difference between the C group towards the HW and HWDO groups, regardless of the jellyfish exposure (Fig.1), highlighting the impact of the temperature alone over the oxygen concentration. At the transcriptomic level, increased temperature was the factor inducing more changes in non-exposed fish, masking the effects of jellyfish exposure. All tested stimuli significantly impacted pathways related to ion homeostasis and extracellular matrix organization. Further studies of stress and immune-related genes will provide details on the specific impact of each stressor and their combined effect.

Increased temperature seems to have a particularly strong effect on fish health, which may aggravate secondary problems like decreased oxygen availability or jellyfish blooms; therefore, understanding the dynamics of how gill microbiota and gene expression are modulated by climate change stressors will be crucial for developing mitigation strategies.

Supported by: Norwegian Research Council (#194050, Insight) and MSCA-ITN EATFISH (H2020 #956697).

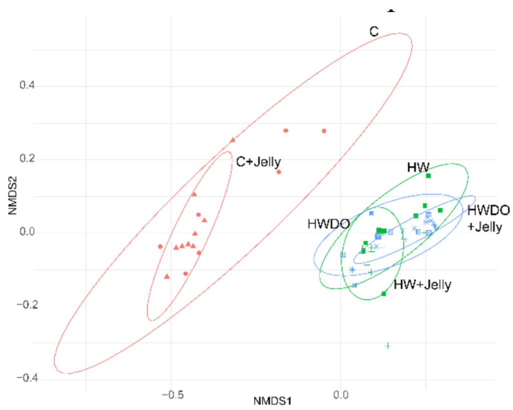

Fig.1: NMDS plot of the microbial populations in the different experimental groups.

HARNESSING COMPUTER VISION TO ASSESS DIET PALATABILITY: THE CASE OF RAINBOW TROUT (*Oncorhynchus mykiss*) FED DIETARY INSECT MEAL

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Introduction
Image processing and computer vision technologies play a crucial role in fish detection, encompassing species identification, population estimation, and behavior analysis (Pennington et al., 2019; Yang et al., 2021). While black soldier fly larvae meal (*Hermetia illucens*) (BSF) has gained traction as a dietary component in laboratory fish trials (Tran et al., 2022), the behavior of fish fed different dietary BSF remains largely underexplored. This study investigated the feed preference of rainbow trout (*Oncorhynchus mykiss*) fed four diets containing 0%, 2.5%, 5%, and 10% dietary BSF meal using computer vision and open-source video analysis.

Materials and methods:
Four isoproteic (47%), isolipidic (17%), and isoenergic (22 MJ/kg) diets were formulated and extruded into 4 mm diameter pellets. Eighty-four rainbow trout (158.9 ± 3.0 g) were randomly assigned to twelve 50 L tanks (7 fish/tank) and fed one of the four diets (three replicates per diet) for five days. Twelve digital cameras were installed above each tank to record feeding activity. Feed intake, pellet count, and heatmaps depicting fish and pellet movement were generated from the video recordings.

Results
Heatmaps generated from video recordings revealed similar patterns of fish and pellet distribution across all dietary groups (Fig. 1). There were no significant differences in feed intake among the experimental diets. Fish in all groups consumed the delivered feed entirely within 25 seconds of feeding activation, and no significant variations were observed in pellet consumption time (Fig. 2).

There was no significant difference in pellet consumption time among the dietary groups (P = 0.19) (Fig. 3).

Interestingly, pellet consumption time was positively correlated with dietary BSF inclusion level (P = 0.042, Adjusted *R-squared*: 0.282, *F-statistic*: 5.33) (Fig. 4). This suggests that fish took longer to consume diets containing BSF compared to the control diet.

Conclusion
This study demonstrates that despite comparable feed intake, fish fed BSF-containing diets exhibited a delayed feeding response compared to those fed the BSF-free diet. This suggests that BSF may be less palatable than fishmeal. Our findings propose an innovative, time-saving, and user-friendly approach to assess feed palatability in fish, surpassing conventional methods based solely on feed intake measurements. This approach holds potential implications for optimizing feed formulations and feeding practices.

Reference:
Pennington, Z.T., *et al*. ezTrack: An open-source video analysis pipeline for the investigation of animal behavior. Scientific Reports. 9, pp. 19979, (2019).
Tran, H.Q., *et al*. Systematic review and meta-analysis of production performance of aquaculture species fed dietary insect meals. Reviews in Aquaculture. 14, pp. 1637-1655, (2022).
Yang, L., *et al*. Computer Vision Models in Intelligent Aquaculture with Emphasis on Fish Detection and Behavior Analysis: A Review. Archives of Computational Methods in Engineering. 28, pp. 2785-2816, (2021).

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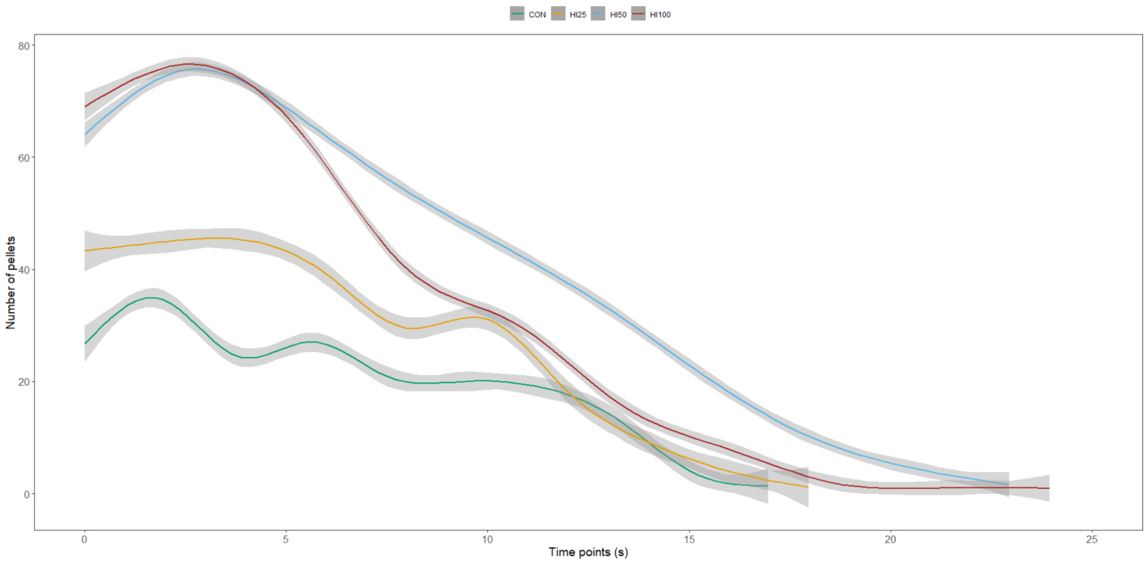

Figure 2. Pellet consumption of fish over time points

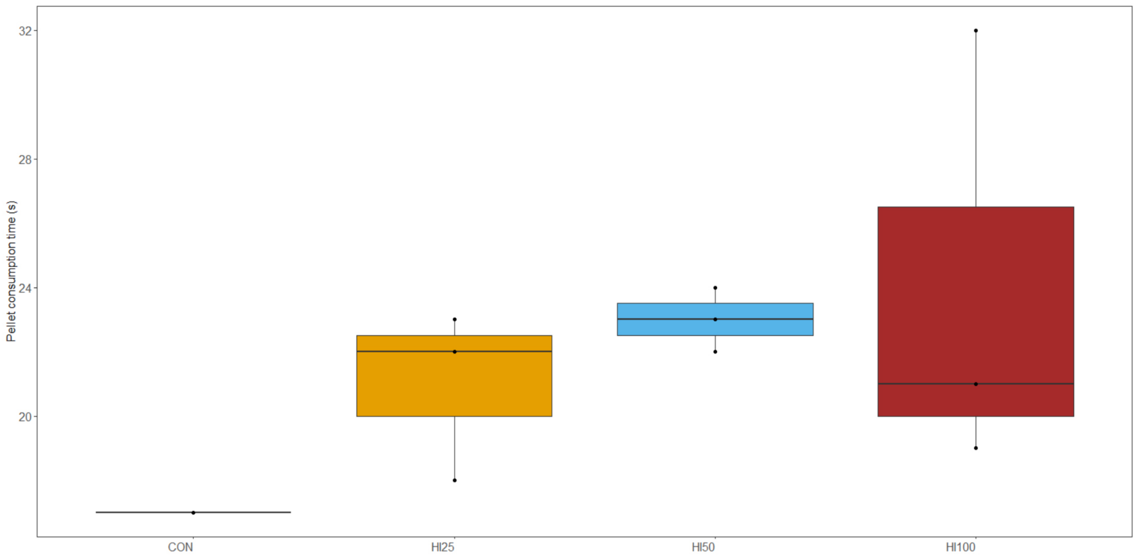

Figure 3. Pellet consumption time of fish fed four experimental diets

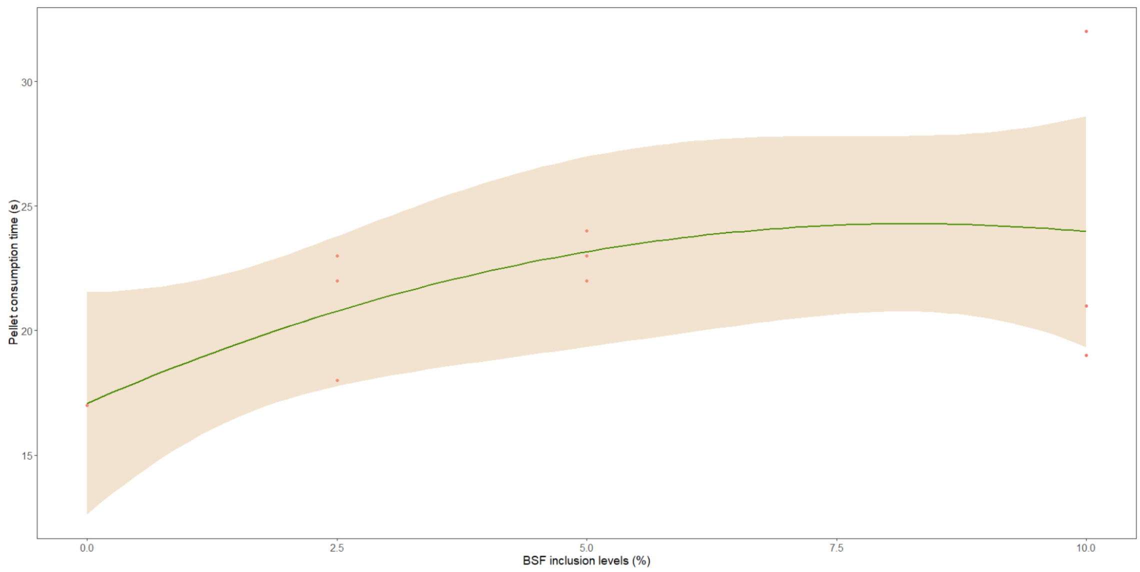

Figure 4. Regression model between dietary BSF and number of consumed pellet

EXPLORING THE IMPACT OF SUBSTITUTING DIETARY FISH OIL WITH RAPESEED OIL ON GROWTH, BODY COMPOSITION, AND GUT HEALTH IN NILE TILAPIA (*Oreochromis niloticus*)

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Introduction

In the face of escalating global demands for fish and the simultaneous decline in capture fisheries, the urgency to fortify aquaculture production has become increasingly apparent. With a rising global demand for fish and a concurrent decline in capture fisheries, the imperative to bolster aquaculture production becomes evident. However, the high cost associated with fish feed, primarily attributed to expensive fish oil and fish meal, presents a significant challenge. To mitigate this, researchers have explored the substitution of fish oil with alternative lipid sources, including vegetable oils such as (e.g., rapeseed oil, canola oil) and terrestrial animal fats (e.g., pork lard) in fish feeds has been widely evaluated in a great deal of researches (Mozanzadeh et al., 2016; Peng et al., 2016). This study aims to comprehensively investigate the impact of replacing fish oil with rapeseed oil on the performance of Nile tilapia (*Oreochromis niloticus*). Nile tilapia, a pivotal species in aquaculture, serves as an exemplary subject for such research, given its economic significance and widespread cultivation.

Materials and methods

Three iso-nitrogenous and iso-energetic diets were meticulously formulated, maintaining a balance of 54% crude protein and 13% crude lipid on a dry matter weight basis. The control diet and two experimental diets were structured with soy and pea protein concentrate in a 1:1 ratio, replacing 50% of fishmeal (FM) as the primary protein source. The experimental diets varied only in their alternative lipid source, with rapeseed oil (RO) replacing fish oil (FO) at 50% (RO50) and 100% (RO100). In June 2023, approximately 1200 juvenile tilapia, each with a mean weight of 4 g, were randomly allocated to twelve 500 L circular tanks. The experimental setup included continuous feeding and dimmed light settings in accordance with best commercial practices. Biometrical data, encompassing weight, total length, and height, were recorded at the initiation and conclusion of the 42-day experiment. Additionally, samples for chemical composition (whole fish), fatty acids, and gut histology were collected to assess the dietary effects on growth, chemical profile, and digestive health of Nile tilapia.

Results

Throughout the 42-day experimental period, the tilapia exhibited a remarkable six-fold increase in body weight, with no observed mortality, indicating the overall good health of the fish. Growth trajectories were generally consistent among groups, with the exception of the RO100 group, which displayed significantly lower growth compared to the other groups. Ongoing analyses include further investigations into chemical composition, fatty acids, and gut histology, and the comprehensive results will be presented.

References

Mozanzadeh, M. T., Agh, N., Yavari, V., Marammazi, J. G., Mohammadian, T., &
Gisbert, E. (2016). Partial or total replacement of dietary fish oil with alternative lipid sources in silvery-black porgy (Sparidentex hasta). *Aquaculture*, *451*, 232-240.

Peng, X., Li, F., Lin, S., & Chen, Y. (2016). Effects of total replacement of fish oil on growth performance, lipid metabolism and antioxidant capacity in tilapia (Oreochromis niloticus). *Aquaculture international*, *24*, 145-156.

A PERSPECTIVE USES OF GENOME EDITING IN AQUACULTURE

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Aquaculture is the fastest growing food production sector and is becoming the primary source of seafood for human consumption. Selective breeding programs allow genetic improvement of production traits, such as disease resistance, but progress is slow due to the often limited heritability and complex genetic composition of the traits as well as long generation intervals. New breeding technologies, such as genome editing using CRISPR/Cas9 have the potential to expedite genetic improvement which can contribute to sustainable solutions for aquaculture. Genome editing can rapidly introduce favorable changes to the genome, such as fixing alleles at existing trait loci, creating de novo alleles, or introducing alleles from other strains or species. The high fecundity and external fertilization of most aquaculture species, including Atlantic salmon can facilitate genome editing for research and application at a scale that is not possible in farmed terrestrial animals. We have in this context used gene editing in Atlantic salmon, with the long-term aim to address major bottlenecks of the industry. One of these is the genetic impact of escaped farmed salmon on wild populations, which is considered the most relevant long-term negative effect on the environment. The solution to this problem is the use of sterile fish. Here, we are working on methods for induced sterility involving gene editing. There are also sustainability issues associated with increased use of vegetable-based ingredients as replacements for marine products in fish feed, lowering the omega-3 content of the cultured fish. This transition comes at the expense of the omega-3 content both in fish feed and the fish filet of the farmed fish. Reduced fish welfare represents another obstacle, and robust farmed fish is needed to avoid negative stress associated phenotypes such as cataract, bone and fin deformities, precocious maturity, and higher disease susceptibility. Gene editing could solve some of these problems as genetic traits can be altered to create phenotypes of interest including disease resistance, sterility, and healthier fish fillets.

FISH BIOMASS MODELING IN 3D: AUTOMATING BODY WEIGHT ESTIMATION FROM UNDERWATER VIDEOS FOR BETTER FISH HEALTH

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Introduction
Accurate biomass estimation is crucial for effective fish farm management, facilitating optimized feeding, health assessment, disease control, and resource allocation. Manual counting and weighing are labor-intensive, leading to the rise of computer vision solutions powered by artificial intelligence. However, hardware costs and computing power remain limiting factors, particularly for three-dimensional information requirements. While advanced methods like 3D-image synthesis and stereo-vision cameras are effective for intensive industrial farms, a more accessible solution is needed for widespread adoption in the aquaculture industry. In collaboration, the Zurich University of Applied Sciences and Urban Blue have initiated the AWACS (Animal Welfare Assessment and Control System) innovation project, funded by the Innosuisse agency. The aim is to develop an automated assessment system for fish farms, enabling continuous monitoring, automatic assessment, and visual analysis of fish health and welfare.

Biomass modeling in a 3D environment
A biomass model using computer vision was developed to calculate fish weight based on single frames extracted from underwater camera videos. The third-dimensional information was derived from the camera's focal length. Increasing the camera's aperture reduces the depth of field, causing objects outside a certain distance to appear blurry. Object detection identifies fish in the frame and determines their blur value, excluding fish outside the focus area and retaining only those at a known horizontal distance from the camera lens. The reduction of data from a video to a subsample of several hundred high-quality single frames with fish suitable for the biomass model increases the quality of the biomass estimation and reduces the computing power and digital storage necessary.

Test results show the applicability of the algorithm
During purging, a 10 m3 RAS tank with 850 rainbow trouts was videotaped the day before slaughtering, and the weight was taken of each fish at slaughtering. The biomass model was calibrated with lateral pictures of 40 of these fish, and the video was analyzed with the algorithm. The estimated body weights were compared with the weights measured with the scale, and preliminary analysis shows a good fit of the two datasets, indicating the functionality of the method. This low-cost, low-computation, and easy-to-install setup improves real-time biomass estimation in aquaculture, both in research and industrial settings.

CONSUMERS' INTENTION TO BUY LOW TROPHIC AQUACULTURE PRODUCTS: AN EXPLORATORY STUDY OF EUROPEAN FOOD-RELATED LIFESTYLE SEGMENTS

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Given the rising global importance of low-trophic level aquaculture products (e.g. seaweed and mussels) for sustainable food supply, this study examined consumer intentions towards low-trophic level aquaculture products among European consumers. This study focuses on the influence of food-related lifestyles, health consciousness, food neophobia, and food quality attributes on consumer intentions for low-trophic level aquaculture products. We conducted an online questionnaire survey in Denmark, the UK, and Spain (500 respondents per country). The consumer segmentation analysis, employing clustering techniques, revealed five distinct segments characterized by Innovation, Responsibility, and Involvement, namely 'Adventurous', 'Uninvolved', 'Foodies', 'Rational', and 'Conservative', each reflecting unique consumer behavioral patterns.

Then, setting-up structural equation modelling to test the conceptual model revealed that attitude significantly influences consumer behavior, with subjective norms and attitude. This dual approach explains the model's predictive power while identifying targeted segments for sustainable aquaculture product marketing, ensuring the distinction between the model's test and the subsequent segmentation analysis is clearly articulated. To enhance the adoption of low-trophic aquaculture products, marketers should primarily target the 'Foodies' segment, characterized by high involvement and innovation, by emphasizing health consciousness and food quality attributes, while mitigating food neophobia and leveraging subjective norms.

CELLULOSE NANOMATERIALS: A NOVEL ADJUVANT AND DELIVERY SYSTEM FOR AQUACULTURE VACCINE APPLICATIONS

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Disease outbreaks are a major impediment to aquaculture production and are forecasted to continue as the industry grows and the climate warms. Vaccines are integral for disease management in aquaculture but they can be expensive, vary in effectiveness, and come with adjuvant-induced adverse effects causing fish welfare issues and negative economic impacts. The goal of this interdisciplinary project is to develop a new generation of vaccines for sustainable aquaculture. Our project uses novel nanomaterials produced from renewable wood fiber as depots/adjuvants in vaccine formulations to modulate the immune response of Atlantic salmon in a biocompatible, environmentally friendly, and cost-effective manner.

Our interdisciplinary research team is elucidating the role of cellulose nanomaterials (CNM) as a vaccine depot and mobile immunostimulant, the extent of CNM migration *in vivo*, and the efficacy of CNM bound antigen as an immunostimulant for protection against two Atlantic salmon pathogens. To accomplish this, we have prepared and conducted *in vitro* characterizations of CNM shear-thinning hydrogels and CNM/antigen (vaccine) formulations by scanning electron microscopy and rheology of CNM variants (CNM) and *in vivo* migration using histopathology. Additionally, we assessed safety/toxicity and immunogenicity of CNM shear-thinning hydrogel formulations *in vivo* as a vaccine depot in Atlantic salmon by quantifying the antibody kinetics in vaccinated fish serum using enzyme-linked immunosorbent assays and gene expression. The next phase of our work will involve conducting *in vivo* studies to evaluate the efficacy of the CNM vaccine(s) in protecting against *Vibrio anguillarum* in Atlantic salmon by performing a pathogen challenge study.

Our results to date will be reported and discussed.

GOVERNMENT MANDATED PARTNERSHIPS IN OFFSHORE SALMON AQUACULTURE

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Marine aquaculture production in open cage systems benefits from inflow of large quantities of sea water, but the open cage technology also means that it is simultaneously a potential source of fish diseases and environmental pollution. Aquaculture companies operating in a common ocean pool thus have potential negative external effects on each other, as well as other stakeholders. In principle, externalities can be regulated by government through taxes or quotas on environmental emissions. However, for fish health externalities such instruments may be less effective. This paper analyses government mandated partnerships as an alternative tool for achieving socially efficient or desirable outcomes. The case we analyze is offshore salmon aquaculture which, as illustrated by the figure below, is structurally different from conventional inshore aquaculture with respect to several important characteristics. We argue that the combination of externalities, large-scale investments and risks provide a rationale for mandated partnerships in offshore aquaculture.

Several market failures and other factors can influence the rationale and design of partnership agreement in an offshore aquaculture area: Externalities (e.g. fish diseases and sea lice), common infrastructure (e.g. vessels and marine surveillance), large-scale value chain investments, risk aversion and financial risks, and access to financing. Rationale of mandatory as opposed to voluntary partnerships is (1) asymmetric information between firms and (2) transaction costs in designing ankd enforcing a contract.

By utilizing lessons from offshore petroleum extraction and knowledge about the specific characteristics of offshore aquaculture we discuss design and propose parameters for a partnership agreement. This includes roles, responsibilities, and share of costs and revenues among the partners in a license. The agreement covers several critical aspects, which we discuss in greater detail: (a) Scope of agreement wrt. assets, investment activities, operational activities, incidents, (b) cost and revenue sharing, (c) operational roles and responsibilities of operator and partners, (d) decision-making processes and procedures, (e) compliance with license obligations (e.g. environmental and safety regulations, and reporting requirements), and (f) conflict resolution mechanisms between the partners.

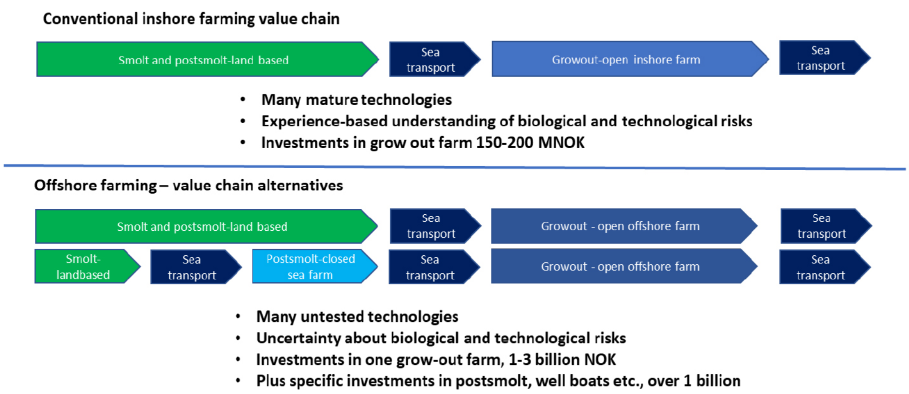

Figure. Conventional inshore vs offshore aquaculture value chain (1EUR=12 NOK)

EFFECTS OF FISHMEAL SUBSTITUTION BY FERMENTED SOYBEAN MEAL ON METABOLISM AND IMMUNE RESPONSES OF EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Soybean meal is widely used as an alternative to fishmeal in aquafeeds due to its high protein content, availability, and competitive price. Fermentation technology of soybean meal is considered an effective method to reduce anti-nutrient factors and enhance nutrient digestibility, palatability, and immune function. The present study aimed to explore the impact of partial replacement of fishmeal by fermented soybean meal (FSBM) focusing on the underlying mechanisms involved on the immune responses of European seabass, an economically important fish species in European and Mediterranean aquaculture.

Five experimental diets containing increasing levels of fishmeal substitution by fermented soybean (FSM15, FSM30, FSM50, FSM70 and FSM80) and a control diet (CD) were produced by extrusion. European seabass juveniles were assigned to experimental tanks and each diet was allocated in triplicate groups. The feeding trial lasted for 93 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 aseptically and stored at – 80 ∞C. All procedures related to animal handling and sampling were carried out in compliance with the regulations and laws of both Greece and the European Union. Serum samples were used for biochemical and immunological parameters assessment. Total protein content, glucose, hemoglobin, nitrite ions (NO_2^-), lysozyme, myeloperoxidase, proteases and proteases inhibitors were studied. Total RNA was extracted from fish immune organs and real-time PCR assays were carried out to analyze the expression pattern of different immune-relevant genes (interferon pathway genes, cytokines, immunoglobulin, and T-cell markers).

Replacing fishmeal with fermented soybean meal in aquafeed had no significant effect on the immune system of European seabass when the replacement level was lower than 70 %. There was a moderate effect on some of the parameters studied when the degree of substitution was increased by more than 70%. Overall, the fishmeal substitution with increased levels of fermented soybean meal did not induce statistically important immune responses that would indicate adverse effects on the fish physiology.

The study was funded by the U.S. Soybean Export Council.

DEVELOPMENT AND VALIDATION OF A MODEL TO EVALUATE THE ENVIRONMENTAL IMPACT OF FINFISH FARMING IN METROPOLITAN FRANCE AND OVERSEAS TERRITORIES: THE MOCAA PROJECT

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Models are important tools to address sustainability challenges associated with developing aquaculture at farm, regional and global scales[1]. The objective of the MOCAA project (*Modeling ecosystem assimilation capacity for a sustainable aquaculture*) is to develop a chain of modeling tools to assess the environmental impact of land-based and marine cage fish farming, in metropolitan France and overseas territories. The MOCAA decision-support tool considers the production systems characteristics (species, production, type and farm management, etc.) and the characteristics of the ecosystem (bathymetry, hydrodynamics, sensitivity of benthic ecosystems, etc.) to simulate the emission, the fate and the impact of aquaculture on the environment. The project involves aquaculture stakeholders to ensure that the tool will meet multi-stakeholder needs. The project is divided into three phases. For each phase, a review of existing tools has been realized, in order to integrate the most accurate existing ones. Workshops are organized in collaboration with fish farmers, public authorities and other stakeholders to present the approach, define needs and to co-construct the tool.

From 2021 to 2023, two modules have been developed and coupled: 1) a farm-scale module (integrating a dynamic bioenergetics model[2] and a farm management model) that simulate fish growth rates and emission of solid and dissolved waste for 7 marine fish species: Seabass (*D. labrax*), seabream (*S. aurata*), red drum (*S. ocellatus*), meagre (*A. regius*), trout (*O. mykiss and S. trutta*) and atlantic salmon (*S. salar*) and 2) a lagrangian dispersion model[3] that simulate the dispersion and dilution of particulate and dissolved waste in the water column. The dispersion module uses outputs of hydrodynamic models or measured water currents. In 2024, the validation of the growth model and farm model are carried out. Outputs of the growth model for each species have been confronted to published data (xx publications). Mean relative errors (MRE) depend on the fish species. The lowest MRE is observed for seabass with 0.14, indicating good accuracy of the model, followed by seabream and salmon species (MREs of 0.21 and 0.29 respectively). In contrast, the trout species showed the highest MREs (0.44 and 0.39). Validation at farm scale is carried out in fish farms within contrasted environment encompassing temperate (Brittany) and tropical (Martinique) conditions. The rigor of the approach is ensured by taking into account all stakeholders from construction to transfer of the modelling tool.

[1] Chary Killian, Brigolin Daniele, Callier Myriam (2022) Farm-scale models in fish aquaculture – An overview of methods and applications. Reviews in Aquaculture 12695, 1-32

[2] Kearney, M. R., & Porter, W. P. (2020). NicheMapR – an R package for biophysical modelling: The ectotherm and Dynamic Energy Budget models. Ecography, 43(1), 85–96. https://doi.org/10.1111/ecog.04680)

[3] Delandmeter, P and E van Sebille (2019), Geoscientific Model Development, 12, 3571–3584

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PRODUCT ATTRIBUTES AND BUYING BEHAVIOR OF PRAWN IN BANGLADESH: AN INVESTIGATION ON CONSUMER PREFERENCE AND CONSUMPTION PATTERN'

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The study delves into prawn attributes and buying behavior in Bangladesh, pivotal for the prawn industry's stakeholders. Understanding consumer preferences and consumption patterns is vital to bridge existing knowledge gaps, enabling businesses to align their offerings with market demands for enhanced effectiveness and sustainable growth.Top of Form

The study was undertaken to examine the prawn attributes and consumers' buying behavior of prawn and investigate consumer preferences and consumption patterns in four districts (i.e., Dhaka, Mymensingh, Khulna and Bogura) of Bangladesh.

120 respondents were interviewed based on stratified random sampling using a structured questionnaire to collect primary data. A combination of descriptive (i.e., sum, average, percentages, ratios, etc.), mathematical (i.e.,Gini coefficient, Lorenz curve, Angel curve, Willingness-To-Pay analysis) and econometric technique (i.e., Logit regression model, 5-point Likert scale) was used to analyze the data and to achieve the objectives.

The study depicts that majority of the respondents belong to the age group of 30-55 years old and they had a medium size family. A significant demand was found for prawn with the majority of consumers purchasing 3–5 times in a month. Notably, consumers with higher income tend to buy larger quantity suggesting a positive correlation between income and consumption. Moreover, the study reveals a 4.3% increase in prawn expenditure with a 10% rise in consumer income indicating income elasticity in prawn consumption. Consumer preferences for prawn are multifaceted driven by factors encompassing taste, year-round availability and suitability for various occasions. Moreover, attributes such as freshness, color, size, texture, appearance and family income significantly influence purchasing decisions. Statistically significant differences were observed among consumer segments highlighting variations in characteristics, consumption habits, awareness, and purchase intentions. The study reveals a positive correlation between product awareness and purchase intention underscores the importance of consumer education. Furthermore, consumer willingness-to-pay is negatively impacted by high prawn prices suggesting price sensitivity among consumers. Integrating consumer attribute preferences and Willingness-To-Pay (WTP) insights can inform the development of innovative prawn products to meet consumer demand better.

This study provides valuable insights into consumer behavior and preferences in the prawn industry, offering actionable implications for producers and policymakers alike. The study recommended monitoring prawn farms and product quality while regulating market prices to enhance consumer satisfaction and ensure market efficiency.

FISH ACOUSTIC TELEMETRY: CAUSAL STOCHASTIC SYSTEM AND MARKOV MODEL ANALYSIS

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Introduction
Fish welfare is a central concern in aquaculture, particularly regarding the physical well-being of fish and their behavioral patterns. This study explores fish telemetry data to analyze behavior and transition dynamics. The telemetry data, collected using various tags and transmitters, provides insights into fish movement, feeding habits, and habitat use. Through statistical analysis and modeling, this study aims to understand the underlying behavior patterns and their implications for fish welfare.

Dataset and Methods
The dataset comprises time-series data on fish acceleration levels, covering a period of 148 days with measurements taken approximately every 2 minutes. Analysis of the telemetry data involves constructing a stochastic causal system, with states defined by acceleration levels and transitions governed by conditional probabilities. Markov modeling is employed to analyze state transitions and their probabilities, offering a framework for understanding fish behavior dynamics.

Visualization techniques, including state trajectories and transition matrices, provide insights into the patterns and trends in fish behavior. The Perron-Frobenius theorem is applied to analyze the stability and convergence properties of the system. By evaluating the eigenvalues of the transition matrix, the long-term behavior and stability of the system are assessed. The steady-state distribution of the system is determined, offering insights into the long-term probabilities of being in each state.

Results and Discussion
The analysis reveals notable patterns in fish behavior, including frequent transitions within certain acceleration ranges and stability in others. The transition matrix highlights the conditional probabilities of transitioning between states, with certain states exhibiting higher probabilities of transition. Visualization of the transition dynamics through cobweb diagrams aids in understanding the causal relationships between states.

Overall, by understanding the behavior dynamics of fish populations, more informed decisions can be made to promote their well-being in captive environments.

Acknowledgment
The study was _supported by the Ministry of Education, Youth and Sports of the Czech Republic - project CENAKVA (LM2018099), the CENAKVA Centre Development (No:CZ:1:05/2:1:00/19:0380) This project also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 871108 (AQUAEXCEL3.0). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein

References
Urban, J., Lastovka, D. 2024. Fish acoustic telemetry as causal stochastic system and Perron-Frobenius analysis of its Markov model. In International Work-Conference on Bioinformatics and Biomedical Engineering. Springer, Cham.

EDUCATIONAL AND TRAINING INNOVATIONS IN THE AQUACULTURE SECTOR OF HUNGARIAN SECONDARY AGRICULTURAL EDUCATION

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The study applies a deductive approach to specific areas of education and training within the general trends in aquaculture, covering the main traces of PILOT training in fisheries as a niche discipline. Current paper, targeted at educators, focuses on the results of a questionnaire survey of 94 educators. Our main objective was to assess the presence of aquaculture-related knowledge content and associated methodologies in the classroom and the general digital literacy of educators.

Our questionnaire study was structured around the following research questions:

Q1: To what extent do the surveyed teachers deal with the aquaculture sector, which includes pond fish farming, intensive (precision) fish farming and natural aquatic fish farming (including angling), in their teaching?

Q2: What digital competences do respondents have and how do they integrate digital skills into their learning and teaching processes?

Q3: Based on your experience as an educator, what traditional and modern methodologies/tools are most effective in engaging learners?

Q4: Based on the teachers' perceptions, how could sector professionals help promote aquaculture among educators?

According to the teachers who responded, the initiation of discourse and the establishment of cooperation between the professional actors of the sector and teachers working in agricultural education is crucial for the positioning and attractiveness of the aquaculture sector for young people. Companies should also put more emphasis on creativity and marketing, by producing online content, organising technical presentations and factory visits.

The work is supported by the Ministry of Agriculture under grant project HAGF/212/2023; and special thanks to the teachers who filled in the questionnaire.

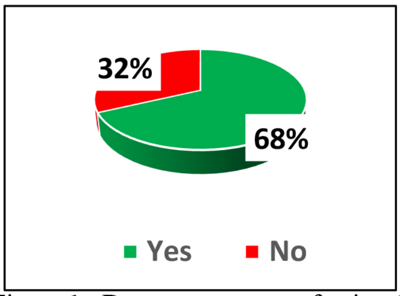

Figure 1.: Do you use any professional documents produced by the aquaculture sector when preparing your teaching materials?

TECHNICAL INFRASTRUCTURE ASSESSMENT OF THE HUNGARIAN AQUACULTURE SECTOR - DEVELOPMENT DEMANDS AND CHALLENGES

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In the Hungarian aquaculture sector, continuous improvement of mechanisation and technical standards is key to maintaining competitiveness and ensuring sustainable production. However, it is important that these improvements not only increase efficiency, but also consider ecological sustainability and minimise environmental impacts.

The increasing demand for aquaculture products, the deteriorating labour market situation, and the need to maintain the economic viability of fish production and fish farming are driving the aquaculture sector to improve the technologies used and to reduce exposure and risks from external influences by introducing smart solutions.

In recent years, there has been an increasing trend towards the use of automated systems and IT solutions in the aquaculture sector. Such technologies can be used to optimise production processes, monitor water quality, and monitor fish health.

The initial results confirm our hypothesis that the sector is aware of the potential of technical improvements and that, overall, technical improvements in all the areas studied are considered important for the reproduction, breeding, production, and marketing processes.

Based on the surveys, the intention to strengthen the infrastructure base (buildings, facilities, energy supply) and the determination to upgrade the technologies based on it to the most advanced digital level are perceived as priorities.

The analysis of some 300 sub-questions in the questionnaires, linked to the different areas of expertise, will make it possible to identify the internal links needed to formulate technical development strategies, thus making the survey a complete one.

The work is supported by the Ministry of Agriculture under grant project HAGF/213/2023; and special thanks to the companies who filled in the questionnaire.

CHALLENGES AND SOLUTIONS FOR CARP-DOMINANT POND AQUACULTURE IN HUNGARY TODAY

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Carp-dominated pond aquaculture in Hungary faces several challenges that impact productivity, sustainability, and economic viability. During our investigations, we identified the following challenges: i) Environmental, ii) Biological, iii) Economic, iv) Regulatory, v) Technological, vi) Social and Cultural and vii) Competing Land Uses.

To address these challenges, a multi-faceted approach is necessary:

- Integrated Water Management: Implementing efficient water use practices and enhancing water quality management can mitigate environmental impacts.
- Disease Prevention: Developing comprehensive disease management plans, including biosecurity measures and vaccination programs.
- Economic Diversification: Exploring value-added products and alternative markets to stabilize income and reduce dependency on single revenue streams.
- Policy Advocacy: Engaging with policymakers to create supportive regulatory frameworks and access to subsidies or financial incentives.
- Technology Integration: Investing in technology and training to enhance productivity and sustainability.
- Community Engagement: Building awareness and acceptance of aquaculture through education and outreach programs.

By addressing these challenges through collaborative efforts involving farmers, researchers, policymakers, and industry stakeholders, the sustainability and profitability of carp-dominated pond aquaculture in Hungary can be significantly improved.

The work was supported by the Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences.

HOW TO IMPROVE STATUS AND WELBIENG OF EEL *Anguilla anguilla* IN SERBIA?

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Introduction
European Eel (Anguilla anguilla) – a fish of Osteichthyes (bony fishes) taxonomic order, Anguillidae family. It dwells in fresh water and dies in salt water. The significant alterations to the Danube River's course through the Djerdap Gorge occurred after the construction of the Iron Gate I and Iron Gate II dams, in 1970 and 1984. This led to an interruption of the Danube's longitudinal continuity and to the curtailment of migration routes of anadromous fish species that migrate from the Black Sea up the Danube for spawning. In addition to having a negative effect on anadromous and potamodromous species, dams also have a negative effect on catadromous species such as the eel (Lenhardt et al., 2018). Generally, it is obvious a lack of data about research and the status of eel in Serbia, as well as any official statistical data in the previous period. Because, until 1968 the Statistical Office of Serbia collected catch data for species (incl. eel) recorded in rivers, channels, and lakes by both recreational and commercial fishermen together. Following that period, eels are only occasionally present in the catch of commercial fishermen, but there is no continuous monitoring of the species (Smederevac–Lalic et al., 2011). Worldwide, the status of European eel remains critical. The recruitment of European eels strongly declined from 1980 to 2011. Time series from 1980 to 2023 show that eel recruitment remains at a very low level (ICES, 2023). Based on the Law on the protection and sustainable use of fish stocks (Anonymous, 2014) in 2015, the Serbian Ministry of Agriculture and Environmental Protection introduced a permanent fishing ban on eel, that came into force in the same year through the Regulation for Conservation and protection of fish stocks (Anonymous, 2015).

Materials and Methods
The abovementioned data encouraged us to make a short analysis of available data about the status and endangerment of eel in Serbia incl. research results as well as public sources (media and newspapers).

Results and discussion
Besides the rare scientific publications about the biology and ecology of eel in Serbia, which are older than 10 years, it is interesting to mention some scientifically unverified data from local newspapers. So, it was found that in October 2009 one sports fisherman from Novi Sad caught a 90-centimeter-long eel on the Danube with a remark that "a catch that has not been recorded on the banks of a large European river in Novi Sad for half a century". Four years later (2013) it was announced that two fishermen from the city of Uzice caught a 1.10-meter-long eel on Zlatar Lake (an artificial lake located among the mountains of Zlatibor and Zlatarin the Southwest part of Serbia). Local authorities from the Special Nature Reserve "Uvac" (to which Zlatar Lake belongs) supposed that "eel was born and raised in the Uvac canyon, and judging by its length, it led a secret life in the mud of the reservoir for more than 15 years". Besides that, in the official report of the joint EIFAAC/ICES/GFCM working group on eels, WGEEL (ICES, 2023) it was published that a joint operation coordinated by Europol, involving law enforcement authorities across the globe, has dealt with a major blow to organized crime groups engaged in international glass eel trafficking. During the period from 2016 to 2023, more than 30 countries (including Serbia) participated in "Operation LAKE VII" led to the arrest of 256 persons responsible for the illegal trafficking of 25 tonnes of live eels worth around EUR 13 million.

The findings of this study suggest that there is a certain space for improvement of the status of eels in Serbia. Certainly, a necessary precondition for any action to be implemented would be to design extensive research about the current biology and ecology of eels in Serbia. Simultaneously, it is recommended capacity building in the local and governmental authorities about this threatened species. Besides the Serbian Ministry of Environmental Protection, there are two important institutions, which are in charge of concrete action in the field: the Institute for Nature Conservation of Serbia in Belgrade and the Institute for Nature Conservation of Vojvodina Province in Novi Sad. Indeed, according to our findings, there are only a few persons (experts) in these two institutes who are in charge of implementing measures to protect all endangered fish species (incl. eel) for the whole territory of Serbia. An additional activity would be a promotional campaign to raise awareness of the importance of the eel as an endangered species for target groups of schoolchildren and students in Serbia.

(Continued on next page)

This research was funded by the Ministry of Education, Science and Technological development of Serbia on the basis of the contracts for the realization and financing of scientific research work in 2024 (The Contract No. 451-03-65/2024-03/200117).

Reference:

1. Anonymous (2014) Law on protection and sustainable use of fish stocks. "Officiale Gazette of RS" No. 128/2014, 95/2018 (*In Serbian*)
2. Anonymous (2018) Regulation on measures for the conservation and protection of fish stocks. "Officiale Gazette of RS" No. 56/15, 94/18 (*In Serbian*)
3. ICES (2023). Joint EIFAAC/ICES/GFCM Working Group on Eels (WGEEL). ICES Scientific Reports, 5(98), pp.7-63.
4. Lenhardt M. , Djikanović V., Hegedis A., Visnjic Jeftic Z., Skoric S., Smederevac-Lalic M. (2018) Qualitative and quantitative changes in the ichtyofauna of the danubian reservoirs after the construction of the iron gates hydropower plant dams. Ecological and Economic Significance of Fauna of Serbia, 171, pp.143-172.
5. Smederevac-Lalic M., Visnjic-Jeftic Z., Pucar M., Mickovic B., Skoric S., Nikcevic M., Hegedis A. (2011) Fishing circumstances on the Danube in Serbia. Water Research and Management, 1(4), pp.45-49.
6. Zonn I.S., Kostianoy A.G., Semenov A.V., Joksimovic A., Djurovic M. (2021) European Eel (Anguilla anguilla). The Adriatic Sea Encyclopedia, pp.124-126.

GROWTH PERFORMANCE, BLOOD PROFILE, AND GONADAL DEVELOPMENT OF AFRICAN CATFISH *Clarias gariepinus* FED SOURSOP *Annona muricata* LEAF MEAL

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Intensive practices in aquaculture, like high stocking density, create stressful environments and disease outbreaks, leading to increased reliance on antibiotics. Plant-based alternatives like Soursop (*Annona muricata*) leaves that are rich in bioactive compounds provide a cost-effective and eco-friendly solution. Despite its recognized health benefits, their potential in aquaculture remains unexplored. This study investigates the potential of *A. muricata* leaves in African catfish (*Clarias gariepinus*) by analyzing their biochemical composition and assessing their effects on growth, blood parameters, and gonadal development in catfish juveniles.

For this 56-day feeding trial, fresh soursop leaves were obtained, authenticated, processed into a meal (SLM), and assessed for biochemical components. A total of 180 juveniles were randomly divided into four groups (control, T1, T2, and T3) of 45 fish, each with three replicates. Fish in the respective groups were fed diets (38% crude protein, 2.7 mcal/kg ME) containing SLM at 0%, 2.5%, 5%, and 7.5%, respectively. Feeding was done twice daily at 5% body weight. Weekly body weight and length measurements were taken. Blood samples and female ovaries were collected for further analysis at the end of the study.

SLM exhibited favourable nutritional profiles, rich in Fe, Zn, and vitamin E. Significant differences in growth performance were observed, with the control group showing the highest feed intake, final weight, and specific growth rate, comparable to T1 but differing from the T3 group, which exhibited the least performance (Table 1). Hematological and lipid profiles remained unaffected across the groups. Serum estrogen levels increased with SLM inclusion, peaking in T3, indicating a dose-dependent effect. Ovarian development varied, with enhanced maturation observed in the control and T3 groups (Table 2). Incorporating SLM at up to 2.5% in catfish diets maintained growth performance and physiological status, while higher levels may influence reproduction, necessitating further investigations for broodstock management in aquaculture.

Table 1. Growth performance of African catfish fed varying levels of Soursop leaf meal

Parameters	Treatments				
	Control	T1	T2	T3	P-Value
Final Weight (g)	339.20 ± 3.16^{a}	334.23 ± 8.43^{ab}	306.34 ± 7.90^{bc}	299.62 ± 7.83^{c}	< 0.01*
Weight Gain (g)	191.20 ± 6.35^{a}	183.72 ± 2.44^{a}	150.70 ± 5.96^{b}	149.27 ± 2.16^{b}	< 0.01*
SGR (%g/day)	1.50 ± 0.07^{a}	1.42 ± 0.03^{ab}	1.21 ± 0.15^{c}	1.23 ± 0.03^{bc}	< 0.01*
Total Feed Intake (g)	597.05 ± 6.65^{a}	606.13 ± 10.21^{a}	571.24 ± 6.35^{ab}	541.06 ± 20.39^{b}	0.022*
Feed Conversion Rate	3.13 ± 0.14^{b}	3.30 ± 0.03^{ab}	3.80 ± 0.15^{a}	3.62 ± 0.12^{ab}	0.016*
Condition Factor, K	1.00 ± 0.01^{a}	1.25 ± 0.2^{a}	1.07 ± 0.03^{a}	1.07 ± 0.07^{a}	0.257^{NS}
Survivability	100 ± 0.0^{a}	93.3 ± 5.77^{a}	100 ± 0.0^{a}	96.7 ± 5.77^{a}	0.219^{NS}

*row means with different superscripts $^{(a,b,c)}$ differ significantly ($p < 0.05$); ^{NS}: Non-Significant; SGR: Specific Growth Rate

Table 2. Ovarian development of African catfish juveniles fed varying levels of Soursop leaf meal

Parameters	Treatments				
	Control	T1	T2	T3	P-Value
BW (g)	368.60 ± 5.	302.40 ± 9.06^{bc}	276.85 ± 18.37^{c}	340.95 ± 4.85^{ab}	< 0.001
OW (g)	29.60 ± 5.(	7.50 ± 0.70^{b}	10.95 ± 1.82^{b}	19.35 ± 1.93^{ab}	< 0.001
GSI	7.92 ± 1.4	2.46 ± 0.26^{b}	3.80 ± 0.45^{b}	5.70 ± 0.64^{ab}	0.002
E_2 (ng/mL)	14.99 ± 0.:	17.60 ± 1.22^{bc}	20.62 ± 1.16^{ab}	22.39 ± 1.10^{a}	< 0.001

^{a,b,c} Row means with different superscripts are significantly different ($p < 0.05$); OW: Ovarian weight; GSI: Gonadosomatic index.

MODELLING AND MAPPING CARBON CAPTURE POTENTIAL OF FARMED BLUE MUSSELS IN THE BALTIC SEA REGION

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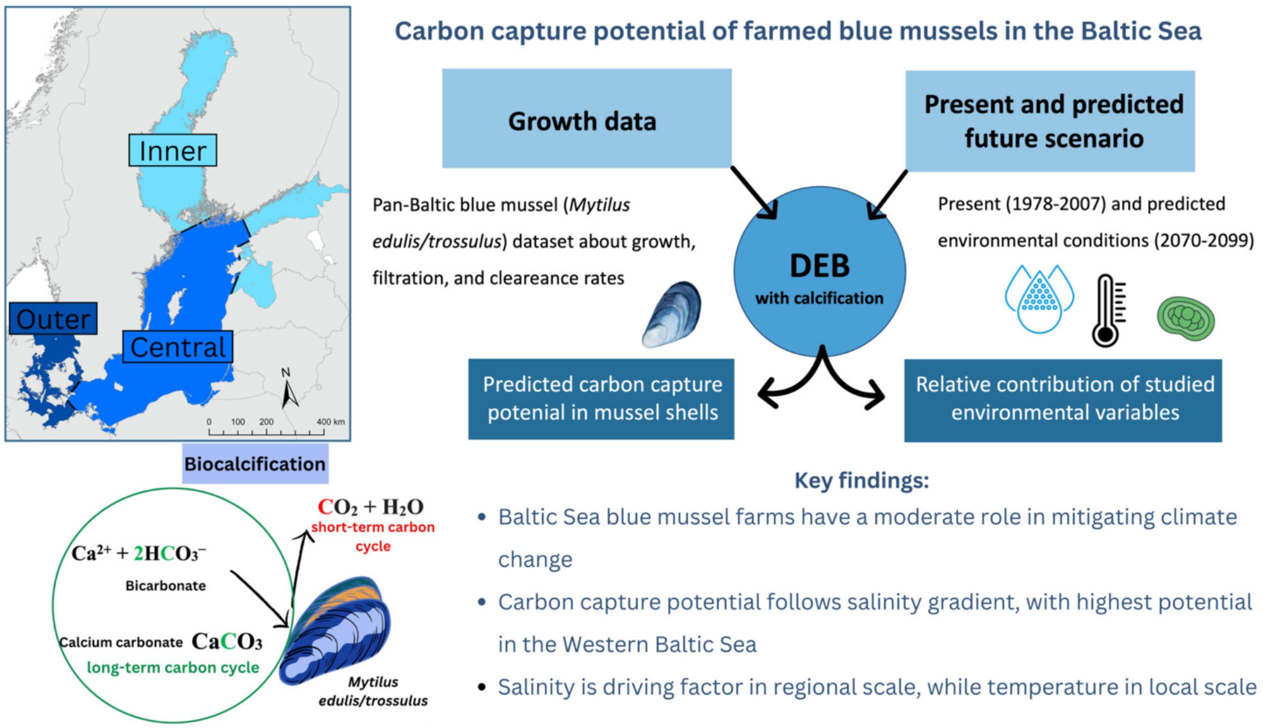
This study applies a regional Dynamic Energy Budget (DEB) model, enhanced to include biocalcification processes, to evaluate the carbon capture potential of farmed blue mussel (*Mytilus edulis/trossulus*) in the Baltic Sea. The research emphasizes the long-term capture of carbon associated with shell formation, crucial for mitigating global warming effects. The model was built using a comprehensive pan-Baltic dataset that includes information on mussel growth, filtration and biodeposition rates, and nutrient content. The study also examined salinity, temperature, and chlorophyll *a* as key environmental factors influencing carbon capture in farmed mussels. Our findings revealed significant spatial and temporal variability in carbon dynamics under current and a plausible future scenario. The tested future predictions are grounded in current scientific understanding and projections of climate change effects on the Baltic Sea. Notably, the outer Baltic Sea subbasins exhibited the highest carbon capture capacity with an average of 55 tonnes (in the present scenario) and 65 tonnes (under future environmental conditions) of carbon sequestrated per farm (km^2) over a cultivation cycle – 17 months. Salinity was the main driver of predicted regional changes in carbon capture, while temperature and chlorophyll *a* had more pronounced local effects. This research advances our understanding of the role low trophic aquaculture plays in mitigating climate change. It highlights the importance of developing location-specific strategies for mussel farming that consider both local and regional environmental conditions. The results contribute to the wider discourse on sustainable aquaculture development and environmental conservation.



INDICATORS FOR QUANTIFYING ENVIRONMENTAL REMEDIATION OR POLLUTION OF AQUACULTURE SYSTEMS

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Aquaculture can remediate or pollute the surrounding environment according to the system design. The following indicators were developed to quantify these aquaculture services or disservices to ecosystem integrity. Positive values indicate pollution mitigation, whereas negative values indicate that the aquaculture system causes pollution. Loads are measured in mass. Effluents may be the outlet water from ponds and tanks, or the water after passing through farms comprised of net-cages, pens, long-lines, trays, etc.

1. Eutrophication (E_N and E_P)
Mariculture: E_N = (Load of nitrogen in source water - Load of nitrogen released in effluents) / mass or units produced
Freshwater Aquaculture: E_P = (Load of phosphorous in source water - Load of phosphorous released in effluents) / mass or units produced
2. Oxygen depletion (O_d)
O_d = (BOD_5 in source water – BOD_5 released in effluents) / mass or units produced
3. Organic Pollution (OP)
OP = (Load of organic matter in source water – Load of organic matter released in effluents) / mass or units produced
4. Siltation (S)
S = (Load of total suspended inorganic solids in source water – Load of suspended inorganic solids released in effluents) / mass or units produced
5. Global Warming (GW)
GW = (Load of greenhouse gases absorbed – load of greenhouse gases released to the atmosphere) / mass or units produced
Greenhouse gases = mass of CO_2 + CH_4 + N_2O, measured in CO_2 equivalents
6. General Chemical Pollution (GCP)
GCP = (Load of chemicals in source water – Load of chemicals in effluents) / mass or units produced
Chemical products = mass of herbicides, insecticides, anti-algae, antibiotics, and other chemicals applied
7. Pollution by Heavy Metals (PHM)
PHM = (Load of heavy metals in source water – Load of heavy metals in effluents) / mass or units produced
8. Changing α-biodiversity (CB)
CB = 100*($S\text{-}W_d$ – $S\text{-}W_s$) / mass or units produced
In which:
$S\text{-}W_d$ = Shannon-Winner diversity index obtained in a similar place not impacted by the farm
S-Ws = Shannon-Winner diversity index obtained surrounding the farm

The above indicators were successfully validated on different freshwater fish, marine shrimp and bivalve farms in Brazil.

(Aquavitae-Horizon 2020; FAPESP; CNPq)

EFFECT OF TEMPERATURE ON LYMPHOCYTE IMMUNE POPULATIONS IN ASYMPTOMATIC ATLANTIC SALMON (*Salmo salar*) NATURALLY INFECTED WITH *Piscine orthoreovirus* (PRV) IN A MARINE FARM

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Piscine orthoreovirus (PRV) is a double-stranded RNA virus responsible for heart and skeletal muscle inflammation (HSMI), an emerging disease in Atlantic salmon (*Salmo salar*) in aquaculture. PRV is highly prevalent in Chile, and it is common to find phenotypes with asymptomatic infection without associated lesions. PRV's persistence can involve the release of anti-inflammatory cytokines and reduced cell populations associated with the Th1 response. The persistence phenotype of PRV in Atlantic salmon under productive conditions has not been evaluated. Also, it has been shown in fish that water temperature significantly impacts the immune response of fish to viral infection, which could modify the response of salmon to PRV in the different seasons.

This work analyzed the cell populations of 15 salmon from a culture center in the Magallanes Region, Chile. Blood samples were collected in autumn, winter, and spring. The diagnosis by qPCR of several pathogens (including PRV) was ruled out. Peripheral blood lymphocyte populations were characterized by flow cytometry based on the expression of IgM, CD4, and CD8 surface markers in PRV (-) and PRV (+) fish. In the case of the PRV (-) fish, a significant decrease in $IgM^-/CD4^-$ cells along with a significant increase in $CD8^+$ cells was detected in autumn (Fig. 1A). In winter, an increase in $IgM^-/CD4^-$ cells and a decrease in $CD8^+$ cells were observed (Fig. 1B). No PRV (-) fish were detected in spring (Fig. 1C). By contrast, PRV (+) fish showed in autumn a significant increase in IgM-/CD4- cells and a significant decrease in $CD8^+$ cells. Importantly, these patterns were consistent in all the seasons evaluated. No IgM^+, $IgM^+/CD4^+$ cells were detected in the PRV (+) fish in spring (Fig. 1C). These results contribute to the knowledge of fish immunity to PRV infection and how temperature may compromise its modulation in a marine productive environment.

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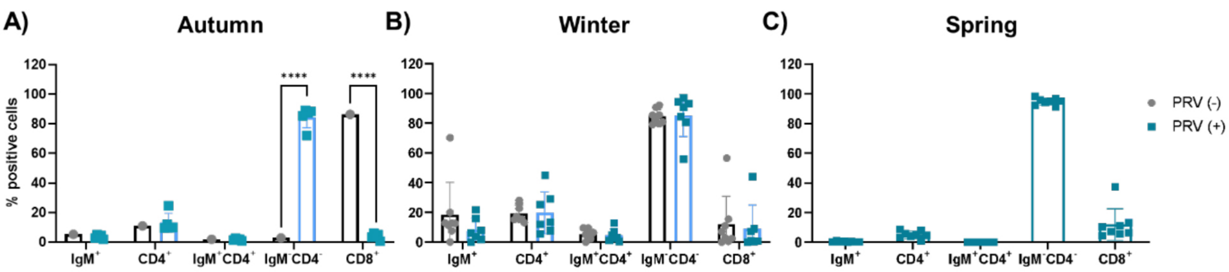

Fig. 1: Effect of PRV on blood cell populations in asymptomatic fish in different seasons using surface markers. A) autumn, B) winter, and C) spring. Statistical differences were represented as follows: P<0.05(*); P<0.01 (**); P<0.001 (***); P<0.0001 (****).

TWO METHODS FOR ELECTRICAL STUNNING OF FARMED PANGASIUS

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To avoid severe discomfort in fish at slaughter or killing, it is key to protect these animals by stunning them, i.e. rendering fish unconscious and insensible first. This is a general provision in the Council Regulation (EC) No. 1099/2009. This provision is met when stunning induces an immediate loss of consciousness and sensibility in fish, which lasts until death.

It is known that most farmed fish are produced in Asia. Since 2014, Vietnam has been the third exporter of farmed fish. Vietnam has become by far the world's leading producer and exporter of farmed pangasius. We, therefore, selected pangasius in our experiments.

In our experiments, we aimed to establish the specifications for two electrical stunning methods for farmed pangasius, i.e. in water and after dewatering. We also assessed whether cutting the ventral aorta could prevent recovery of the stunned animals.

Electrical stunning in water. For electrical stunning in water, a field strength of 7.2 V/cm (50 Hz ac) was applied in (140-200 µS/cm) to pass sufficient current through the brains of each fish, using EEG registrations and behavioural observations. Individual pangasius were exposed to a one-second stun to establish the conditions for an immediate loss of consciousness and sensibility. Cutting the ventral aorta after an exposure of 20 s to the electricity resulted in death in the individual fish without recovery, using EEGs and behavioural observations.

Electrical stunning after dewatering (Fig. 1). Our data showed that 200 V 50 Hz ac across the electrodes for 1 second is sufficient for an immediate stun in pangasius, using EEG registrations and behavioural observations. When the fish were exposed for 15 s to the electricity followed by cutting the ventral aorta, an immediate loss of consciousness and sensibility was achieved without recovery, using EEGs and behavioural observations.

To conclude, our data show that both electrical stunning in water and after dewatering can serve as an effective stunning method for farmed pangasius. Recovery of the stunned animals can be prevented by cutting the ventral aorta.

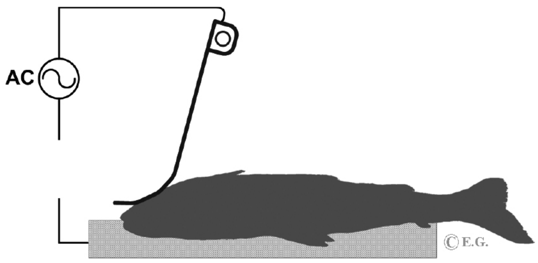

Figure 1: Electrical stunning after dewatering

THE SOCIAL SCIENCE OF OFFSHORE AQUACULTURE: UNCERTAINTIES, CHALLENGES AND SOLUTION-ORIENTED GOVERNANCE NEEDS

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Aquaculture technology is on the move, enabling production in more open and exposed ocean environments around the world. These new systems offer solutions to environmental challenges facing conventional aquaculture, yet new technologies also create new social challenges and potentially exacerbate, or at minimum recreate, others. Offshore aquaculture research and governance are still in early stages, as is our understanding of the social repercussions and challenges associated with development.

This paper provides an evaluation and reflection on offshore aquaculture from a social science perspective and is based on findings from a modified World Café group discussion method including the thoughts and experiences of social science experts.

Key challenges and uncertainties including a lack of an appropriate regulatory framework, societal perceptions of offshore aquaculture, and offshore aquaculture's contribution to society were identified. Among the identified challenges are conflicts of space and concern of social equity outcomes. These identified challenges and uncertainties coincide with long-standing challenges of conventional aquaculture. The governance implications of these challenges are discussed as well as the need for social sciences to address these challenges through transformative and transdisciplinary approaches that bridge science and society.

In conclusion, we observe that the technological changes in offshore aquaculture challenge conventional governance and require transformed and disrupted solutions that intersect not only science and society, but also different scientific bodies and disciplines. The development of offshore aquaculture is both a challenge and opportunity for the application of this transformed mode of research and understanding. In this regard, transdisciplinary research approaches are warranted. This implies a different orientation of science and its role in governance in the 21st century. The character of this new (transformative) orientation of science is only now beginning to emerge, but will need to accommodate new opportunities for science and research, in tandem with society. Only then can we forge a collective meaning on how to manage the complex challenges for offshore aquaculture.

THE EFFECTS OF SEAWEED INTEGRATION INTO RECIRCULATING SHRIMP AQUACULTURE: SHRIMP PERFORMANCE AND MICROBIOME

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As the global shrimp aquaculture industry rapidly expands, monitoring animal health during production becomes increasingly critical. Productivity is closely tied to animal health, and the gut microbiome is emerging as a key factor for successful cultivation. The gut microbiome or gut microbiota refers to the microbes that colonise the gut. This microbial community plays a crucial role as they contribute to the digestive and immune system of the host. This relative new insight offers a promising alternative to antibiotics still widely used for disease management. Supplementing microbiota has shown to enhance growth and survival rates in various commercial species.

In this study efforts were made to alter shrimp microbiome in two ways; (i) by cultivating seaweed alongside shrimp production within the same system and (ii) by introducing a reformulated shrimp feed containing seaweed in low dietary inclusion rates. The aim is to evaluate the effect of shrimp performance; in terms of algal biotransformation, shrimp growth and robustness, and microbiome modulation in shrimp and culture water. For this purpose Zilt prepared three recirculating aquaculture systems (RAS). A control RAS (CTRL) operated under optimal production conditions and fed with a conventional formula. A RAS suitable for integrated multi-trophic aquaculture (IMTA), in this case *Caulerpa lentillifera* - *Litopenaeus vannamei,* also fed with a conventional formula referred to as T2. Lastly, a RAS was operated under optimal conditions but fed using the same feed formula but with 1% dried algae incorporated. This treatment is referred to as T1. The three systems were operated in parallel over a period of 28 days. Among daily monitoring major sampling rounds were planned at day 0, day 9 and day 28 to collect shrimp gut, hepatopancreas and culture water samples.

Some preliminary results are presented in Table 1. The treatment with seaweed included feed (T1) performed poorly in terms of shrimp robustness with an average survival of only 50%. During the trial leftover feed and cannibalism was observed, indicating little affinity to the adjusted feed. No definitive conclusion can be drawn whether seaweed cultivation in T2 has a positive or negative effect on shrimp performance. More definitive answers will follow once gut microbiome is mapped using the BlueRemediomics Discovery Platform (aided by European Molecular Biology Laboratory) Additionally, mass spectrometry will be used to understand any microbial differentiations in culture water between different treatments.

Acknowledgments: Funding: BlueRemediomics-Grant No.101082304, EU Horizon Europe Programme. Farmed shrimp were sourced from white panther, Austria

ROLE OF POND FARMING IN THE EUROPEAN AQUACULTURE SCENE – REALITY AND VISIONS

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Fish production in ponds is the oldest type of aquaculture that has a more than two-thousand-year-old history both in Asia and Europe. However, raising finfish and other species in constructed earthen ponds is by far the most widespread culture method globally even nowadays. Pond fish farming is a diverse subsector of the world aquaculture with the wide range of species produced and colourful production method from the most intensive ones to the extensive way. The most significant species produced in ponds are Cyprinids. These species give the 48% of the global aquaculture production. Although the Common carp is the third most abundant species with 4,236.3 thousand tonnes production on global level, European pond fish farming, which is based on the Common carp production is less than 10% of total EU aquaculture production (FAO 2022).

In the EU pond fish farming is a special segment of the diverse aquaculture sector. Even if traditional pond fish farming left behind from other systems in volume, pond aquaculture has diverse and complex environmental benefits. Maintaining 250,000 ha of man-made wetlands in the EU, pond farms highly contribute to preserving wetland related biodiversity, especially those located in Ramsar- and NATURA200 areas (FDFS & HAKI 2020). Most of these fish farms comprise not one pond but entire systems of ponds, including extensive canal and/or embankment systems. We should emphasise however, that the mentioned benefits of fishponds would not exist if there were no well managed fish farming in the ponds. Pond farming requires low levels of inputs, strongly integrated into natural environment so have a low impact on the environment (Koushik et al 2020). Ponds are also contributing to increase the climate resilience with carbon sequestration, retention of water as well as the soluble and floating compartments of supply (Knösche et al. 2004). Pond farming assists in the better water management. Pond aquaculture provides one of the most complex web of different ecosystem services connected the human food production systems (Willot et al. 2019, Palásti et al 2020, Färber et al. 2020).

The cultural value of traditional pond fish farming has also been recognised by various international schemes such as the FAO Globally Important Agriculture Heritage Systems (GIAHS) or the UNESCO Intangible Cultural Heritage (ICH) (FAO 2024). The operation of fishponds is a source of livelihood for many people in rural areas, where employment options are limited. The multifunctional characteristics of pond fish farms is also increasing (Békefi & Váradi 2007, Popp et al. 2018, 2019).

Preserving the complex environmental and social benefits of pond aquaculture and maintaining its traditions is an indisputably important task in which supportive policies must play an eminent role. However, in addition the preservation of this European cultural heritage, space must be open to increase viability of pond farming through innovations. Sustainability level of traditional pond fish production can be further increased by applying new systems and technologies. Fishponds can be important components of Combined Intensive Extensive systems for sustainable intensification as well as enhancing the circular approach integrated with other food production systems, either animal husbandry or plant production. These innovations could make aquaculture a key player in the freshwater blue bioeconomy. The application of early integrated systems (e.g. fish-cum-duck or fish-cum-rice) can also be reconsidered, using new methods, equipment and facilities that are now available as a result of research and technology development. There is a very wide range of possible utilization of fishponds, from extensive to intensive production function according to their size, location, environmental characteristics, and other factors. There is a need for further research and innovation to explore appropriate use of existing fishponds to meet economic, environmental and social challenges, without losing, but rather enhancing specific values that a fishpond can provide. This requires the introduction of a much more targeted research and innovation funding framework than is currently existing.

FIELD BASED OBSERVATIONS ON THE POTENTIAL IMPACT OF KRILL MEAL ON INTESTINAL RESILIENCE IN ORGANIC EUROPEAN SEABASS, *Dicentrarchus labrax*

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Antarctic krill meal and oil are well known sustainable and functional ingredients in aquaculture feeds, rich in essential aminoacids, omega-3s, phospholipids, micronutrients, and antioxidants such as astaxanthin, shown to enhance growth, health, and fillet quality in different fish species. The present findings are part of a wider field trial with seabass aimed at evaluating possible biological performance benefits of partial fish meal replacement by krill meal while using isonitrogenous and isocaloric diets in organic production of European seabass. A 12-week field trial, with juveniles weighing 4.5 grams, was carried out at Galaxidi Marine Farm in Greece under commercial organic fish farming conditions. Two identical and proximal sea cages were used, each housing approximately 170,000 fish. Performance data, including feed consumption, FCR and mortalities were recorded during the monitoring period and also at the end of the trial. When the final weights of fish reached approximately 60g, ten fish from each group were randomly selected, and sampled for liver and intestine histology. The histological status of the livers and the intestines were assessed using a qualitative scoring system. During the trial, an outbreak of vibriosis caused by *Vibrio harveyi* occurred affecting the section of the fish farm where the trial cages were situated, resulting in mortalities among the population, with no treatments administered. The control group fed fish meal-based diet exhibited clinical signs of severe enteritis, with intestines showing loss of normal tissue architecture, reduced villi length, pronounced reduction of supranuclear vacuoles, epithelial sloughing, and inflammation. In contrast, the intestines of the fish fed the krill including diet maintained normal tissue architecture, displaying a normal histological appearance with well-developed villi, particularly rich in supranuclear vacuoles, especially in the distal gut area.

Although no statistically significant differences in biological performance data recorded were observed between the groups, the histological observations indicate that incorporating krill meal into organic fish feeds has the ability to strengthen intestinal resilience in seabass and expedite the healing process of lesions associated with bacterial enteritis. These findings are in agreement with other published studies indicating the advantageous effects of krill meal enriched diets in wound healing and tissue restoration. Of particular note is the possibility of developing further the inclusion of krill meal in functional fish diets aimed at facilitating recovery post-bacterial enteritis infections under commercial farm conditions.

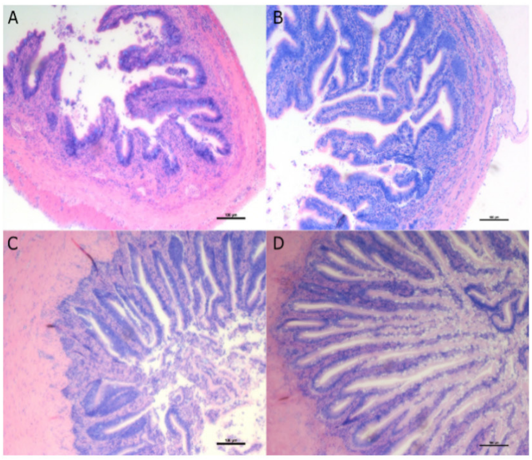

Figure 1. A & C. Midgut and distal gut of control fish, **B & D.** Midgut and distal gut of fish fed krill-diet.

USE OF SOLID STATE FERMENTATION TO IMPROVE THE NUTRITIONAL VALUE OF FORAGE LEGUMES AS FEED INGREDIENTS IN EUROPEAN SEA BASS NUTRITION-*IN VIVO* EVALUATION

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The European Union (EU) is dependent on imports of soya beans to meet its domestic needs. In this respect, the development of alternative endemic feed ingredients is key to decrease its dependence. Legumes such as *Lupinus albus*, with their high protein content and balanced amino acid profile, are promising candidates. However, the presence of anti-nutritional factors (ANF) that inhibit enzymatic activity and the absorption of other nutrients restricts their use in feeds. Solid-state fermentation is a novel technology in the field of animal nutrition for utilising plant ingredients. Microorganisms, such as *Saccharomyces cerevisiae*, have the ability to produce enzymes and bioactive metabolites and reduce ANF's during fermentation (Ihtifazhuddin et al., 2016, Dileep et al., 2021). The aim of the present study was the replacement of soybean meal by Lupin meal fermented with *Saccharomyces cerevisiae*, in European sea bass nutrition.

Lupin seeds were fermented by inoculation with *Saccharomyces cerevisiae* at a concentration of 1^10E6/g of substrate. Four diets were formulated, one control containing only soybean meal and three diets replacing soybean meal by fermented lupin meal in three different levels 67, 83 and 100%. Juvenile sea bass (18.9 g initial weight) were fed the experimental feeds *ad libitum* three times per day for a period of 71 days.

In the current study inclusion of fermented lupin meal, revealed positive results. Specifically, the population fed diets with fermented lupin showed higher final weight (62.5-63.8g) in contrast to the population fed control diet. Even though FCR did not show significant differences, a trend (P=0.09) for lower FCR was observed for fish treated with FRL2 compared to those treated with control diet. Significantly higher digestibility of protein observed for the groups fed diets FRL2 and FRL3 compared to control group. These results showed that fermented lupin is a promising alternative to soya for Mediterranean aquaculture.

Table 1. Formulation of experimental feeds (g/100g)

	CTRL	FRL1	FRL2	FRL3
Fish meal	20	20	20	20
Krill meal	3	3	3	3
Blood meal	8	8	8	8
Wheat meal	12.5	12.9	13.1	13.3
Wheat Gluten	3	3	3	3
Corn gluten	15.1	15.2	15	15
SPC	7	7	7	7
Soybean meal	**15**	**5**	**2.5**	
Fermented Lupin		**10**	**12.5**	**15**
Fish Oil	13.2	12.6	12.5	12.3
Others	3.2	3.4	3.4	3.4
Nutrients				
Protein	46.1	46.5	46.4	46.5
Fat	16.9	17	17.1	17

Table 2. Growth parameters and digestibility of protein

	CTRL	FRL 1	FRL 2	FRL 3
Final Weight g	61.8	63.8	63.1	62.5
stdev	1.8	1.6	2.6	0.4
Weight increase g	42.8	44.9	44.2	43.6
stdev	1.7	1.6	2.6	0.4
FCR	1.2	1.2	1.2	1.2
stdev	0.0	0.0	0.0	0.0
SGR	1.7	1.7	1.7	1.7
stdev	0.0	0.0	0.1	0.0
% consumption	2.1	2.1	2.1	2.1
stdev	0.1	0.0	0.0	0.0
Dig. Protein %	91.1^{a}	92.0^{ab}	92.4^{b}	92.9^{b}
stdev	0.3	0.2	0.5	0.4

CONVENTIONAL FISHMEAL VS FISHMEAL PRODUCED BY *Lagochepalus sceleratus-IN VITRO* EVALUATION

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Fishmeal is recognised as a premium protein source in aquaculture diets. However, the high reliance on it, as the main protein source in aquafeeds, has led to over-exploitation of marine resources, resulting in sustainability and economic issues. Efforts to reduce unsustainable catches have resulted in a reduced supply of marine ingredients for aquafeed production. In addition, several plant ingredients have been investigated as alternatives to fishmeal, with controversial results. A new potential source that does not compete with human food supplies and has no economic value, are invasive species such as *Lagocephalus sceleratus* (Gmelin, 1789). These have rapidly established themselves in the Eastern Mediterranean, with various impacts on the ecosystem and human welfare, and may provide an alternative marine protein source to conventional fishmeal. The aim of the current study was to evaluate *in vitro* the fishmeal produced by *Lagocephalus sceleratus* compared to conventional fishmeal.

Fishmeal from *Lagocephalus sceleratus* (FmL) was obtained after homogenisation, cooking, pressing, drying and grinding. A commercial conventional fishmeal (FmC) was used for comparison with FmL in terms of nutritional value using *in vitro* analyses (Table 1).

In vitro evaluation of fishmeal showed that FmL is high in nutritional value relative to FmC. The protein content was found to be higher and the fat content lower. The amino acid profile was found to be balanced with elevated levels of lysine and methionine. It is worth noting that all essential amino acids were present in higher concentrations in FmL in relation to FmC. Regarding essential fatty acids, FmL is deficient in EPA but rich in DHA. Moreover, arachidonic acid (ARA) and docosapentaenoic acid (DPA) are found in higher quantities in FmL. Additionally, it is a rich source of zinc (Zn) and iron (Fe) but is less abundant in copper (Cu) and manganese (Mn). Overall, the nutritional profile of fishmeal produced by *Lagocephalus sceleratus* (FmL) can be considered as an alternative protein source.

Table 1: Nutritional value of fishmeal

Macro/micro components	**FmC**	**FmL**
Protein g/100g	66.0	73.2
Fat g/100g	10.0	8.8
Moisture g/100g	8.0	2.3
Ash g/100g	15.0	17.7
Arginine g/100g	3.0	4.4
Histidine g/100g	0.5	0.6
Isoleucine g/100g	3.3	4.2
Leucine g/100g	4.5	5.1
Lysine g/100g	7.4	9.6
Methionine g/100g	1.6	1.8
Phenyalanine g/100g	4.1	4.8
Threonine g/100g	2.5	2.6
Valine g/100g	3.3	3.8
Saturated g/100g	3.2	3.5
Monounsaturated g/100g	2.3	2.2
n9 g/100g	1.1	1.4
n6 g/100g	0.3	0.7
n3 g/100g	3.0	2.4
ARA g/100g	0.1	0.5
EPA g/100g	1.4	0.2
DHA g/100g	1.2	1.9
DPA g/100g	0.2	0.4
HUFA g/100g	2.6	2.0
Ca g/100g	2.4	5.2
Cu mg/kg	8.9	2.2
Fe mg/kg	216	330
Zn mg/kg	70	388
Mn mg/kg	8.5	3.5

INSECT MEAL, INSECT FRASS AND HYDROPONIC BY-PRODUCTS FED TO COMMON CARP (*Cyprinus carpio*) WHICH WAS CO-CULTURED WITH LETTUCE (*Lactuca sativa)* IN A CLOSED-LOOP AQUAPONIC SYSTEM: AWARE PROJECT

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Aquaponics is recognized as an innovative and environmentally friendly production system. The aim of this study is to evaluate a) the growth performance of *Hermetia illucens* larvae fed with wheat bran-based substrates supplemented with 25, 50, 75 and 100% of hydroponic by-products (vegetative parts of tomato and cucumber plants, as well as low quality, non-marketable cucumbers and tomato fruits) and b) the effect of feeding insect meal (*H. illucens)*, insect frass and hydroponic by-products as a replacement of fishmeal on the growth performance of common carp (*Cyprinus carpio*) and on the growth and nutrient uptake of lettuce (*Lactuca sativa*) in a laboratory closed-loop aquaponic system (CLA).

In a first trial, fifty (50) six-day-old *H. illucens* larvae were introduced in the vials together with 15 g of each feeding substrate composed of 6 g of solid substrate and 9 ml of water using separate vials for each substrate. Wheat bran-based feeding substrates with different percentages of hydroponic by-products, i.e., 0, 25, 50, 75 and 100% were evaluated. The inclusion of hydroponic by-products at high rates (50 and 75%) significantly hindered larval growth. In another trial, a total of 297 *C. carpio* individuals were obtained from a local fish hatchery and were distributed in 9 CLA systems. In the hydroponic part of the system, 108 *L. sativa* individuals were placed in the hydroponic baskets. Three diets (3 replicates/dietary treatment) were formulated, the Control diet (C) containing 15% of fishmeal, the *H. illucens* diet (B), were the fishmeal protein of the control diet was replaced at 50% by *H. illucens* meal and the plant residues (F) diet were the fishmeal protein of the control diet was replaced at 50% by a mix of *H. illucens* meal, *H. illucens* frass and hydroponic by-products at a 2:1:1 ratio. Growth performance indicators of common carp (Table 1) showed no statistically significant differences across the three dietary treatments ($p>0.05$) during the 45 days of the experimental period. All 9 CLA systems effectively cultivated lettuce with optimal root-to-shoot ratios. These results aim to contribute to the enhancement of the sustainable profile of *H. illucens* larvae produced as aquafeed ingredient.

	C	B	F
Weight Gain (g)	5.04 ± 1.83	5.01 ± 1.74	4.53 ± 1.71
SGR (%/day)	2.08 ± 0.11	2.15 ± 0.11	2.16 ± 0.12
Total Leaf Number	34.34 ± 0.86	36.08 ± 0.77	36.92 ± 1.27
Plant fresh weight (g)	78.01 ± 7.89	104.10 ± 8.06	89.80 ± 10.57
Plant height (cm)	21.67 ± 0.36	22.40 ± 2.64	21.65 ± 3.07
Root-to-shoot ratio	0.19 ± 0.02	0.16 ± 0.008	0.19 ± 0.032

Table 1: Growth performance of carp and lettuce (means ± standard error).

METHIONINE NUTRACEUTICAL ROLE AGAINST VIRAL HEMORRHAGIC SEPTICEMIA VIRUS (VHSV) INFECTION IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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The European aquaculture of rainbow trout (*Oncorhynchus mykiss*) is severely affected by pathological events such as outbreaks of viral hemorrhagic septicemia virus (VHSV). Aiming to minimize this issue, numerous efforts have been made to find preventive and therapeutic strategies. Dietary supplementation of methionine (MET) has been recently demonstrated to have beneficial effects on fish resistance to bacterial infections. However, there are no studies regarding the ability of this essential amino acid to modulate host antiviral responses. The present study focused on the ability of dietary MET to modulate the immune response of trout against VHSV. Juvenile trout (5.9 ± 0.9 g) were fed a control diet (CTRL) or a methionine-supplemented (MET, 2x the requirement for the species) diet for a period of 4 weeks and then sampled. Thereafter, the remaining fish were bath challenged with VHSV (10^5 ml^{-1} $TCID_{50}$) and sampled in a time-course manner. Skin and gills were collected for viral load and RNAseq analyses, before (0 h, n = 16) and post-challenge (24, 72 and 120 h, n = 9). By assessing tissue viral load, the peak of infection was recorded at 72 h for both dietary treatments. Curiously, at that time, MET-fed fish showed up-regulation of significant immunological pathways, indicating a positive interaction of this diet at the peak of infection. Particularly in the gills, the MET diet at 72h displayed a strong up-regulation of immune pathways of the general immune response, bacterial recognition agents, DNA and RNA polymerase, pattern recognition receptor pathways and regulatory transcription. These pathways are important against VHSV infections. On the other hand, MET treatment showed to down-regulate pathways involved in cell cycle regulation, biological process and stimulation and apoptosis. In the skin, despite having less positive regulation of differentially expressed genes (DEGs), enrichment of type I interferon related pathway was observed, which is part of the host's innate immune response against viruses. Some of the most significant DEGs in both tissues have viral immune response functions. In short, MET dietary supplementation allowed an improved response of rainbow trout juveniles against VHSV infection, with the up-regulation of GO terms associated with an improved immune response, despite the similar viral load between the two diets at the peak of infection.

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CARBOHYDRATE MANAGEMENT IN PONDS AND BIOFLOC SYSTEMS

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Nutrient availability drives production in pond-based aquaculture systems. The principal nutrients considered in this study are carbon (C), nitrogen (N) and phosphorous (P). The challenge is to make these nutrients available in the right amounts and ratios to the farmed species. The nutrient use efficiencies, measured as the fraction of available nutrients retained in de novo fish production, provide insight in the total amounts of nutrients accumulating, volatilizing or discharged from the pond. The environmental sustainability of pond farming depends on how well the nutrients that were not retained in fish biomass can be recycled.

Carbohydrates (CHO) are the most abundant type of molecules available in nature, the majority of which are non-starch polysaccharides (NSP) (Fig.1). Higher animals, including fish and shrimp, cannot digest NSPs, but bacteria and fungi in ponds can. Globally, inland surface waters emit annually 6 times more CO_2 by degrading NSPs than the burning of fossil fuels (doi:10.3389/fenvs.2022.904955). In nature, these CHOs are the fuel used to break down organic matter and provide the N and P that drive natural food production in aquatic ecosystems.

With pond feeds we do the opposite. Compared to terrestrial NSP-rich wastes, aquaculture feeds are super-rich sources of N and P, but also a poor energy (C) source. Post-feeding aquaculture waste contains insufficient energy to recycle the N and P waste through the food web. In outdoor ponds, extra carbon is added through algae production, but in intensive systems the algae production is too small to recycle all the post-feeding N and P waste. For this reason, nutrients are removed, often by discharging to surrounding surface waters, or extra CHO is applied, as in biofloc systems, to partially recycle the waste and to maintain water quality.

Recent research results will be shared showing that the type of CHO administrated affects production in ponds and biofloc systems. In extensive and semi-intensive systems, including up to 40 – 45% of feed ingredients like wheat bran or rice bran rich in lignocellulosic and hemicellulosic compounds (Fig.1) in pelleted feed did not lower production compared to conventional feed in tilapia ponds, and resulted in slightly less production in whiteleg shrimp biofloc systems.

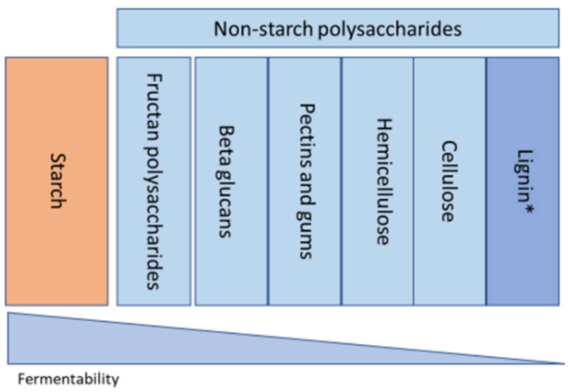

Fig. 1: Classes of dietary carbohydrates from terrestrial plants

HOW SELECTED FISH LINES CONTRIBUTE TO RESEARCH: THE CASE OF EUROPEAN SEABASS *Dicentrarchus labrax* LINES DEVELOPED IN IFREMER PALAVAS

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Selected lines represent a valuable tool, particularly in the field of genetics, where they are employed to investigate the influence of genetics on specific traits. In addition to this, divergent lines and population lines can also be used for other animal studies, such as those pertaining to physiological and behavioural functions.

Over the past 25 years, Ifremer has maintained natural populations of European seabass from the Atlantic to the East Mediterranean and has developed several selected lines in its research station of Palavas-les-Flots (France). The experimental selection program of Ifremer naturally started by selecting growth traits in the years 2000. Then, more complex traits have been investigated, including feed efficiency, adaptation to plant-based diets, resistance to diseases, and processing traits (e.g. fillet yield). For the majority of those traits, the genetic variability was first assessed on the three natural populations (Atlantic, East and West Mediterranean). Based on these investigations, we developed and maintained several lines: G+ (maximized growth line); FD- (low weight loss under Feed Deprivation); FD+ (high weight loss under Feed Deprivation); Eff+ (feed efficient), Eff- (feed inefficient); Noda+ (resistant to nodavirus); Noda- (sensitive to nodavirus); FEM+ (sex ratio biased to females); and ALBI (homozygous albino). These lines have been selected from one to four generations.

These European seabass populations and lines contribute to research in various research fields, including quantitative genetics, population genetics, physiology, ethology, nutrition and zootechny. A total of 28 research projects have made use of those lines. These projects have led to the writing of 107 scientific articles using the seabass lines so far. The populations and lines were employed to investigate a range of aquaculture traits and their interrelationships. These included growth at various thermal regimes or various diets (Boucher et al., 2012; Rodde et al., 2020; Montero et al., 2023)the use of fish meal and fish oil has been widely challenged, leading to the rapidly increasing use of plant-based products in feed. However, high substitution rates impair fish health and growth in carnivorous species. We demonstrated that survival rate, mean body weight and biomass can be improved in rainbow trout (Oncorhynchus mykiss, processing yields (carcass%, fillet%, viscera%, muscle fat), feed conversion ratio (Grima et al., 2010a, 2010b; Daulé et al., 2014; Besson et al., 2019; Montero et al., 2023)and selecting fish to genetically enhance this trait would be highly valuable. However, no selective breeding program specifically targeted to feed efficiency exists for farmed fish, mostly because of the difficulty of measuring individual feed intake. However, a negative phenotypic correlation between feed efficiency and weight loss at fasting has been previously demonstrated in sea bass submitted to feed deprivation (FD, hypoxia tolerance (Ferrari et al., 2016; Rodde et al., 2021; Nati et al., 2023)fish from a full facto-rial mating (10 females x 50 males, NNV resistance (Doan Q. et al., 2017; Griot et al., 2020, 2021; Barsøe et al., 2021; Delpuech et al., 2023)also called viral encephalo- and retinopathy (VER, or sex (Anastasiadi et al., 2018; Faggion et al., 2018, 2021; Geffroy et al., 2021; Goikoetxea et al., 2022; Clota et al., 2024;)which grow faster and mature later than males. However, typical rearing practices often result in populations with a high proportion of males. To test whether photoperiod, temperature and fasting impact the sex ratio, two distinct experiments were conducted. In the first one, we tested the effects of two photoperiods (12L:12D vs 10L:14D. These findings provide key insights for ongoing selective breeding programs, which contribute to the EU blue economy. Additionally, they offer insights into aquaculture's potential to adapt to climate change. The natural populations also constitute valuable genetic material for exploring the phenotypic divergence between Atlantic and Mediterranean European seabass and understanding the underlying evolution mechanisms (Duranton et al., 2018, 2020)"uris":["http://zotero.org/users/1953436/items/SPPKBHBM"],"itemData":{"id":767,"type":"article-journal","abstract":"Understanding how new species arise through the progressive establishment of reproductive isolation (RI.

Most of the selected lines developed in Ifremer facilities with the support of DGAMPA (French Directorate General for Maritime Affairs, Fisheries and Aquaculture) are open to collaboration, do not hesitate to contact us, especially using the AQUAEXCEL3.0 and AQUASERV transnational access programs.

RESILIENT SUPPLY CHAINS UNDER PRESSURE: INVESTIGATING HOW SHOCKS IMPACTED THE ENVIRONMENTAL FOOTPRINT OF NORWEGIAN SEAFOOD PRODUCTS

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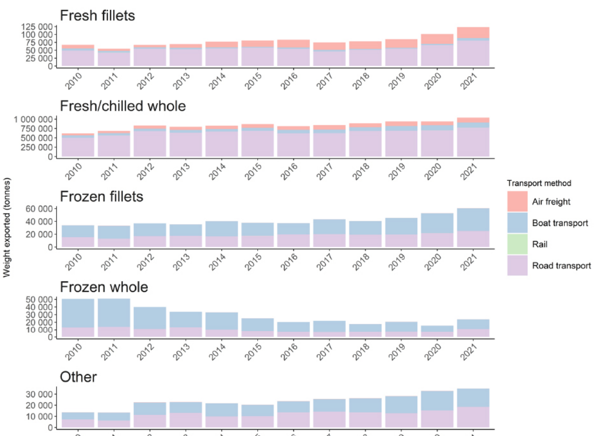
Introduction

Norway is a global leader in fish production from both fisheries and aquaculture. Advocates for Norwegian seafood products claim many products, such as farm-raised salmon, can play a pivotal role in reducing the environmental footprint of food production. However, as seafood is highly perishable, seafood supply chains are vulnerable to global economic shocks which disrupt the processing and shipment of products. In this study, we focused on how different economic shocks in the previous 10 years (specifically, China's ban on Norwegian salmon products from 2010-2014, Russia's ban on Norwegian seafood products from 2014-present, trade restrictions due to the global COVID19 pandemic 2020-2021, and Brexit's impact on Norwegian mackerel fisheries due to a lapse in the Common Fisheries Policy in 2021) impacted the logistics network of salmon, cod, mackerel, king crab, and snow crab products. These product groups were selected due to their relevance to the Norwegian seafood market's environmental footprint.

We used trade data from 2010-2021 (purchased from the Norwegian toll office) to quantify the volume seafood products traded, their mode of transport, and destination country and openly available export data through the European Market Observatory for Fisheries and Aquaculture to track where countries that processed the largest volume of fish. Further, we conducted semi-structured interviews to contextualize our data and analyses. We found that the Norwegian seafood supply chain has been resilient to economic shocks in the previous 10 years. Volumes of products remained high throughout economic shocks. However, disruptions to the supply chain resulted in shifts in mode of transport and in exporting countries. Economic shocks also negatively impacted the environmental footprint of products due to changes in packaging requirements by importers (i.e., during the COVID19 pandemic), which countries processed the most fish by volume, and changes in mode of transport for different product groups. Bans or trade restrictions did not result in decreases to volumes of fish traded; rather, fish were traded in different markets where bans or restrictions were not in place.

FIGURE 1: Norwegian salmon exports across modes of transport broken up by product group.



INTEGRATED MULTITROPHIC AQUACULTURE IN BIOFLOCS: A 13-YEAR STORY AT THE MARINE SHRIMP LABORATORY

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The biofloc system is characterized by low water exchange and high productivity, made possible by the complex microbial community that maintains stable water quality parameters. However, aiming to increase system efficiency, the Marine Shrimp Laboratory (LCM) began studies 12 years ago on integrating shrimp farming (*Penaeus vannamei*) with other species. This story began in 2011 when the first seedling of *Salicornia neei* was collected from the mangrove at Daniela Beach (Florianópolis) and brought to LCM. The first aquaponics experiment demonstrated that it was possible to produce double the shrimp biomass in plants. The second species to be integrated was tilapia (*Oreochromis niloticus*), where it was shown that integration with fish increased both productivity and nutrient retention in the system (nitrogen and phosphorus). Subsequently, all three species were integrated, further increasing productivity. A later study showed that integrating shrimp, mullets (*Mugil liza*), and macroalgae (*Ulva lactuca*) also increased productivity and nutrient retention. Additionally, the inclusion of microalgae (*Scenedesmus obliquos*) in the integrated shrimp and tilapia system also promoted increased productivity. In a recent study, it was observed that integrating shrimp, tilapia, and Ulva ohnoi macroalgae cultivation also increased productivity and nutrient retention. Another halophyte plant used in aquaponics is sea rosemary (*Batis maritima*), which also shows good productivity. Based on the experimental results, LCM has just completed a cultivation unit to validate the results on a commercial pilot scale. The unit consists of a 900m2 greenhouse with 8 50,000L tanks for shrimp farming, 8 5,000L tanks for fish farming, two 4,000L tanks for macroalgae farming, and beds with halophyte plants (salicornia and sea rosemary) that receive solids removed from the system. The greenhouse also has a rainwater collection system for evaporation replacement and a photovoltaic system for energy generation. The products from the pilot system, such as shrimp, halophyte plants, and macroalgae, are already being used in local cuisine and are sought after by various chefs in Santa Catarina. This project aims to demonstrate the technical, financial, and environmental feasibility of using integrated AMTI technology with bioflocs, contributing to sustainable food production.

Figure 1: System installed at UFSC for production of shrimp in IMTA system with biofloc.

SALMON FARMING IN 2050: WHAT WILL THE SUSTAINABILITY PERFORMANCE OF THE EMERGING TECHNOLOGIES FOR SALMON FARMING BE?

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As the Norwegian salmon aquaculture faces sustainability challenges and growth restrictions due to high mortality, escapees and negative environmental impacts, there is immense development going on towards innovative technologies to replace and complement open net pens for sea farming. Many new concepts have been designed and there is currently a push towards implementing new concepts in commercial fish farming. Research and development of new technologies is expensive and slow. To make good decisions on distributing resources effectively a better understanding of the impacts bound to applying and scaling up these technologies is needed. This research aims to contribute to this goal.

The objective of this work is to identify the sustainability impacts associated with some emerging technologies for salmon farming and to evaluate the environmental impacts of some future scenarios employing these technologies. The technologies considered in this analysis are semi-closed, closed, land-based, submerged and offshore. Future hypothetical scenarios including a mix of different technologies, or one or two dominating technologies are described. The environmental footprint of 1 kg salmon at farm gate is calculated, including the inputs to the operations and equipment of the farm. By applying the results from the environmental footprint analysis per technology to the future scenarios, rough estimates of the environmental impacts in the different future scenarios for 2050 are quantified. In addition to the environmental impacts, the socio-economic impacts of the future scenarios are also evaluated.

Methodological approach: The future scenarios are built based on some key drivers for change such as regulatory framework, technology innovation, access to renewable energy and others. Limitations for future growth such as reduced access to marine area, climate change impacts, ecosystems carrying capacity are also integrated into the future scenarios. The drivers for change and limitations are identified through workshops with key actors from the Norwegian Aquaculture industry. The total production volume in 2050 and the dominating technology or a mix of technologies as a result of the different drivers and limitations is described in each scenario. The sustainability performance of the different scenarios is evaluated by applying environmental, economic and social indicators. These indicators include both quantitative indicators such as GHG emissions (in CO_2-equivalents), marine eutrophication, fresh water and marine ecotoxicity, number of jobs and value creation as well as qualitative indicators such as fish welfare. Relevant indicators have been identified through literature review, stakeholder engagement and semi-structured interviews with industry representatives.

Results and discussion: The potential impacts of the different scenarios are calculated across the indicators and compared to those in the baseline scenario. The results helps to answer whether future growth in aquaculture is possible without a proportional increase in negative impact. The different scenarios give a picture of the potential consequences of the implementation of the different technologies across several sustainability dimensions like the climate, environment, ecosystem and society. This gives a good knowledge base for future policy decision making processes and for the most feasible technology development path.

This work is funded by the Norwegian Seafood Research Fund, Project number: 901833.

ENHANCING NILE TILAPIA GROWTH AND HEALTH: HARNESSING PROBIOTIC CONSORTIA OR SINGLE STRAIN FOR IMPROVED PERFORMANCE, GUT HEALTH, AND IMMUNITY

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Intensive aquaculture operations often face significant economic losses due to stress-induced disease outbreaks. In response, antibiotics have been extensively utilized to combat infections. However, this widespread use of antibiotics, especially in frequent or low doses, elevates the risk of antibiotic-resistant bacteria emergence. Consequently, there is a pressing need to explore alternative strategies. Thus, this study focuses on assessing the probiotic potential of single and multistrain consortia isolated from the intestinal microbiota of tilapia (*O. niloticus*).

Bacteria were isolated and underwent an extensive screening procedure to determine their probiotic capabilities. This involved assessing their ability to withstand low pH conditions, tolerate bile salts, display hydrophobic properties, and exhibit antimicrobial activity against prevalent pathogens like *Streptococcus agalactiae* and *Aeromonas hydrophila*. From the isolated strains, *Lactococcus lactis* A12, AMB7, *Priestia megaterium* M4, and *Priestia* sp. M10 emerged as promising candidates (see Figure 1).

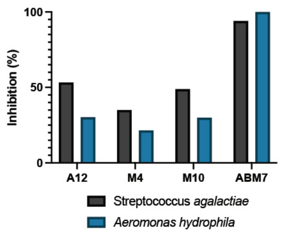

Figure 1. Antibacterial activity of the extracellular products of probiotiotics A12, M4, M10, AMB7.

Potential probiotics were also evaluated *in vivo* by oral administration of single or multistrain bacterial consortia to Nile tilapia alevins. Growth performance, intestinal histology, microbiome composition, resistance to *S. agalactiae* during an experimental challenge, and immune response was studied by transcriptomics analysis.

All treatments improved growth performance, microvillus length, and survival during experimental infection with *S. agalactiae*. Moreover, they showed modulation of immune system-related pathways such as Neutrophil extracellular trap formation, Fc gamma phagocytosis, leukocyte transendothelial migration, and natural killer cell activity, among others (Figure 2). These findings demonstrate synergistic modes of action beneficial for fish.

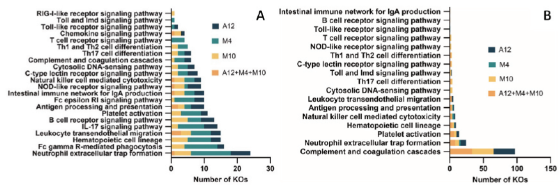

Figure 2. Change in expression genes during the administration of probiotiotics A12, M4, M10, A12+M4+M10: a mixture. Upregulated (A) and Downregulated (B) pathways related with immune system.

References

Melo-Bolívar, J. et al. (2023). Fish and Shellfish Immunology
Melo-Bolívar, J. et al. (2022). Microorganisms

MODELLING LARGE-SCALE LOW-TROPHIC AQUACULTURE IN THE DUTCH NORTH SEA UNDER CLIMATE CHANGE SCENARIOS

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Within the EU, there is a strong incentive towards developing sustainable, low-trophic aquaculture. At the same time, climate change leads to changes in temperature, freshwater inputs and stratification in the marine environment, affecting nutrient and primary production patterns. This can subsequently affect the system's carrying capacity for low-trophic, extractive aquaculture. As part of the FutureMARES EU Horizon H2020 project, we investigated the potential for large-scale sugar kelp and mussel aquaculture in the Dutch North Sea, and how it might be influenced by climate change.

We used a 3D coupled hydrodynamics, water quality and ecology model of the North Sea. Within the model, we also computed sugar kelp and mussel dynamics, together with their respective feedbacks on the environment. We simulated hypothetical large-scale cultivation scenarios for both species, separately and combined. In the Netherlands, future offshore aquaculture will be co-located in wind farms. In our simulations, we therefore placed the aquaculture farms within planned offshore wind farms, based on their suitability for cultivation of the simulated species. These upscaling scenarios were run for a historical state, and for 2050 and 2100 conditions according to a global sustainability scenario and a more pessimistic scenario with respect to climate change.

Our results show that sugar kelp cultivation yields close to a 1 kgDW/m^2 target can be achieved in every farm of the historical state. For the most sustainable scenario, yields achieved in 2100 are 2-10% higher than in the historical state, due to higher winter nutrients along the Dutch coast. In the least sustainable scenario, temperatures simulated in 2100 are significantly higher and offshore nutrients in the Dutch EEZ are lower. Consequently, simulated sugar kelp yields are 2-16% lower compared to the historical state. Results from mussel cultivation scenarios show that final cultivation yields vary much more from one farm to another and are more sensitive to the cultivation density, than for sugar kelp. Highest yields are achieved in the most near-shore farms within the Rhine region of freshwater influence, receiving high nutrient loads and exerting higher primary production. When large-scale mussel cultivation is combined with large-scale sugar kelp cultivation, the production yields for both species are not significantly altered.

Additional runs were performed including flat oyster beds in areas potentially suitable for restoration, in addition to large-scale low-trophic aquaculture. Results show that the presence of oyster beds might affect mussel cultivation yields negatively, but they have very little effects on sugar kelp yields. Using the same model, we also investigated the effects that cultivation scenarios have on the environment (e.g. nutrients and phytoplankton). In all cases, cultivation at such large scales lead to significant decreases in phytoplankton biomass, which may impact the marine food chain.

ASSESSMENT OF THE EFFECTS OF PFAS ON BLUE MUSSEL *Mytilus edulis* USING QPCR, BIOCHEMICAL AND LIPIDOMIC APPROACHES - IMPLICATIONS FOR COASTAL MONITORING AND ENVIRONMENTAL RISK EVALUATION

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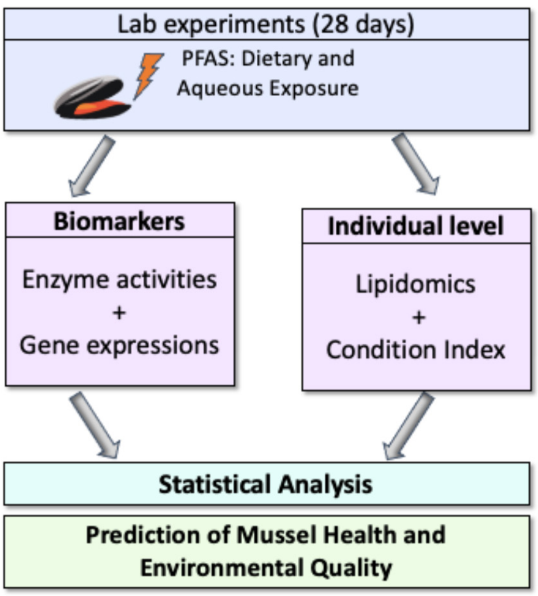
Per- and polyfluoroalkyl substances (PFAS) are highly persistent, harmful chemicals with complex environmental transport behaviour. PFAS have emerged as a concerning class of contaminants, with over 4000 non-naturally occurring chemicals displaying widespread environmental presence. The long lasting, bio accumulative, and toxic properties of certain PFAS compounds, notably PFOS and long-chain PFCAs, have regulatory action. However, the use of alternative compounds with poorly understood environmental behaviours continues. Despite growing calls for improved management, there remains a significant knowledge gap regarding biota exposure to PFASs.

In this research, the impact of PFAS is assessed by exposing the mussels to PFAS mixtures, mimicking environmental contamination through water and food in controlled tanks for 28 days, followed by 14 days depuration phase. By using distinct ^{13}C isotopic labelling, the study traced the sources of contamination. The analysis compared control mussels with PFAS-exposed ones from various tank conditions, evaluating health effects through using oxidative stress biomarkers (CAT, SOD, LOOH), a detoxification marker (GST), and a neurotoxicity marker (AChE). Early molecular responses were investigated by measuring the relative expression of genes associated with cell proliferation, apoptosis, cellular stress, and energy metabolism through qPCR analysis. Furthermore, the condition index of individual mussels was assessed to evaluate overall health and condition, and a lipidomic analysis was performed to investigate alterations in lipid profiles in response to varying exposure conditions.

This research sheds light on the biochemical and molecular responses of blue mussels to simultaneous exposure to PFAS through waterborne and foodborne routes. By enhancing our understanding of the risks associated with these contaminants, the findings contribute significantly to the field of ecotoxicology and aid in the development of more effective management strategies.



CAN EELGRASS *Zostera marina* WIN ITS LONG BACTERIAL CLIMATE WAR?

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For over a century, researchers detailed how eelgrass *Zostera marina* could sequester organic matter in shallow water. In 1915, P. B. Jensen of the Danish Biological Station determined that eelgrass was the source of over 100 grams of organic matter per square meter along the Danish Wadden sea coast. In 1925, The Treatise on Sedimentation (National Research Council USA) sought to bring international perspectives to a new concern about marine soils. A second effort organized in 1935 included the emerging research areas of sedimentation, bacteriology, chemistry and coastal processes. These areas were represented by papers authored by Lüders, Häntzschel, Strøm and Zobell circa 1938. The outbreak of World War II postponed publishing until Recent Marine Sediments with Parker D. Trask as editor in 1955. Kaare Münster Strøm's summary of research in Norwegian fjords reviews coastal stagnation as how it relates to a "super abundance" of organic matter and the presence of hydrogen sulfide. The study period, however, was during a largely negative NAO, a period recognized for severe storms and colder temperatures. The NAO, North Atlantic Oscillation, was associated with changes in the wintertime polar circulation which changed in 1980 (Hurrell and H. Van Loon 1997).

Inshore fishers in New England USA also noticed significant habitat change in areas of tidal restrictions. Warmer winters created similar stagnation conditions described by Strøm (1938) although in much smaller systems than Norwegian fjords. Waterford, CT (USA) winter flounder fishers described sulfur smelling muck to town officials in 1981. Inshore fishery observations detailed a second major eelgrass die off in Niantic Bay between 1981 and 1985 following the records of Cottam – US Fish and Wildlife Service of a previous die off of eelgrass 1930 to 1935. Accounts from the shellfish industries (oyster, soft shell clam, bay scallop and hard clam) all mention eelgrass gaining habitat coverage with a transition of once firm or hard bottoms to these much softer and with the presence of hydrogen sulfide. Industry observations include areas of high sulfide with dead or dying shellfish with the presence of "off flavors" watery meats and poor-quality meats in general (Galtsoff 1937).

In 1985, Donald Rhoads of Yale University Department of Geology, described the formation of sapropels during prolonged heat and low dissolved oxygen. He termed them mono-sulfidic muds rich in sulfides with high organic contents (EPA-NOAA Workshop 1987) in western Long Island Sound. Long Island Sound is known for alluvial deposits containing high amounts of iron and organic matter capable of sustaining sulfate-reducing bacteria, a source of sulfide compounds known to weaken or kill submerged aquatic grasses (Pedersen et al. 2004), making warmer temperatures and high organic deposition key climate change indicators for eelgrass soil health and sulfide formation near its roots.

Sulfide toxicity is reviewed for west and east coast Atlantic eelgrass introducing sulfide toxicity as a response that indicates both being derived from the same parent root stock.

REMOVAL OF DISSOLVED PHOSPHORUS FROM AQUACULTURE EFFLUENTS USING CRUSHED CONCRETE

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Practical and cost-effective solutions for the removal of dissolved phosphorus from fish farm effluents are lacking. Calcium-rich crushed concrete has proven to be effective at removing phosphates from other types of wastewater, and this study therefore examined if crushed concrete can be effective at treating aquaculture wastewater, too. The phosphate sorption capacity of crushed concrete was investigated in laboratory batch experiments as well as in flow-through filters in the laboratory and in field experiments.

The laboratory batch experiments showed that crushed concrete achieved phosphate removal capacities above 200 g PO_4-P/kg concrete due to calcium phosphate precipitation at high pH in addition to P binding to concrete. Furthermore, the studies showed that the P sorption capacities of crushed concrete were highly dependent on P concentrations in the water as described by Langmuir adsorption isotherms.

The laboratory flow-through column tests showed that crushed concrete filters operated at different hydraulic retention times (HRT) removed phosphates at rates of up to ~9 g P/m^3/d at an HRT of approximately 4 hours. Furthermore, the tests showed that high effluent pH values from the concrete were effectively neutralized by downstream woodchip bioreactors.

The field experiments were carried out at a recirculated trout farm in Denmark and showed that crushed concrete filters run over 20 weeks were able to remove up to 3 g P/m^3/d at an HRT of 7h without showing signs of filter clogging.

THE UNDERLYING BENEFITS OF IMPROVED FARM PERFORMANCE USING NOVAQPRO® MICROBIAL BIOMASS FOR TIGER SHRIMP *Penaeus monodon*

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Balancing the competing demands of increased feed costs and reduced farm gate prices with lowering marine capture resources and nutrient inputs is critical to ensure the long-term profitability and sustainability of shrimp farming. The use of least-cost formulations may seem attractive, but it often negatively impacts animal health and performance, reducing yields, water quality, and nutritional condition. Poor feed utilisation in turn affects water quality and lead to excessive nutrient emissions from the farm. The inclusion of a microbial biomass commercialised as NovaqPro® has enabled greater flexibility in formulations, for instance the reduction of protein and marine resources in the diet of shrimp. Most of the research to date has been carried out at laboratory scale and in order to characterise the benefits at the commercial scale, a replicated pond experiment was conducted at Australian Prawn Farms, Queensland, Australia in 2022. Black tiger shrimp (*Penaeus monodon*) were fed a standard commercial diet Eco (n=6; CP 40%) against Propel-G diet (n=6; CP 37%) that contained NovaqPro® from day 70 to harvest (130 days). The use of NovaqPro® enabled a commercial feed formulation with reduced protein (just meeting requirements) and fishmeal that improved growth by ~5%, harvestable yield by 2.5t ha^{-1}, and survival rates by ~10% compared to a traditional control diet. Furthermore, feed conversion ratio improved by ~6% and critically total ammonia nitrogen (TAN) production per tonne of biomass harvested decreased by ~40%. In support of this, the retention of both N and P were improved by 32% and 23% respectively over the initial period day 70-130 and by 23% and 13% respectively over the entire experimental period (day-70 to harvest). Gut microbial alpha diversity was unaffected by diet and was shown to reduce towards the end of the culture period. There was a corresponding 11% increase in the nutritional condition indicator Brix measured in the hemolymph of sampled prawns at day 150, that has been shown to be associated with larger moult increment and energy reserves and supporting growth performance improvements. Furthermore, hemolymph proteomics revealed significant reductions in antioxidant proteins glutathione peroxidase, superoxide dismutase and ProPO activating enzymes, supporting reduced activation of antioxidant stress mechanisms in NovaqPro® fed prawns. Importantly, the net revenue from the ponds fed low protein diets containing NovaqPro® improved by ~16% and the farm was able to meet environmental emissions targets. Overall, these trials demonstrate how the use of NovaqPro® microbial biomass can help farmers achieve greater yiels and profitability while reducing the use of marine resources and protein in commercial diets. By improving retention efficiency of shrimp, ponds contained lower levels of dissolved nitrogen, this approach being essential for reducing environmental impact and enhancing the long-term sustainability of the shrimp farming industry.

MEASURING THE IMPACTS OF HIGH TEMPERATURE STRESS ON ATLANTIC SALMON AND NUTRITIONAL INTERVENTION STRATEGIES

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Marine heat waves are becoming increasingly common as an outcome of climate change. Increased seawater temperatures beyond 18°C directly affects marine and aquaculture fish with detrimental effects on metabolism, physiology and homeostasis. In southern Australia, salmon farming operations observe a reduction in feed intake, along with compromised osmoregulation, flesh decolouration, condition factor and hepatosomatic index. Dietary macronutrient composition impacts energy and oxygen requirements during digestion, leading to the hypothesis that a highly digestible diet can be beneficial during periods of reduced feed intake at high temperature.

This presentation summarizes recent efforts in Australia to understand the biological impacts, develop high-throughput methods to measure thermal stress and details several dietary manipulation experiments that seek to address the impacts of thermal stress in salmon. A combined discovery and targeted proteomics and metabolomics approach was used to generate a panel of biological thermal stress indicators. These were applied to investigate the impacts of dietary carotenoid source or macronutrient (protein or lipid) energy source replicated thermal stress challenges.

The effects of diets during chronic thermal stress on fish performance (survival, specific feed rate (SFR), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR)), flesh pigmentation, whole fish nutrient retention (protein, lipid and energy) and energetics (metabolic rate). The impact of diet at the tank level was assessed on post-digestion oxygen consumption rate (routine metabolic rate; RMR) and acute critical thermal tolerance (CTmax). Performance of individual fish was tracked using intraperitoneal tags and is highly recommended to overcome and understand high levels of individual variation.

Diets containing carotenoids from natural sources (bacteria and algae) significantly increased feed intake during peak thermal stress and significantly reduced liver heat shock and antioxidant biomarkers, but with no subsequent benefit on flesh pigmentation. Fish fed a higher proportion of energy in the form of protein resulted in significantly reduced performance (WG, SGR, FCR) with associated reduction in visceral fat. Fish fed high protein diets at high temperature had significantly reduced protein and energy retention, coupled with significantly reduced RMR but without impact on Ctmax. Lastly, diet significantly reduced liver proteomics biomarkers linked with the heat shock response and antioxidant pathways.

Along with tools to quantify stress levels in Atlantic salmon and measure the potential beneficial effects of diets deployed during peak thermal stress periods, this study supports several nutritional intervention strategies (fortification, support and recovery) that can be effective in mitigating the impacts of thermal stress and supporting physiological homeostasis and fish welfare.

CO-OCCURRENCE OF EMERGING PATHOGENS ISOLATED FROM INFECTED FARMED TILAPIA IN THE LAKE VICTORIA REGION, UGANDA: *Aeromonas sp, Edwardsiella sp and Francisella sp*

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Tilapia production from aquaculture is growing fast in Uganda to meet the increasing demand for fish. Farm yields and profits are increasingly reduced by periodic disease outbreaks in land and water-based systems. High mortalities experienced within hatcheries and cage systems with infected fish presenting signs of lethargy, exophthalmia, granulomatous spleen, fin rot, ulcerations and haemorrhages. Histopathological reports showed gill hyperplasia with epitheliocystis, and granulomatous head-kidney, spleen, liver with melanomacrophages. However, asymptomatic fish samples showed recovery stages of populations as exhibited in granulomas spleen and kidney. Molecular identification revealed unique strains of *Aeromonas veronii*, *A. hydrophila* of *Edwarsiella ictaluri, E. anguillarum, Francisella philomiragia, F. noatunensis and F. tularensis* from infected farmed tilapia in Uganda. These were resistant to Ampicilin and Amoxicillin, erythromycin and sulphamethoxazole trimethoprim, respectively. Identification of co-occurrence of these emerging pathogens presents strategies to reduce further economic losses in the tilapia industry.

DEVELOPMENT OF AN ENTIRELY PLASMID-BASED REVERSE GENETICS SYSTEM FOR TILAPIA LAKE VIRUS (TiLV)

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Tilapia lake virus (TiLV) is a 10-segmented single-stranded negative-sense RNA virus first identified in 2014. TiLV can cause up to 90% mortality in tilapia and poses a severe threat to the global tilapia industry. However, scientific knowledge and research relating to TiLV are very limited. The unavailability of a reverse genetics system (RGS) has made it challenging to elucidate the molecular mechanisms underlying TiLV replication and pathogenesis. Firstly, we identified that TiLV can infect and replicate in the Vero E6 cells after susceptibility testing of many mammalian cell lines. Based on this discovery and through sequence analyses of the TiLV genome, we successfully generated recombinant TiLVs. This was achieved by transfecting 10 synthetic plasmids containing the complete TiLV reference genome (GCF_001630085.1, TiLV isolate Til-4-2011) into Vero E6 cells that were co-cultured with fish-derived E11 cells. Sequence analysis of the genome of the rescued virus and comparison with parental TiLV genomic segments confirmed the successful rescue. We then sequenced the complete genome of a wild-type TiLV strain originating from Israel (named TiLV-Israel-HK), the new recombinant TiLV (TiLV-Israel-HK) was also successfully recovered. Furthermore, we used our system to generate reassortant viruses of TiLV. All TiLVs rescued using the RGS caused a clear cytopathic effect in E11 cells, which was indistinguishable from wild-type virus. This RGS will allow in-depth characterization of this emerged virus and facilitate the development of vaccines and antiviral therapeutics.

In summary, we have established the first RGS for TiLV. We believe this RGS will bring a lot of convenience for studying TiLV and accelerate research relating to TiLV.

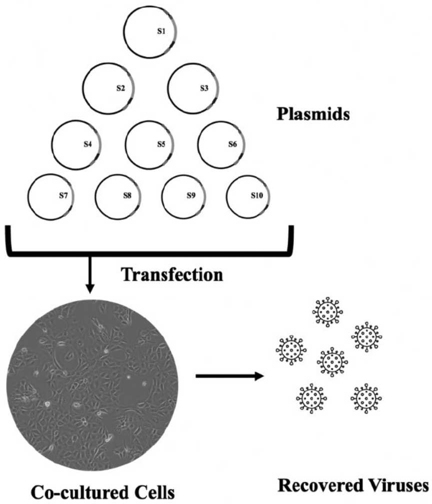

Figure 1. Schematic diagram of the reverse genetics system for the rescue of TiLV.

FUNCTIONAL ANALYSIS OF LEPTIN IN GROUPER *IN VIVO* BY IMMUNO-INHIITION WITH ORAL DELIVERY SYSTEM

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Grouper is one of the major economic fish in Taiwan. Recently, the grouper farming production is restricted due to the long duration of culturing a fish to size for sale. Therefore, it is important to develop grouper with higher growth rate. Leptin is a kind of hormone, which is also a suppressor of orexigenic gene. Disruption of leptin signaling significantly improve weight-gaining ability of mouse. In this study, we will use immune-inhibition technique to establish leptin-inhibited grouper by oral delivery system. We noticed the PEIa-gL6E level achieved induction limitation with 0.1mM IPTG. After purification, we determine the dosage of target protein in oral inhibitor. In animal test, the fish were fed by three different concentrations of oral inhibitor, and the body weight, length, and food intake were recoreded. The weight of grouper gained 7-11% with the oral inhibitors feeding, while the length only showed slight changes (<3%). Besides, the food conversion ratio of group fed with oral inhibitors were higher than control. In conclusion, leptin may affect the accumulation of body weight instead of body length. This provides us insights to investigate the orexigenic and anorexic responds that leptin involved in.

SEX DETERMINATION IN YELLOW PERCH: TRANSCRIPTOME PROFILE OF KEY SEX-RELATED GENE EXPRESSION AND EFFECTS OF *CYP19A1* siRNA-SILENCING

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Yellow perch (YP, *Perca favencens*) demonstrate female-biased size dimorphism, with females having larger body size and faster growth compared to males, making the production of mono-sex female populations crucial for enhancing YP industry development. In this study, we first sequenced the whole genome of the yellow perch and screened transcripts of brain, liver, and gonadal tissues from fish of different sexes. By comparing known female sex-related genes in fish such as *cyp19a1* and *dmrt1* with YP genome, we obtained full-length sequences through genomic homology analysis. Then an experiment was conducted to evaluate the effects of *cyp19a*-siRNA interference on gonadal development, sex differentiation and determination in YP. Transcriptome analysis revealed that the gonadal tissue of female YP exhibited predominantly and differentially up-regulated expression of genes than males, and *cyp19a1* was predominantly expressed in the gonads while *cyp19a2* showed predominant expression in the brain. KEGG pathway enrichment analysis illustrated the metabolic pathways of the top 25 enriched genes and confirmed CYP19 (P450) pathway in yellow perch.

For the *cyp19a1*-siRNA interference experiment, four intermittent in vivo injections of siRNA were administered to interfere with *cyp19a1* gene expression in an all-female group of YP. The results revealed that the siRNA interference resulted in an incomplete masculinization and hermaphroditism of all-female mono-sex YP, displaying the presence of male spermatocytes along with female oocytes. In contrast, tissues from the blank control and negative control groups only contained oocytes. The appearance of the gonads in the experimental group appeared more irregular, lacking the smooth surface and typical histoarchitecture observed in the blank control and negative control groups. In addition, the *cyp19a1*-siRNA treatment group had significantly ($P<0.05$) lower body weight than the control groups, indicating that the interference with *cyp19a1* might have affected the expression of estrogen, leading to a trend similar to the growth of males; similarly, the gonad weight and GSIs of the *cyp19a1* group were significantly ($P<0.05$) lower than those in the control groups, suggesting that the *cyp19a1*-siRNA interference might have led to a trend of male type of gonadal development. Furthermore, the expression level of the *dmrt1* in the treatment group was significantly ($P<0.05$) upregulated compared to the control groups, suggesting the correlation roles between these two genes in the regulation of sex development.

These findings suggested that the *cyp19a1* plays a crucial role in the sex determination mechanism of yellow perch, while it is likely that other genes were also involved in the process. Regulation of the *cyp19a1* gene could be used to promote growth and the generation of intersex cells. Further research is needed to determine whether knockout or interference of *cyp19a1* could lead female to male transformation in yellow perch.

EFFICIENT GENOME EDITING IN AN OREOCHROMIS MOSSAMBICUS CELL LINE USING Cas 9 RIBONUCLEOPROTEIN COMPLEXES

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Infectious and parasitic diseases pose significant challenges to tilapia aquaculture, causing notable economic losses and adversely affecting the welfare of these fish species. Utilizing cell lines as experimental models plays a pivotal role in advancing our comprehension of infectious diseases, providing a valuable platform for studying the intricate interactions between pathogens and the host. In combination with of genome editing technologies, such as CRISPR/Cas systems, it becomes possible to assess the specific functions of genes within these systems. This innovative approach allows researchers to explore and manipulate the genetic landscape, in this case allowing research into potential targets for enhancing resistance to diseases such as Tilapia lake virus (TiLV) in tilapia aquaculture. The effectiveness of CRISPR/Cas editing using ribonucleoproteins (RNP) in Omb (Mozambique Tilapia brain) cell lines. In the current study, a method of genome editing of OmB cell lines using RNP complexes was optimized and tested. Through optimization, we determined that the optimal concentration of RNP is 2 μM, and the best electroporation settings are 1700V, 15ms, and 2 pulses. In tests for the knockout of endogenous genes, we achieved over 60% high knockout efficiency. This optimization scheme will facilitate functional genetic studies in the Mozambique tilapia brain cell line.

STRATEGIES TO REMOVE NITRATE IN *Penaeus vannamei* BIOFLOC SUPERINTENSIVE CULTURE SYSTEM: DENITRIFICATION PROCESS

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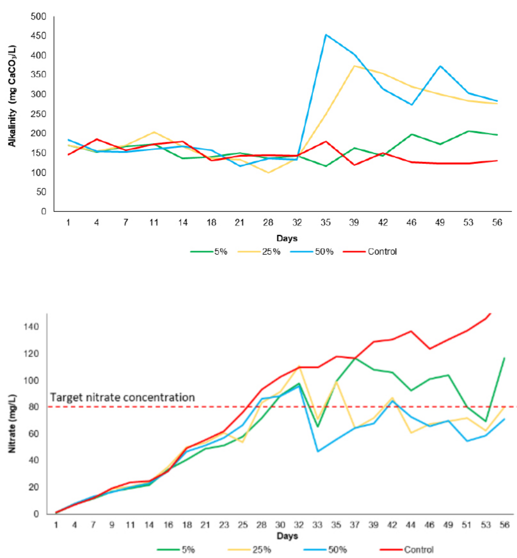
In the last few years biofloc technology system is used for the purpose of preserving water quality in intensive aquaculture systems. In this system, the production of heterotrophic bacteria helps in the formation of bioflocs. The nitrifying bacteria transform ammonia into nitrite and nitrite into nitrate. Nitrate tends to accumulate reaching high concentrations (>100 mg/L), which can cause a decrease in growth and even cause mortalities. To solve this problem, several strategies are applied that involve biological processes such as the denitrification process. Therefore, the present work aimed to evaluate the effectiveness of denitrification in reducing nitrate levels concomitantly with the culture of *Penaeus vannamei* in a biofloc system in 8 weeks.

The experiment consisted of 1 control treatment, where the no denitrification process was carried out, and 3 experimental treatments which denitrifications were carried out in bioreactors of different volumes relative to the volumes of the culture tanks (5%, 25% and 50%). Each treatment was done with 3 replications. *P. vannamei* juveniles were stocked at a density of 400 shrimp/m^3 in 500-liter tanks. Water quality parameters were evaluated daily, such as pH, alkalinity, temperature, salinity, dissolved oxygen, total ammonia nitrogen, nitrite, nitrate, total suspended solids, and settleable solids. Biometrics were performed weekly to estimate shrimp growth. The shrimp were fed twice a day with extruded commercial feed. To carry out the denitrification process, water from each experimental units was pumped to bioreactors without aeration, where organic carbon (sugar) was added in a carbon/nitrogen ratio of 3/1 to stimulate the process. Calcium hydroxide was used to maintain pH levels above 7.4. Denitrification was considered complete when nitrite and nitrate concentrations achieved around 0 mg/L, in denitrification tanks. At the end of the experiment, water quality and zootechnical parameters were submitted to statistical analysis (ANOVA).

Significant differences ($p<0.05$) were observed among the water quality parameters and zootechnical performance in the different treatments. It was confirmed that is possible to denitrify nitrate during trial and keep nitrate in target concentrations.

Figure 1: Alkalinity (a) and Nitrate (b) concentrations in different treatments during experimental period.



DIFFERENCES BETWEEN SEMI-INTENSIVE CONVENTIONAL AND SUPER-INTENSIVE BIOFLOC *Penaeus vannamei* CULTURE

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The semi-intensive culture systems in earthen ponds have been limited by several factors such as disease, weaknesses of the systems or competition by culture area with other industries. Several studies have focused on improving the new technologies necessary to increase shrimp production. The Biofloc Technology Culture Systems (BFT) in raceways is considered a revolutionary system in aquaculture, because its production of microorganisms helps in the maintenance of water quality, reduces feed conversion rates, and increases biosecurity, resulting in high production multiples crops. Additionally, BFT is considered environmentally friendly, with an ability to reuse the same water multiple times, thus avoiding pollution of coastal waters. Technological innovation permits increases in shrimp production capacity per unit area. The addition of new management tools such as air injectors (nozzles), artificial substrate, clarifiers and some procedures may allow unprecedented increases in the load capacity of these systems.

For example, the demand for dissolved oxygen (DO) by shrimp is one of the main concerns as culture systems intensify. In intensive or super-intensive culture of shrimp can be possible if the system's aeration devices can meet the organism's oxygen demand. The number of devices to be used will depend on the water's salinity and temperature, stocking density, shrimp size, and in the BFT systems the amount of suspended solids. For this reason, studies and evaluations of the support capacity of each new aeration technology is important to maximize its effect.

Therefore, several researchers have evaluated the influence of the limitation of different parameters and procedures. These are important challenges to overcome the production when working in BFT with high densities, and different devices and other procedures (Table 1), that can have significant impacts on shrimp development in super-intensive system.

In the present study we evaluate these important changes from conventional to BFT systems, and we discuss the influence of high stocking densities on water quality and on the growth performance of *P. vannamei* in a BFT system with the adoption of these new tools and procedures.

Table 1: Main modifications from conventional shrimp culture systems to Biofloc Technology super-intensive shrimp culture systems.

MAIN CHANGES TO BFT SYSTEMS
FACILITIES: SIZE, FORMAT, MATERIALS
THE USE OF MICROBIAL LOOP: BIOFLOC
NITRIFICATION PROCESS
HIGH PRODUCTION OF BACTERIAS
MANAGEMENT OF ALKALINITY/PH/CO2
HIGH CONCENTRATION OF TSS
AERATION SYSTEMS
USE OF VERTICAL SUBSTRATES
WATER MANAGEMENT
HIGH STOCKING DENSITIES
FEEDING MANAGEMENT
USE OF PROBIOTICS
LOW OR ZERO WATER EXCHANGE
REUSE OF WATER
DENITRIFICATION PROCESS
OTHERS

BIOREMEDIATION IN MARINE RAS: TESTING *Ulva's* CAPACITY FOR LOWERING TOXIC OZONATION BY-PRODUCTS

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Introduction

The maintenance of good water quality is crucial for production success in recirculating aquaculture systems (RAS). Ozone (O_3) has seen an increase in interest among aquafarmers worldwide as a water treatment tool with a wide array of beneficial effects. Besides its germicidal properties, O_3 can enhance solid removal, oxidise toxic nitrogen compounds and degrade a broad spectrum of biogenic and man-made molecules. While its application in freshwater is mostly unproblematic, ozonation of seawater creates by-products termed 'ozone-produced oxidants' (OPO). These can accumulate in a system when process water is recirculated and are detrimental to animal health if species-specific thresholds are exceeded.

Material & Methods

In this experiment, we tested the seaweed *Ulva* sp. for its OPO-bioremediation capacity in an outdoor IMTA (Integrated Multi-Trophic Aquaculture)-RAS-setup with Gilthead seabream (*Sparus aurata*). Effluent seawater was ozonated and subsequently led through an *Ulva* cultivation unit. OPO-concentrations in the seawater were measured before and after the seaweed unit and the decline was compared to controls without *Ulva*. Additionally, the impacts of OPO on growth, metabolic composition and photosynthetic efficiency of *Ulva* were investigated by comparing OPO-exposed seaweed to controls cultivated without ozonation. 16S rRNA gene sequence analyses were performed to monitor the *Ulva*-associated microbiomes in response to ozonation.

Results

While seaweed-containing systems exhibited a significantly higher reduction in OPO, ozonation also diminished *Ulva* growth (32 %) and caused a darker, fringier, and more rigid morphology. Moreover, ozonation elevated chlorophyll -, total phenolic – and flavonoid contents, and shifted the amino acid composition in *Ulva* towards more glutamine, glutamic acid and serine.

Conclusion

With this study we showed that *Ulva* can reduce OPO-concentrations in marine RAS, but with impacts on growth, chemical composition, and morphological properties of algal biomass.

Acknowledgements

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A NOVEL PROTEIN SOURCE FROM LESSER MEALWORM (*Alphitobius diaperinus*) LARVAE MEAL FOR EUROPEAN PERCH (*Perca fluviatilis*): INVESTIGATION ON PELLET CHARACTERISTICS, PRODUCTION PERFORMANCE, SERUM BIOCHEMISTRY, DIGESTIBILITY, HISTOLOGY, SENSORY AND TRAIT OF FILLET, AND ENVIRONMENTAL INDECES

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Our study investigates the substitution of fishmeal with a novel protein source derived from lesser mealworm (*Alphitobius diaperinus*) larva meal (LMW) in the diets of aquaculture-emerging fish - European perch (*Perca fluviatilis*).

Four diets were formulated, in which fishmeal at 21% inclusion level from the control diet was replaced by LMW at 25%, 50%, and 75% and fed to European perch juveniles for 84 days.

Analysis of post-trial serum biochemistry revealed that none of the 11 parameters investigated were affected by dietary LMW. Nevertheless, a significant difference emerged at the 24 h post-trial for phosphate, total protein, albumin, globulin, and alanine aminotransferase. Dietary LMW had a positive impact on the growth performance of European perch, as evidenced by a positive linear model with specific growth rate and survival rate.

Examination of liver lesion severity demonstrated that fish fed the LMW75 diet exhibited less severe liver lesions than those on the control diet.

Overall, this study underscores the suitability of LMW as a protein source in the diet for European perch, positively affecting growth performance, health status, sensory attributes of fillets, and environmental impact indicators. Nonetheless, careful attention should be given to the nutritional profile, particularly essential fatty acids in fish fillets.

AQUA FEED – CHALLENGES AND SOLUTIONS LEARNED FROM 20 YEARS OF IMPLEMENTED CRITERIA

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AQUACULTURE OPERATIONS

Farming of aquatic species in a responsible manner requires a considerable amount of resources, financial as well as human. Specific for species that are farmed and grown with complementary compound feed, appropriate feed management practices at hatchery and grow-out level shall be implemented.

This requires: Legal compliance for feed formulations, including the protein levels based on the species to be fed; Monitoring of feed efficiency: feeding levels in accordance with the needs of the different aquatic species, minimizing feed waste and avoiding unnecessary pollution; Transparency of feed ingredients given from feed manufacturers to aqua farms: from the nutritional point of view, the feed needs to be species specific, address the correct stage of life, information on the fishmeal and fish oil composition, including species and country(ies) of origin, fish-in/fish-out ratio (whole fish from wild catch), records of eFCRs and efficient use of feed monitoring, analysis needed for food safety controls; Traceability systems needs to be in place to trace batches of feed to the batches of the farmed biomass.

COMPOUND FEED MILL OPERATION

Compound feed mills supplying farms aiming for responsible claims shall have in place:
Feed safety, HACCP-based risk assessment of feed ingredients; Traceability and responsible sourcing of feed ingredients through the selection and verification management of suppliers; Feed efficiency: importance of having specific feed for specific species, knowing the feeding behaviour of aquatic species and water quality parameters; Detailed management systems to evaluate, additionally to feed safety for all raw materials, the responsible sourcing of key raw materials from the marine and agricultural sources; Traceability, one step back and one step forward; Appropriate processing and handling of aqua feed; Quality and recall procedures.

CHALLENGES AND SOLUTIONS

Feedback gathered from the aqua feed manufacturing sector for the last 20 years have provided GLOBALG.A.P. as a standard setting organization, a varied palette of information, including challenges and solutions.

The main challenge remains on responsible sourcing for key marine and agricultural ingredients. Responsible sourcing covers legality and traceability. By achieving these aspects means the need to rely on verification tools that will trace the manufactured feedback to the compound feed production process.

Envisaged solutions are successful collaboration of all actors in the feed ingredients supply chain, that not only include the responsible sourcing of raw material but also the welfare of aquatic species targeted.

THE BLUE ECONOMY COOPERATIVE RESEARCH CENTRE: SUSTAINABLE OFFSHORE SEAWEED AQUACULTURE

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The Blue Economy Cooperative Research Center (CRC) is a ten-year project funded by the Australian Government, industry partners and research providers to take aquaculture off shore along-side the development of marine renewable energy. Seaweed aquaculture is an integral part of offshore developments and the CRC has invested in several large research projects in this space. This presentation will outline the findings from our current research projects and discuss the direction of our research for the remainder of the CRC through to 2029.

NATURAL STRUCTURAL ENRICHMENT FOR FARMED ATLANTIC SALMON *Salmo salar* IN COMMERCIAL SEA PENS

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Aquaculture enclosures have typically prioritised ergonomic and economic requirements over concerns for fish welfare. Various integral farming practices, such as handling and crowding of fish stock, also have a high risk of evoking strong stress responses in fish and consequently impacting their welfare. As fish welfare becomes a priority for the salmon farming industry, improving rearing conditions for these fish is critical. Environmental enrichment (EE) is a proven avenue for improving the quality of life of farmed animals by helping captive animals to meet their physiological and behavioural needs. More specifically, structural enrichment (SE) provides this stimulus by adding novel structures / objects into the rearing environment resulting in additional physical complexity / heterogeneity. The benefits of using SE within terrestrial farming has been widely demonstrated, whereas the needs of aquatic farmed animals for such enrichment have comparatively been overlooked. There have indeed been studies on salmonids demonstrating the benefits of incorporating SE (including improved growth, survival, physiological stress responses, and fin conditions of farmed salmon). Previous studies have focused solely on juvenile salmon in freshwater farming, neglecting investigations into potential effects of SE in sea pens on farmed salmon welfare. This study aims to investigate the impact of SE, using kelp-based structures, on farmed Atlantic salmon welfare. Six commercial sea pens (n = 50–60,000 salmon) will be used, with 3 enriched with 2 'Kelprings' (PVC and rope "lantern-like" structures covered in kelp). The remaining three pens (i.e., unenriched/barren), serve as control samples. Following deployment of these Kelprings, 15-minute, bi-weekly video recordings will be captured systematically across all six pens (i.e., immediately following the first feed of every 14th day) over a three-month period. These recordings will be captured using feed cameras to ensure consistent, fixed POV recordings of the salmon behaviour. At the end of these three months, a putatively stressful event (grading) will be conducted on all salmon as part of the husbandry routines. In addition to behavioural analyses (including but not limited to Qualitative Behavioural Assessment [QBA]), fin / skin condition, growth, and mortality will be compared and assessed between enriched and unenriched pens. Expected outcomes are that salmon reared under structurally enriched sea cages will i) Have improved condition indices, and better fin / skin condition (owing to reduced conspecific aggression and abrasion against enclosure perimeters), ii) recover faster from stressful events (i.e., grading), and have shorter startle responses, iii) exhibit significantly more positive valence in their behavioural expressivity, as captured by QBA, iv) have improved mortality rates in the event of an intervention / significant disturbance. Principal Components Analysis (PCA) of the QBA scores will identify the perceived patterns of expressive characteristics across the video recordings of salmon behaviour, and reveal any potential dimensions of salmon expressivity. These components of the QBA scores will then be compared across the different treatments (enriched vs. unenriched salmon) The results from this study will provide insights into the potential benefits of structural enrichment for farmed salmon at the seawater rearing stage (with unique insights obtained from evaluating the emotional states through QBA), which will be used to inform certification schemes on future welfare standards.

NAKED CLAMS TO OPEN A NEW SECTOR IN SUSTAINABLE NUTRITIOUS FOOD PRODUCTION

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A ground-breaking form of sustainable food production turning wood into protein

The concept of Naked Clam ™ aquaculture was initially proposed by Dr Willer in April 2020, and the scoping publication was made in September 2020 (Willer et al, Frontiers in Sustainable Food Systems, https://doi.org/10.3389/fsufs.2020.575416). Interest led to a partnership between Dr Willer at the University of Cambridge and Dr Shipway at the University of Plymouth on Naked Clam aquaculture.

Dr Willer & Dr Shipway subsequently developed the world's first Naked Clam aquaculture system at laboratory scale using a modular wood matrix that facilitates rapid growth and easy extraction, and published this research in November 2023 (Willer et al, npj Sustainable Agriculture, https://doi.org/10.1038/s44264-023-00004-y). This received extensive press coverage including on Sky News, BBC, Radio 4, CBC, ITV, The Guardian, and Australian, US, European and Japanese media.

The research provided the first ever nutritional profile and feeding efficacy assessment of Naked Clams [3]. Naked Clams are rich in the same monounsaturated fats found in olive oil that are attributed to have cardiovascular benefits, are high in protein, and harbour symbiotic bacteria that synthesise B12. Sustainable plant-based diets are deficient in B12, and a serving of just 10 g (dry weight) of Naked Clams per week would meet an individual's entire B12 requirements. The authors also identified how Naked Clams can be fortified with additional essential nutrients including omega-3 using novel microencapsulated feeds (BioBullets) developed at the University of Cambridge.

The team are currently performing further research and development to enable scale-up and commercialisation.

Naked Clams in wooden growth panel

Dr Shipway (left) and Dr Willer (right)

VITAMIN E REQUIREMENT IN ATLANTIC SALMON *Salmo salar* DURING SMOLTIFICATION AND ENVIRONMENTAL CHALLENGE

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Vitamin E acts as a strong inhibitor of lipid peroxidation with RRR-α-tocopherol being the most effective form in animals. For this reason, vitamin E requirements show strong interactions not only to dietary levels of polyunsaturated fatty acids, but also with that of natural or synthetic dietary antioxidants. Considering major changes in raw ingredients for aquafeeds, this study aimed to re-evaluate the α-tocopherol (aTOH) requirement of Atlantic salmon (*Salmo salar*) during smoltification when fed diets following a modern feed formulation. In addition, the fish were exposed to an environmental challenge as dietary antioxidant requirements, including vitamin E, might be higher under stressful environmental conditions as they can occur during seawater transfer.
The experimental design followed a regression model. Seven diets were formulated on a basal feed mix (α-tocopherol: 42 mg/kg, VE42) by supplementation of graded levels of synthetic dl-α-tocophenyl acetate to receive diets of 100 mg/kg, VE100; 200 mg/kg, VE200; 300 mg/kg, VE300; 400 mg/kg, VE400; 600 mg/kg, VE600 and 800 mg/kg, VE800. Sixteen tanks in a flow-through system were stocked with 110 Atlantic salmon (initial weight: 81 ± 2 g), respectively, corresponding to duplicate tanks per treatment, except for VE42 and VE800 that were run in triplicate. Twelve fish per tank were sampled after 12 weeks of rearing in freshwater at standard conditions (12 ˚C, >75 % DO). The remaining fish were subsequently exposed, for an additional 6 weeks, to an environmental seawater challenge under combined hypoxia and temperature stress (14 ˚C, 60 % DO, salinity: 22 ppt) prior to sampling.

Dietary aTOH level had no significant effect on final body weight in freshwater or seawater challenged fish. Liver aTOH levels increased with dietary aTOH levels following a 2nd order polynomial. Fish fed VE800 showed significantly higher liver aTOH levels at freshwater stage compared to seawater challenged fish (Figure 1). In addition, in stressed fish, liver malondialdehyde (MDA) levels were significantly higher in VE42 compared to VE200, VE400, VE600 and VE800.

MDA levels indicate that a dietary aTOH level above 100 mg/kg is required to prevent the accumulation of lipid peroxidation products in the liver of stressed fish.

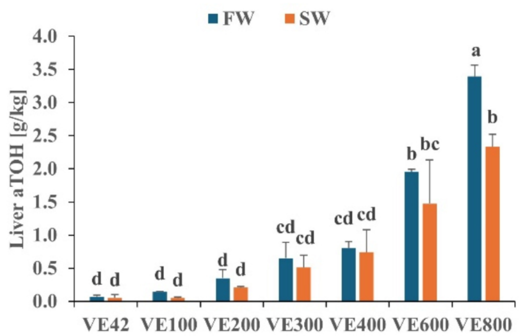

Figure 1. Liver a-tocopherol (aTOH) levels measured in fish fed experimental diets with graded levels of aTOH after 12 weeks of rearing at standard freshwater conditions (FW) or at the end of a 6-week seawater challenge (SW).

BONE MATRIX FORMATION IS INDEPENDENT FROM BONE MINERALISATION. STUDIES ON FARMED ATLANTIC SALMON AND ZEBRAFISH

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A series of studies on farmed Atlantic salmon and the zebrafish model clearly show bone matrix formation and matrix mineralisation as separated processes (1-3). This has consequence for the diagnosis of skeletal malformation and for the evaluation of the consequences of phosphorus deficiency. In salmon and zebrafish matrix formation and mineralisation become uncoupled if the animals receive a low phosphorus (P) diet. Osteoblasts continue bone matrix formation under conditions of low dietary P intake. Likewise the formation of non mineralised scales matrix continues. A limited low P period does not cause an increase of vertebral column malformations (1-3). The no-mineralised bone matrix that is produced during low P intake can fully mineralise if animals receive again a P-sufficient diet (2,3).

Low-P dietary intake, reduces plasma P levels in salmon by 50% but osteoblasts continue regular bone matrix formation. Ultrahigh resolution synchrotron tomography scans show that reduced P intake in zebrafish even increases the amount of non-mineralised bone matrix (Fig. 1) (1). Delayed bone matrix mineralisation has no effect on the expression of osteoblast key genes. In salmon vertebral bodies, the expression of *bgp*, *col1a1*, *enpp1*, *entpd5*, and *opn* remains unaltered. Only plasma *fgf23,* coding for a hormone that decreases renal P re-absorption, is down-regulated under low P conditions (4), indicating higher P retention. If zebrafish and salmon return to a P-sufficient diet, the non-mineralised bone matrix mineralises. Mineralisation does not resume in the vicinity of osteoblasts but far away from the cells connected to the last mineralised matrix (2). This suggests that osteoblasts produce a "mineralisation-ready" matrix but the actual process of matrix mineralisation is cell-independent. Bone matrix mineralisation arguably depends on the availability of plasma P and possibly, as recently suggested, on the composition of the collagenous matrix (5).

A better understanding of the effect of low, regular and high dietary P intake on bone cells and on the growth and the mineralisation of the skeleton can help to optimise P levels in fish diets.

1. Cotti et al. (2020) More Bone with Less Minerals. Int. J Mol. Sci., 21, 5429.
2. Witten et al. (2019) Journal of Experimental Biology 222.
3. Drábiková et al. (2022) Aquaculture 559, 738430.
4. Drábiková et al. (2023). Biomolecules 13, 663.
5. van der Meijden et. al. (2023). Adv. Funct. Mater. 2212339.

THE EFFECT OF COMMERCIAL DRY DIET SUPPLEMENTATION WITH ORGANIC & INORGANIC ACIDS ON COMMON CARP PERFORMANCE

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A 60-day experiment at 25°C with four triplicated feeding groups was designed to assess whether the acidification (supplements of 1.5%) of a commercial diet AF using organic acids and inorganic hydrochloric acid (mimicking the action of gastric juice) may improve various parameters of reared common carp juveniles. A control group was given a non-altered feed.

Survival rates in all groups were 100%. The best rearing results were obtained with dry diet enriched with 1.5% HCl (Table 1). The organic acids did not exert a positive effect on the fish growth though allowed to significantly reduce the occurrence of body deformities. The addition of HCl had a positive effect on the ash and P content in the fish body. No serious histopathological changes were found in all of the studied fish, although the acidification of feed adversely affected intestinal morphology of fish (Table 2).

The use of HCl (much cheaper than the organic acids) brought the best fish growth and reduction of the incidence of deformities so can be recommended for the extended research.

The study was conducted within the National Inland Fisheries Research Institute Project Z-005 and the IMMUNOVICTU project (00004-65-21.1-OR1400002/22/23) funded by the European Union (Fisheries and Sea 2014-2020; Priority 2)

TABLE 1. Growth, incidence of body deformities and body chemical composition of juvenile common carp fed with a commercial dry diet supplemented with 1.5% organic acids and hydrochloric acid

Parameter/Diet	Commercial dry diet AF	AF with citric acid	AF with acetic acid	AF with HCl
TL (mm)	115.1 ± 0.9[b]	114.8 ± 0.9[b]	115.0 ± 1.5[b]	**118.7 ± 1.0[a]**
BW (g)	23.5 ± 0.2[b]	24.1 ± 0.4[b]	24.0 ± 0.6[b]	**25.7 ± 0.4[a]**
Fish with deformities (%)	16.7[a]	**1.1[b]**	**2.2[b]**	**1.1[b]**
Protein (% w.m.)	14.17 ± 1.22	15.36 ± 0.23	14.24 ± 0.59	14.97 ± 0.48
Fat (% w.m.)	8.12 ± 1.46	6.81 ± 0.37	7.88 ± 0.87	7.40 ± 1.87
Ash (% w.m.)	1.89 ± 0.51	2.29 ± 0.48	1.87 ± 0.14	2.10 ± 0.52
Phosphorus (g/kg)	4.03 ± 0.56[ab]	4.10 ± 0.51[ab]	3.48 ± 0.28[b]	**4.55 ± 0.54[a]**

TABLE 2. Selected parameters of histomorphometric analysis of fore-, mid- and hindguts of fish

Parameter [µm]/Diet	Commercial dry diet AF	AF with citric acid	AF with acetic acid	AF with HCl
Foregut fold height	978.1±174.1[a]	847.4±168.8[b]	869.9±165.3[b]	918.6±93.6[ab]
Foregut enterocyte height	51.5±5.2[a]	45.0±6.7[b]	52.9±4.5[a]	42.9±5.9[c]
Foregut supranuclear height	28.3±3.3[a]	22.3±3.39[b]	28.1±3.9[a]	21.7±2.6[b]
Foregut enterocyte microvilli	1.37±0.20[c]	1.55±0.23[a]	1.51±0.21[b]	1.52±0.21[ab]
Midgut fold height	768.3±281.0[a]	634.4±132.7[b]	632.2±99.4[b]	668.5±158.4[b]
Hindgut fold height	814.7±107.8[a]	785.6±119.3[a]	796.4±113.9[b]	763.7±124.2[a]

Tetradesmus obliquus FRACTIONS AS MODULATORS OF GUT HEALTH STATUS

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Tetradesmus obliquus, is a fast-growth microalgae and a natural producer of high-value bioactive compounds such as photosynthetic pigments, polyphenols, fatty acids, and polysaccharides. This characteristic makes them an attractive additive in aquafeed due to their antioxidant, antimicrobial, and immunomodulatory properties (Oliveira et al 2021). Therefore, this study aimed to assess the potential of *Tetradesmus obliquus* (TETRA) fractions as gut health promoters for gilthead seabream (*Sparus aurata*), using an *ex vivo* model. Commercially available TETRA biomass was processed in a multi-product biorefinery pipeline based on aqueous extractions and hydrolysis. The resulting fractions of this algae (F1-7) were analysed using an *ex vivo* screening platform based on gilthead seabream anterior intestine explant following previously standardized optimal conditions. Explants were incubated with each algae fraction (F2-F7) at a single dosage for an initial screening, after which the most promising fractions (F2, F4, and F5) were evaluated at two doses, to refine the explant responses. Both fractions and control treatment (CTRL - no algae addition) were tested in triplicate. Tissue response was evaluated by the expression of a panel of genes related to innate immune response, antioxidant response, and epithelial integrity and permeability. Through a supervised multivariate analysis of the intestinal transcriptional response after incubation with the different algae fractions, it was possible to observe that the soluble fraction TETRA F2, at a higher dosage, seems to stimulate intestinal immune response and this was evident through an up regulation of cytokines *IL1b* and *IL8*. Since TETRA F4 also evidenced immunostimulant properties, data suggest that soluble fractions of the extracts comprehend more bioactivity. Based on the observed response, retrieving the soluble fraction of the first aqueous extraction (F2) could be a cost-effective processing method for TETRA biomass to be used as a functional ingredient to support fish performance and resilience. In conclusion, this study showed the potential of TETRA fractions, in a dose-response manner, on the capacity to modulate gilthead seabream gut response assessed an *ex vivo* model.

References

Oliveira, C. Y. B., Oliveira, C. D. L., Prasad, R., Ong, H. C., Araujo, E. S., Shabnam, N., & Gálvez, A. O. (2021). A multidisciplinary review of Tetradesmus obliquus: a microalga suitable for large-scale biomass production and emerging environmental applications. Reviews in Aquaculture, 13(3),1594-1618.

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IMPACTS OF DIETARY ALGAE SUPPLEMENTATION ON TURBOT POSTLARVAE PERFORMANCE

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Turbot (*Scophthalmus maximus*) is a fast-growing flatfish that has been commercially cultured for more than two decades. Yet several bottlenecks remain in the mass production of high-quality larvae. Early life stages of marine fish development are characterized by variable survival rates, as it is a period extremely sensitive to external conditions. Nutrition in the early stages is a key factor for has a tremendous impacts on the growth, survival, and health status of fish larvae and post-larvae, and also on later in fish life. Algae has proven to be a rich source of structurally diverse and complex compounds exhibiting numerous interesting biological effects. They have been continuously explored as feed ingredients and feed specialties due to their potential to support antimicrobial, anti-inflammatory, immunomodulatory, and antioxidant properties. Therefore, this work aims to provide a nutritional approach, with the supplementation of macroalgae biomasses, to respond to the current challenges of marine hatcheries, exploring their effect on molecular pathways related to fish immune and antioxidant response and epithelium integrity - key biomarkers of fish performance/robustness.

A growth trial was conducted in 51 days after hatching (DAH) turbot that were fed one of the four experimental diets: a commercial-like diet as control (CTRL), and the other three diets supplemented with *Gracilaria gracilis* (GRAC), *Nannocloropsis oceanica* (NANNO) and a blend of the two algae (BLEND). After 28 days of feeding experimental diets, fish were sampled to assess key performance indicators, and the anterior intestine was dissected to analyse transcriptional modulation of the tissue through RT-qPCR, to understand and optimize the application of algae biomass in fish nutrition.

At the end of the growing trial, the zootechnical parameter observed showed similar results between dietary treatments. Fish presented an average of 98.9±0.3% survival rate, 6.8±0.2g final body weight and 0.8±0.1 FCR. Algae inclusion derived a modulatory response on the expression of genes related to immune response (e.g *IL8*, *IL1b*, *COX2*), antioxidant capacity (e.g *SOD*, *GPX*, *NRF2*) and mucosal epithelium integrity (e.g *OCL*, *CLDN12*, *TJP*).

Overall, this work provides evidence that dietary supplementation of algae could be a nutritional strategy to enhance marine fish larvae' robustness at early life stages of development.

EFFECTS OF PROTEIN SOURCES AND EXTRUSION PROCESSING CONDITIONS *VIA* PELLET PHYSICAL QUALITY ON THE FEED INTAKE, GASTROINTESTINAL EMPTYING AND DIGESTION OF SPOTTED SEABASS *Lateolabrax maculatus*

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The diversification of future aquaculture feed formulas is expected to rise due to the increasing fish feed production. Changes in formulas and the corresponding adjustments to processing parameters can create variations in the pellet physical quality (PPQ). Despite these potential changes in PPQ, the impact of varying PPQ on the physiological response of fish is often undervalued and overlooked.

In this study, we investigated the effect of protein sources and extrusion processing conditions through PPQ on the feed intake, gastrointestinal emptying and digestibility of spotted seabass. Four diets were formulated following a 2×2 factorial design, with two protein sources (fishmeal [FM] or cottonseed protein concentrate [CPC]) and two extrusion processing conditions (PC-M, preconditioning water content 30 % and die temperature 120 °C vs. PC-H, preconditioning water content 22 % and die temperature 140 °C). Both experimental diets were similar in macronutrient composition. Fish (46 g) were fed twice daily to apparent satiation for 20 days. Feed intake, nutrient digestibility and fish performance were evaluated. At the end of the experiment, chyme was collected after fish were fed restrictively one meal (0.95 % BW/d) to determine gastrointestinal emptying and water fluxes. Chyme was quantitatively collected from stomach and intestine at 2, 4, 6, 8, 12 and 24 h postprandial.

Altering the protein source affected PPQ as well as nutrient digestibility. CPC-pellets exhibited a longer hydration time, a higher hardness, pellet durability index (PDI) and expansion rate than FM-pellets. CPC-pellets lowered nutrient and energy digestibility compared to FM-pellets. In addition, CPC-pellets led to a decrease in postprandial plasma glucose level and hepatosomatic index than FM-pellets. Processing conditions steered PPQ and influenced the physiological response of fish. PC-M led to a shorter hydration time, a reduced hardness and PDI, but a higher expansion rate than PC-H. PC-M resulted in a 23 % increase in stomach water flux at 4 h postprandial and a 2 % rise in stomach chyme water content than PC-H. Moreover, PC-H led to a respective 0.6% and 2.1% increase in protein and fat digestibility compared to PC-M. Feed intake and gastric emptying of fish remained unaffected by variations in PPQ in this study.

EXPLORING SUSTAINABLE AGRICULTURE: WHEATGRASS *(Triticum aestivum)* GROWTH UNDER DIFFERENT AFRICAN CATFISH (*Clarias gariepinus*) AQUACULTURE EFFLUENTS AND SUBSTRATE CONDITIONS

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Introduction:
Our study investigates the optimal growth conditions for wheatgrass, a plant known for its nutritional value. The study is structured to examine the effectiveness of various irrigation irrigation sources and horticultural substrates, highlighting the sustainability of different cultivation methods in promoting efficient growth and improving nutritional quality.

Materials and Methods:
All studies were conducted under the similar setup (Xu et al., 2022), wheatgrass was cultivated using three distinct irrigation sources: the extensive aquaculture unit (EAU), intensive aquaculture unit (IAU), and regular tap water enriched with fertilizer (Control). Additionally, various horticultural substrates used were: 100% coconut fibers, a mix of 70% coconut fibers and 30% perlite, perlite and vermiculite (50:50), coconut & vermiculite substrate (50:50), and 100% vermiculite. The focus was on measuring and analyzing the growth parameters and nutrient content in wheatgrass under these varied conditions.

Results and Discussion:
The study's findings demonstrated that the improved growth and higher vitamin content of wheatgrass irrigated with water from the aquaculture units was a significant discovery. Particularly, there was a rise in the amount of vitamin B_6 in the wheatgrass that was irrigated with water from IAU. Furthermore, wheatgrass that was irrigated with water from EAU showed a notable increase in vitamin B_5 concentration, which was similar to the wheatgrass in the IAU group. Assumably, those results are attributed to the increased concentrations of dissolved organic matter and beneficial microorganisms in the fish water. Considering substrates utilised, the selection of substrate had a significant impact on the plant's growth characteristics and nutrient content. The study highlights the potential of aquaponics as a sustainable method of producing superfoods and emphasises the necessity for additional thorough research on the microbial composition of aquaculture effluents. These investigations may help elucidate the processes underlying the noted rises in the nutrient content, especially the vitamin content of wheatgrass cultivated in aquaponic systems.

References
Xu L, Ziethen CJ, Appelbaum S, Palm HW, Knaus U. Aquaponics Production of Wheatgrass (*Triticum aestivum* L.) in Different Horticultural Substrates with African Catfish (*Clarias gariepinus*) in Northern Germany. AgriEngineering. 2022 Nov 4;4(4):1076-94. https://doi.org/10.3390/agriengineering4040067

COMPARATIVE ANALYSIS OF GENOME-WIDE SELECTION MODELS BASED ON BODY WEIGHT TRAITS IN LARGE YELLOW CROAKER (*Larimichthys crocea*)

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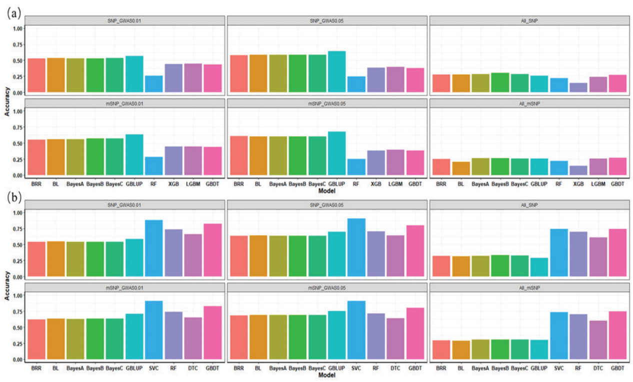
In recent years, genome selection (GS) has attracted widespread attention in the field of genetic breeding of aquaculture species. GS has higher predictive accuracy compared to traditional breeding methods based on pedigree, and has great application prospects in aquatic animal breeding. However, there are few reports on the comparison of genome selection models for aquatic animals, which greatly affects the accuracy and efficiency of predicting breeding values for aquatic animals.

The aim of this study was to evaluate the feasibility of genomic selection for body weight traits in large yellow croakers and compare the accuracy of different models and machine learning methods in predicting body weight traits in large yellow croaker. This study employed 546 large yellow croaker individuals to estimate the heritability of body weight traits, and then compared the predictive accuracy of 10 genome selection models such as GBLUP, Bayes as well as machine learning to identify the most appropriate genome selection models for body weight traits in large yellow croaker.

The results indicated that genomic selection is feasible in the breeding of large yellow croaker. The GBLUP model has the highest accuracy among continuous traits. The SVC model has the highest accuracy in classifying traits (Figure 1). Machine learning is more suitable for binary traits and GBLUP is more suitable for continuous traits. In summary, this study provides an important database and analytical method basis for carrying out genome-wide selective breeding of large yellow croaker.



PANGENOMIC ANALYSIS PROVIDES INSIGHT INTO THE PRECISE IDENTIFICATION OF GERMPLASM IN COMMON CARP

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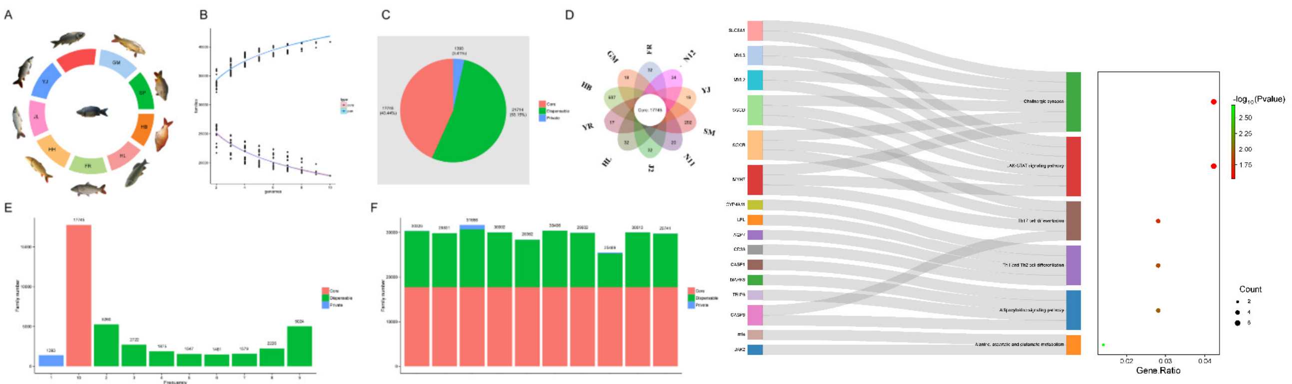
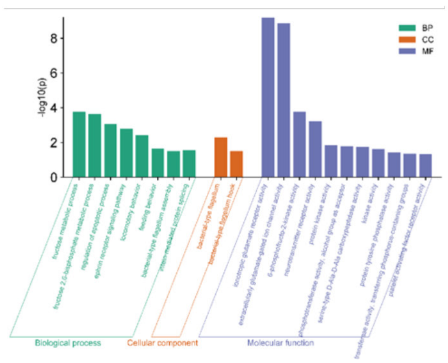
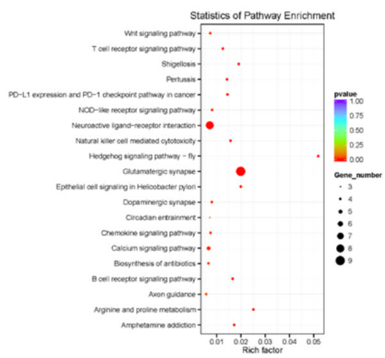
Currently trait dissection and breeding analysis in aquaculture species mainly rely on SNPs generated through aligning sequencing data to a single reference genome. However, it is far from enough to cover all genetic variations of germplasm (especially large structural variations). Pangenomic analysis played a key in recent years in germplasm identification and breeding of plants and livestock animals, which used graphic genomes to accurately screen trait associated genes and variations.

This study constructed 7 chromosome-level genomes of common carp, then annotations and variation identification were carried out. Combined with the existing genomes of Songpu mirror carp, Heilongjiang carp, and Hebao red carp, a high-precision graphic pan-genome covering 10 species of common carp was constructed. Totally 40852 genes were annotated, including core genome of 17745 genes, indispensable genome of 21714 genes, and germplasm specific 1393 genes. Through comparative genomic analysis, annotation of large segment variations, whole genome association analysis, and gene functional identification, functional variations and gene elements related to traits such as disease resistance, intramuscular fat were identified, providing a solid foundation for subsequent breeding of common carp.







APPLICATIONS OF GENOMICS SELECTION FOR SWIMMING PERFORMANCE IN LARGE YELLOW CROAKER

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Large yellow croaker (*Larimichthys crocea*) is the largest fish species produced by marine aquaculture in China. Nevertheless, the croaker aquaculture industry is currently confronted with significant challenges, including the prevalence of severe parasite diseases and the inability of cultured species to tolerate rapid currents. In light of these considerations, we conducted studies investigating disease resistance associations and genomic selection (GS) for swimming performance traits in large yellow croaker. The results demonstrated that: (1) there were individual differences in the critical swimming speed (U_{crit}) of large yellow croaker, with individuals exhibiting superior U_{crit} significantly more resistant to parasite disease than those with inferior U_{crit}; (2) U_{crit} in large yellow croaker is a complex trait controlled by multiple micro-effective genes, with a heritability of 0.26; (3) GS for U_{crit} in large yellow croaker to produce offspring with both improved swimming performance and resistance to parasite disease. This study is the first GS for swimming performance in fish, which proves that this breeding strategy can achieve the genetic improvement of flow resistance in large yellow croaker, and also has the potential of breeding for disease resistance in fish.

CATCH THE WAVE: A WORKFLOW TO IMPLEMENT IMAGES AND ARTIFICIAL INTELLIGENCE FOR SELECTIVE BREEDING

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Introduction

Recent advancements in artificial intelligence (AI) have opened up new possibilities for measuring and monitoring breeding candidates by extensive data collection. Concurrently, automatic phenotyping using non-invasive computer vision techniques has gained significant attention. However, the abundance of methods and data requires a structured workflow to effectively address critical issues in aquaculture breeding. This study introduces a workflow (Fig. 1) specifically designed to maximize the benefits of integrating imaging and AI technologies into both new and existing breeding programs. We demonstrate the effectiveness of this workflow with a case study on critical swimming speed ($U_{crit}U_{crit}$) of rainbow trout, a reliable indicator for swimming performance. We identified four swim traits genetically correlated to $U_{crit}U_{crit}$, and discussed the opportunities that these traits present for selective breeding.

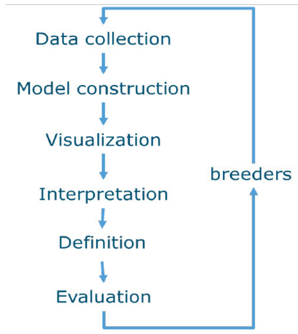

Figure 1. Workflow overview.

Case study

In *data collection*, we captured 3D images of each breeding candidate after conducting swim tests to determine $U_{crit}U_{crit}$. To examine the physical characteristics influencing $U_{crit}U_{crit}$, we utilized a convolutional neural network or *CNN-model* trained on these images to predict $U_{crit}U_{crit}$ values. The result revealed that morphological variance in the fish accounted for only 12% of the variance in swimming performance. *Visualization* with Gradient-weighted Class Activation Maps (GradCAM) helped pinpoint image regions contributing to the predictions (see Fig. 2). With the *interpretation* of fish physiologists, we refined GradCAM into three specific morphological regions in the images with putative biological significance to $U_{crit}U_{crit}$. Four swim traits (see Table 1) were *defined* from these regions, and subsequently *evaluated* for their genetic correlations with $U_{crit}U_{crit}$. Our findings indicated that epaxial muscle volume showed the strongest genetic correlation (-0.48) with $U_{crit}U_{crit}$. Genetically, fish with larger and broader epaxial muscles, larger heads, and smaller caudal fins swim less fast. This suggests that increased fillet yield could be obtained by selecting for lower $U_{crit}U_{crit}$ in trout.

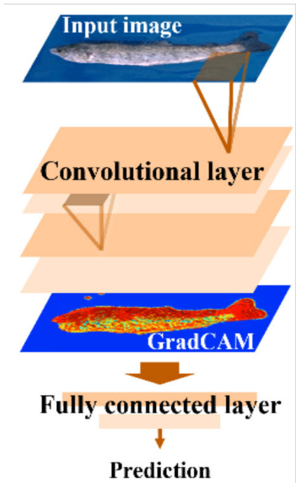

Figure 2. Sketch of CNN and GradCAM.

Table 1. Definitions and image regions of swim

Trait name	Definition	Region in image
Head volume	Volume of the head	
Caudal fin volume	Volume of the tail	
Epaxial muscle volume	Volume of the epaxial muscle	
Epaxial muscle shape	Coefficient of variation (CV) of the depth values of all pixels in the epaxial muscle region	

SYSTEM DYNAMICS MODELLING TO UNDERSTAND IMPACTS OF CLIMATE CHANGE ON SALMON PRODUCTION

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Fish production is influenced by many interacting biological, environmental, social, and economic factors. Robust climate impact assessment at a marine cage site must include the complexities, interrelationships and trade-offs between different natural processes and human interventions. As the fish respond to acute or chronic changes in environmental variables, such as water temperature, pH, dissolved oxygen, salinity, and pathogens, they become stressed. Also, farm operations like delousing and net cleaning can increase stress. However, most studies consider each or a couple of stressors in isolation due to methodological challenges. Stress causes reduced feed intake and increased metabolism, leading to depressed growth potential, poor disease resistance or mortality of farmed fish. Climate change further challenges production through stressors such as increased temperatures and storms. There is a need to better understand how multiple stressors (climate, environment, and production) could impact salmon production in the future.

The present study uses system dynamics to model salmon production in the marine environment. Our model is based on one-dimensional, box modelling approach, divided into three components namely, water quality, fish growth and health & welfare sub-models. The first component deals with dissolved oxygen dynamics, formulated with photosynthesis and air-sea flux as source terms and water column respiration as the major sink term. Three forcing functions drive the dynamics: light and heat from the Sun and wind regime. These are time-dependent inputs with a 1-hour simulation time step to account for daily and seasonal dynamics of the model variables. A baseline simulation of this sub-model describes normal condition of the farm environment in terms of water temperature, dissolved oxygen level, current velocity, etc. Based on this, scenarios of climate-related stressful conditions can be created.

The second and third model components serve together as the response model. Both components interact with each other and with the water quality model and calibrated with farm production data. A flexible nonlinear formulation for fish growth and disease susceptibility is used to allow effective coupling of the three model components. Most growth models of farmed salmon are a function of temperature and body weight, and only few studies have attempted to incorporate the effect of multiple stressors. We aim to leverage on the power of system dynamics to capture complex interrelationships in marine aquaculture system for better assessment of climate change impacts and evaluation of adaptation strategies.

VIRSTATIN AS A PROMISING ANTI-VIRULENCE AGENT TO DISARM BACTERIAL AQUACULTURE PATHOGENS

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To meet the increased demand for animal protein, the aquaculture industry has become the fastest expanding sector in global food production over the past decades. However, the sustainability of this industry is seriously threatened by bacterial diseases, with *Vibrio* species being among the most prevalent and harmful pathogens. Traditional antimicrobials to control these diseases have led to increased multi-drug resistance and environmental concerns, highlighting the urgent need for innovative disease management strategies. A promising novel approach to control bacterial diseases is anti-virulence therapy, which aims at blocking virulence factors, i.e. those phenotypes pathogens need to infect their host. Rather than killing or inhibiting growth, anti-virulence therapy aims at disarming pathogens.

Virstatin, 4-[N-(1,8-naphthalimide)]-*n*-butyric acid, is a small molecule that has been found to block virulence in the human pathogen *Vibrio cholerae* by inhibiting the virulence transcriptional activator ToxT, thus emerging as a promising alternative to target the virulence factors of pathogens without affecting their growth.

In this study, we explored the effect of virstatin on the virulence of *Vibrio campbellii,* a significant aquaculture pathogen, through both *in vivo* and *in vitro* experiments. Our results demonstrate that virstatin significantly increased the survival of brine shrimp larvae challenged with *V. campbellii* at concentrations of 50 µM and 100 µM. Additionally, virstatin also led to a marked decrease in biofilm formation, which is a critical virulence factor of *V. campbellii*, at 8 and 24 hours, respectively. Notably, our data indicate that virstatin did not affect bacterial growth, which suggests that its protective effect is mediated through inhibition of virulence factors rather than bacterial viability. This is important because it indicates that the selective pressure for resistance development will be lower than for conventional antibiotics.

These findings reveal the potential of virstatin as a promising anti-virulence agent to combat bacterial pathogens in aquaculture, making it an effective and sustainable tool in the aquaculture disease management arsenal.

ESCAPE RESPONSES IN EUROPEAN EEL (*Anguilla anguilla*) LARVAE TO MECHANICAL AND VISUAL STIMULI

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Understanding escape behavior in fish larvae is crucial for developing successful aquaculture protocols. This knowledge informs optimal husbandry practices, improves survival and well-being. Studying European eel (*Anguilla anguilla*) larvae presents unique difficulties as field observations of pre-leptocephali in the Sargasso Sea are logistically challenging. This laboratory study utilizes video tracking to investigate the escape behavior of hatchery-reared eel larvae from hatching to 12 days post-hatch (dph). This trial focuses on identifying and characterizing the larvae's escape responses to visual and mechanical stimuli, specifically sudden changes in light intensity and vibrations caused by a ball-bounce near the tank.

Escape variables, such as distance moved in single-frame intervals (D_{esc_40ms}), distance moved in 120 ms (3 frames) (D_{esc_120ms}), initial escape speed (U_{init}), total distance moved in 5 s (D_{esc_05s}), mean speed for the entire response (U_{mean}), maximum speed for the entire response (U_{max}) were computed to quantify the short- and long-term larval escape behavior. As shown in Figure 1, light-flash stimuli elicited a stronger escape response compared to ball-bounce (mechanical) stimuli, especially considering the long-term escape behavior parameters, suggesting that larvae may become accustomed to repeated mechanical stimuli. Moreover, escape response was strongest in 3 dph larvae and decreased with age up to 12 dph, showing the quick development of the sensory system, with younger larvae being less able to discriminate between potential threats and random perturbances of the environment around them. Additionally, while repeated stimulations are common in escape response studies, excessively short intervals may underestimate escape performance due to insufficient recovery time in larvae.

In conclusion, this study highlights the need for optimized hatchery rearing practices during the early stages of European eel by minimizing the use of sudden light intensity changes during the first few days after hatching, as well as minimizing water turbulences to avoid excessive mechanical stimulation. These measures can contribute to improved welfare and potentially enhance survival rates of European eel larvae in hatchery settings.

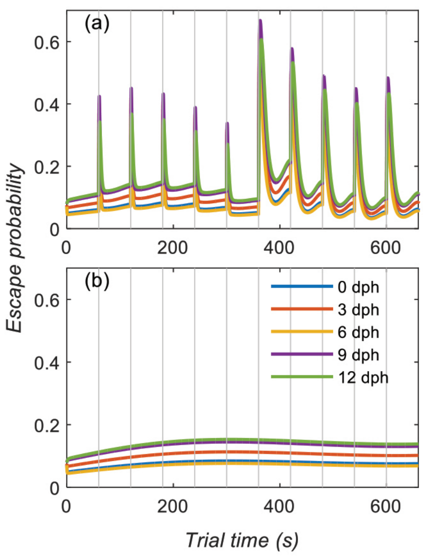

FIGURE 1. Estimated probability of escape behaviors in (a) the escape trials, and (b) the normal trials at different dphs.

RECOMBINANT NANOVACCINE BASED ON HYBRID CHITOSAN-AGNPS NANOPARTICLES AGAINST VIRAL NERVOUS NECROSIS IN GROUPER

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One of the biggest challenges in grouper farming is the attack from various parasites or intracellular viruses, such as *Viral Nervous Necrosis* (VNN). This virus targets grouper during the larval and juvenile stages and can cause mortality rates of up to 100%. The aim of this research is to study the impact of administering a nanovaccine with recombinant protein from Chlorella vulgaris based on hybrid Chitosan-AgNPs nanoparticles in enhancing the growth, survival rates, Relative Percent Survival, and immune response of cantang grouper infected with VNN through the blood cellular response. The treatments are divided into 8 groups: K+ (Fish infected with VNN), K- (Healthy fish), T1 (Cantang Grouper + nanovaccine 33 μl), T2 (Cantang Grouper + nanovaccine 66 μl), T3 (Cantang Grouper + nanovaccine 112 μl), T4 (Cantang Grouper + nanovaccine 33 μl + VNN), T5 (Cantang Grouper + nanovaccine 66 μl + VNN), and T6 (Cantang Grouper + nanovaccine 112 μl + VNN). Parameters observed in this study include growth, instantaneous growth, relative growth, and total growth over 28 days. Observations of survival rate, Relative Percent Survival, and blood immune response, including measurements of erythrocyte and leukocyte counts, hemoglobin levels, and leukocyte differentials (lymphocytes, monocytes, basophils, and neutrophils).

The study shows that administering the recombinant nanovaccine, based on silver nanoparticles (C-AgNPs) derived from C.vulgaris, significantly boosts immune gene expression in cantang grouper post-challenge. Treatment 4 (Cantang grouper + nanovaccine 33 μl + VNN virus) yields the best outcomes: a survival rate (SR) of 88.9%, enhanced growth rates, and notable blood cellular responses. Survival rates for 28-day cultured cantang grouper range from 16.7% to 94.4%, with the highest in Treatment 1 (T1) and Treatment 4 (T4). Relative Percent Survival (RPS) ranges from 56.3% to 87.5%, with T1 and T4 showing the highest values.

Notably, T4 exhibits the best leukocyte and erythrocyte counts, as well as hemoglobin levels. In conclusion, the administration of the recombinant nanovaccine from Chlorella vulgaris based on silver nanoparticles (C-AgNPs) in vivo shows a preventive response to Viral Nervous Necrosis virus infection. The optimal nanovaccine dosage is 33 μl, representing an effective vaccine immunogenicity level in enhancing the immune system of cantang grouper. This is evidenced by the growth response, survival rates, Relative Percent Survival, and blood cellular response of cantang grouper.

Table 1. Response of Blood Cells.

Parameters	K(+)	K(-)	T1	T2	T3	T4	T5	T6
Erythrocyte (10^6cells/mm^3)	2.28	2.55	2.65	2.62	2.61	2.55	2.52	2.49
Leukocyte (10^3cells/mm^3)	1.56	1.13	1.78	1.71	1.68	1.80	1.73	1.65
Hemoglobin (g/dL)	5.2	6.3	7.4	7.1	7.0	6.7	6.2	5.9
Neutrophils (%)	15	7	20	20	17	22	19	17
Lymphocytes (%)	83	78	88	87	84	89	87	83
Monocytes (%)	15.17	9.17	23.33	17.00	16.33	23.33	17.67	16.17

DMRT1-siRNA INTERFERENCE LED TO INCREASED FEMALE NUMBER AND LARGER SIZE IN YELLOW PERCH

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Yellow perch (*Perca flavencens*) exhibit sexually dimorphic growth, with females growing faster, maturing later, and reaching a larger size than males. Production of mono-sex fish using sex control technology has become essential for the improvement of aquaculture productivity. In this study, effects of *dmrt1* siRNA-silencing on gonadal development, sex differentiation and determination were evaluated. Despite administering the injection at a later stage (58-61 days after hatching), a partial feminization of all-male fish was achieved. The results showed that the *dmrt1* gene interference led to an 20% increase in the female number and larger size compared to the control group. The percentage of females in the experimental group that received *dmrt1*-siRNA treatment significantly increased to 70.09%, compared to the 46.79% observed in the negative control group that also received *dmrt1*-siRNA treatment. The expression level of *dmrt1* exhibited a negative correlation with the body size and growth rate of yellow perch. The interference also exerted a detrimental effect on the process of sperm production and maturation. No abnormal gonadal development or hermaphroditism were observed in both females and males.

The findings suggested that *dmrt1* might be a key gene in the male sex determination mechanism of yellow perch. It was likely that other genes were involved, and further exploration was necessary. Meanwhile, the negatively influences of *dmrt1* on the growth rate of yellow perch suggests that regulation of the *dmrt1* gene could be used to promote growth. Further research is needed to determine whether fully knockout or silent of *dmrt1* could lead male to female transformation in yellow perch.

SALINE-ALKALINE AQUACULTURE IN CHINA

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China has 99.13 million hectares of saline-alkaline land and 45.87 million hectares of saline-alkaline water resources in 19 provinces, municipalities and autonomous regions, and most of them belong to Athalassic saline water. Saline–alkaline waters present a challenging environment for most organisms with the characteristics of high pH value, high carbonate alkalinity, high ion coefficient, many types of water quality have serious imbalance of main ion ratio. With more than 30 years efforts, through the research and development of saline-alkaline water quality improvement and control technology, selecting of saline-alkaline-tolerant aquaculture organisms, and the construction of saline water aquaculture and fishery comprehensive utilization model, we have demonstrated the application of saline-alkaline water aquaculture and fishery comprehensive utilization model in 11 provinces and cities in China. Here, we provide a summary of the development of saline-alkaline aquaculture in china, including the characteristics of saline-alkaline water and soil, stress associated with high saline-alkaline environments, strategies for survival in high saline-alkaline environments, saline-alkaline aquaculture models in china with typical cases and future directions.

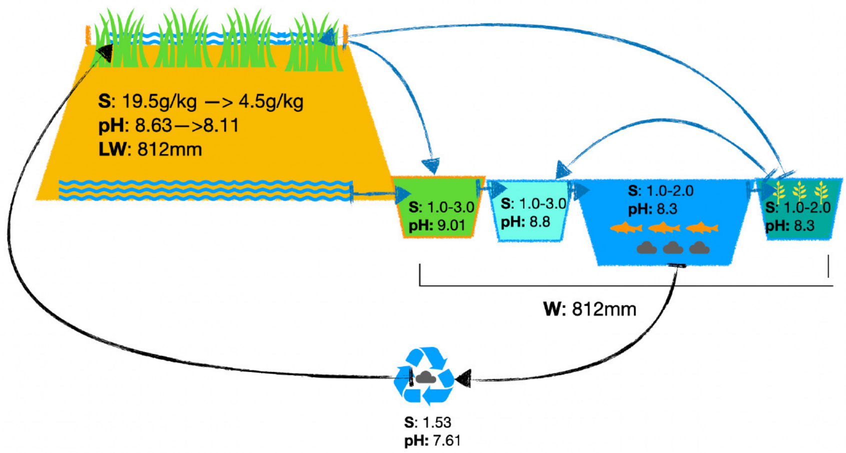

Figure 1 New model of reclaiming saline-alkaline water and soil (S:salinity, LW:Leaching water, W: collected water)

PRELIMINARY STUDIES ON THE DETECTION AND PRESENCE OF LYPHOCYSTIS DISEASE VIRUS (LCVD) IN SEA BREAMS (SPARUS AURATA) RAISED IN AEGEAN SEA

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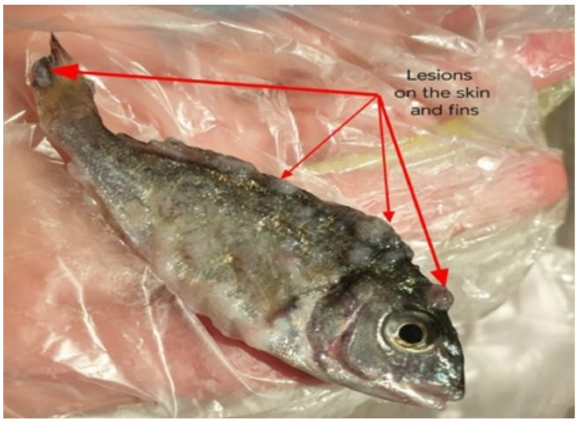
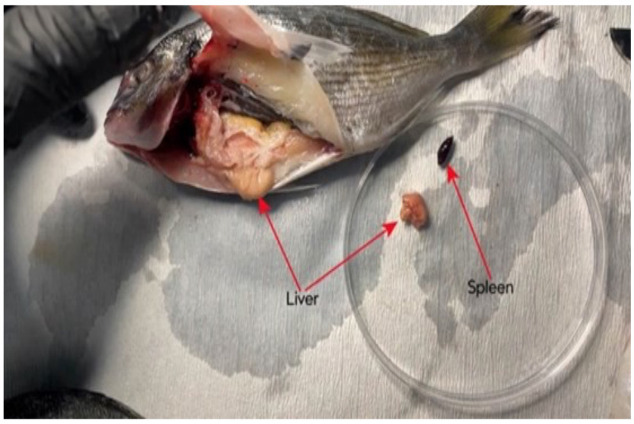
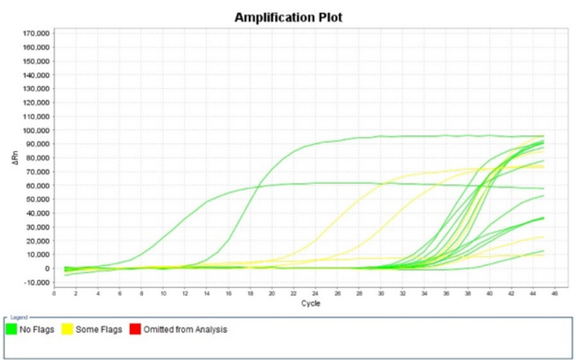
Lymphocystis disease (LCD) is the most frequently reported viral infection in sea bream farms in the South Atlantic and Mediterranean regions. Therefore, in this study, the presence of lymphocystis disease virus (LCVD) which is the causative agent of LCD was investigated in sea bream (Sparus aurata) farm in the Aegean region. The 78 fish samples, 40 of them showing fin/skin lesions characteristic to LCD and 38 fishes without skin lesions were collected. Samples from skin lesions and spleen and livers were taken from the fishes without skin lesions. The samples pooled were analyzed for the presence of LCDV by SYBR-Green real time PCR. All samples were found to be positive by real time PCR, but an amplification was seen only in 1 sample by conventional PCR. Sequence analysis has indicated that nucleotide sequences were belong to capsid gene of LCDV. In conclusion, this study shows that LCDV is present in Türkiye and causes serious health problems in sea bream in Izmir, Türkiye. Screening of fishes for LCDV by real time PCR is very crucial especially in fishes without skin lesions. Sequence analysis helps to determine circulating strains and variants of the virus in Türkiye

This study shows that LCDV is present in Türkiye and causes serious health problems in Izmir, Turkey. Screening of fishes for LCDV by real time PCR is very crucial especially in fishes without skin lesions. Conventional PCR helps for sequencing the virus to determine circulating strains and variants of the virus. This will input data in vaccine preparation and vaccination strategies







REDUCING LAND-BASED MARICULTURE ENVIRONMENTAL RISK BY IN-SITU ELECTRO-CHLORINATION FOR DISINFECTION OF EFFLUENT

Uri Yogev [1,2*], Or Ohayon[2], Yuval Avni[2], Tanya Karginov [2], Noam Yogev[2], Iris Meiri[2], Rosa Sterm[2]

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Land-based mariculture operations often discharge seawater into the sea. proliferation of microorganisms in the farm potentially causes environmental harm. To mitigate these risks, stringent regulations mandate the disinfection of mariculture effluent, traditionally achieved through UV disinfection. However, UV disinfection entails high energy consumption and maintenance costs. In this study, we explored the feasibility of on-site electro-chlorination as a cost-effective and environmentally sustainable alternative to UV radiation for disinfecting mariculture effluent.

Effluents from the Israeli National Center for Mariculture, characterized by approximately 41 ppt salinity, were used in this study. Effluents were analyzed for nutrients, total organic carbon (TOC), and chlorine demand. Sand-filtered effluents were utilized for the experiment and circulated through an electro-chlorinator (Magen, Israel) to produce chlorine (hypochlorite) on-site. The chlorine solution generated was then applied to evaluate the disinfection efficacy of the effluents at various doses. Disinfection efficiency was evaluated using total plate counts, and water samples were further analyzed for trihalomethanes (THM) concentration using quadrupole gas chromatography-mass spectrometry (GC/MS).

Results indicated On-site electro-chlorination effectively reduced microbial loads in the effluent, and a chlorination dose of 5 min·mg/L is satisfying. Total THM concentrations below 22 ppb (Fig 1), much lower than drinking water standards. Notably, the use of on-site electro-chlorination substantially minimized the risk of chlorine leakage and operational costs compared to traditional UV disinfection methods. Based on these findings, a disinfection design incorporating sand filtration for parasite removal and electro-chlorination for bacterial and viral disinfection was proposed (Fig 2) and subsequently approved by Israeli regulators for mariculture effluent disinfection before discharge into the sea. This study underscores the potential of on-site electro-chlorination as a viable and sustainable disinfection method for mariculture effluent, offering significant cost savings and environmental benefits.

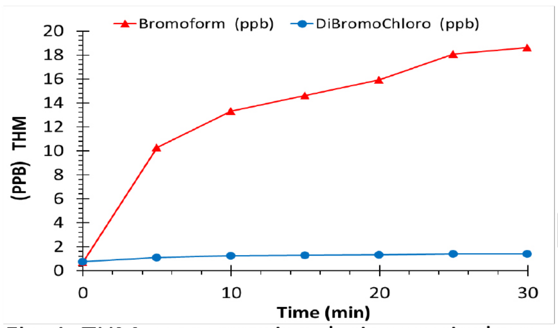

Fig. 1. THM concentration during mariculture chlorination. Chloroform and Dichloromethane were not detected

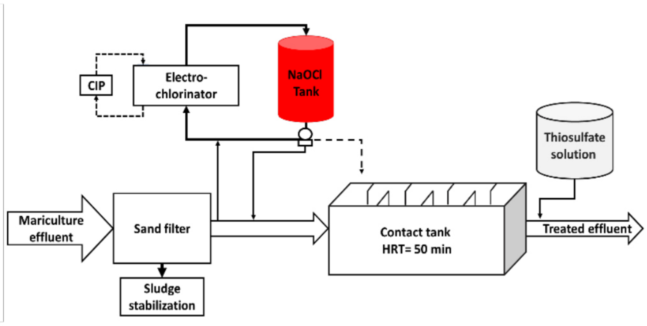

Fig. 2. Schematic of the disinfection design for mariculture chlorination

UPSCALING AND EVALUATION OF A COMMERCIAL-SCALE TREATMENT (DENITRIFICATION) FACILITY FOR LAND-BASED MARINE RAS EFFLUENTS

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At the current stage of commercial land-based marine recirculating aquaculture systems (RAS) production, effluent discharge is inevitable. The effluent primarily comprises fish metabolites and a small portion of uneaten feed, accounting for approximately 70% of feed nutrients and 50% of feed carbon. Typically, due to its high salt content, this stream is released into the sea. However, environmental regulations are becoming increasingly stringent, necessitating treatment prior to discharge. In general, RAS systems consist of a solid separation filter and a nitrification biofilter. Therefore, in addition to sludge, the primary dissolved nitrogen form is nitrate. Denitrification is typically employed as the solution, with extensive work, mainly on small and pilot-scale systems. However, a commercial denitrification treatment for marine RAS is currently lacking. This study aimed to upscale and test a semi-commercial marine RAS effluent treatment facility for nutrient and sludge removal.

The facility was designed to treat the effluent of marine RAS receiving up to 500 kg fish feed per day at a maximum flow rate of 30 m^3/h. It is based on the activated sludge concept with an intrinsic carbon source (fish sludge) for denitrification, and an anaerobic digestion pond for sludge stabilization (Fig. 1). The system comprises a 34 m^3 mixed tank (operating under anoxic conditions) that receives RAS effluents, followed by a 32 m^3 pressurized solid separator (Soliquator, Odis Filtering, Israel). Enhanced sedimentation is achieved through flocculation. The clear water passes through a pair of sand filters before being discharged into the sea. Most settled biomass is recirculated back to the mixed tank, while a small portion of excess biomass outflows to a 400-600 m^3 anaerobic pond. The supernatants from the pond are pumped into a second 34 m^3 mixed tank operating under aerobic conditions (aeration by diffusers) before retreatment in the anoxic tank. Water samples were collected weekly during the study and analyzed using standard methods.

Overall, the system operated for over a year, achieving nitrogen and phosphorus removal efficiency of 60-70%, primarily attributable to suspended solids removal. These results are relatively lower than previously published for pilot scale systems, and the system is currently undergoing optimization. The solid separation efficiency in the Soliquator increases with total suspended solids (TSS) concentrations, with flocculation polymer having a significant impact. Imbalance and high polymer addition resulted in sand filter clogging, necessitating media replacement. It was found that online continuous monitoring and control are critical factors in upscaling and require full attention. Economic analysis revealed that the initial investment in facility installation is the primary cost component, followed by energy consumption. Successful operation of commercial effluent treatment will enhance the feasibility and sustainability of the land-based mariculture industry, representing a significant milestone for its growth potential.

Fig. 1. Picture of the semi commercial marine-RAS effluent treatment facility

FIRST REPORT OF *Parvatrema duboisi* INFECTION IN WILD MEDITERRANEAN MUSSEL *Mytilus galloprovicialis* IN BULGARIAN WATERS OF THE BLACK SEA

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In several European countries, industrial-scale production of local and introduced mussels and oyster species is organized. The main cultivated species is the blue mussel or the Mediterranean mussel (*Mytilus galloprovincialis*) is native to the Mediterranean coast and the Black and Adriatic Seas. During the last decade, there has been an increased interest towards the cultivation of Mediterranean mussels in Bulgaria. More than 50 farms have been created but production is unevenly distributed along the coast like the Northern Bulgarian Black Sea coast produces over 85% of the total production. Regardless of the use of ecologically friendly cultivation technologies and final products that meet safety requirements, collection and consumption from wild populations of mussels continue. In the absence of veterinary sanitary control on wild populations, these organisms are an unexplored potential human health risk factor. Since there are significant gaps in knowledge about the health status of wild mussels in Bulgarian waters of the Black Sea, this gives us an apparent motivating reason to arrange the current study. In the present study, we present the first record of the trematode parasite *Parvatrema duboisi* infection in wild Mediterranean mussels (*Mytilus galloprovincialis*) along the Bulgarian Black Sea coast**.**

Mature mussels (*Mytilus galloprovincialis*) were obtained in a natural bed on the North Bulgarian Black Sea coast near Cape Shabla. The samples were collected monthly by scientific scuba diving in the period between December 2022 and November 2023. The biometric measurements were undertaken with a digital calliper (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 240 specimens have been analyzed to check health status and for evidence of potential pathogenic organisms by macroscopic diagnosis and 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 a 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).

In macroscopic observation of samples (n=100) from the winter period of 2022/2023, some changes were found in the shell's surface, as well as irregular carbonate deposition and metacercariae (formation of pearls) in the mantle of a small number of infected mussels. In the subsequent histological examination among the analyzed internal organs, parasite metacercariae were observed in the mantle and hepatopancreas (Figure 1a, b, c) the effects on the examined mussels presented hemocyte infiltration around the metacercariae. The parasites were identified as *Parvatrema duboisi* based on their distinguished trematode structure and large oral sucker with cyst.

Marine bivalve molluscs are as first intermediate hosts and molluscs (bivalve or gastropod) or polychaetes as the second intermediate hosts for *Gymnophallid trematodes*. Based on observations, we concluded that the number of metacercariae increases occur during a period of decreasing marine water temperature, have been reported in the Black Sea mussels previously.

Conclusion
Despite the wide distribution of *Mytilus galloprovincialis*, their wild populations have hardly been studied, and there is no report of health status from Bulgaria. To our knowledge, this is the first record of natural *Parvatrema duboisi* infection in wild Mediterranean mussels (*Mytilus galloprovincialis*) along the Bulgarian Black Sea coast. Results obtained here have the potential to provide a valuable background for future studies.

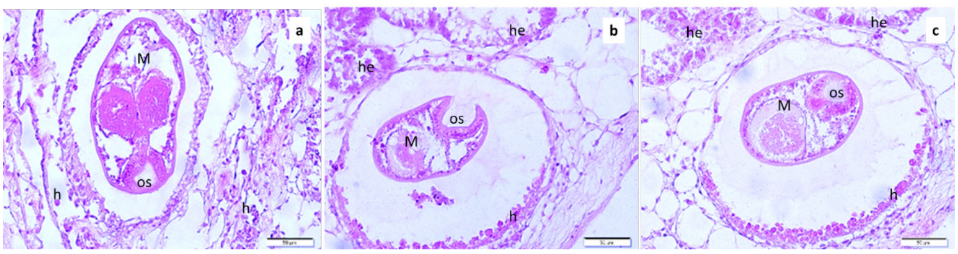

Figure 1. Metacercariae of *Parvatrema duboisi* from *M. galloprovincialis* **a:** mantle; **b,c:** hepatopancreas (os: oral sucker, h: hemocytes, he: hepatopancreas, Metacercariae) (H&E)

MULTIPLE STRESSORS RELATED CLIMATE CHANGE HAVE COMPOUNDING EFFECTS ON FISH HEALTH

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A record 62.7 million salmon died in Norwegian net-pens in 2023. High mortalities in Norwegian salmon farming are caused by e.g., pathogens, parasites, ulcers, gill diseases and intense handling operations, such as delousing procedures. As the effects of climate change become more prominent and impacts intensify, environmental conditions may increase as a risk factor to fish health.

Using downscaled temperature projections based on the Intergovernmental Panel on Climate Change (IPCC) climate projection (Shared Socioeconomic Pathways, SSPs), we have analysed potential future temperatures at selected marine fish farms in Norway. Based on the regional climate projections, we designed experimental trials with different temperature profiles reflecting the near future scenarios. Then we tested the robustness of Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*) after exposure to sub-optimal temperatures by exposing them to jellyfish fragments and reduced pH, respectively.

Results from the temperature projections show that the farming area may experience increased temperatures the next 10–15 years, including more days with temperatures above what is considered optimal for the species that are currently farmed in the areas. Sub-optimal environmental conditions induced micro-damages at the molecular and cellular levels in several organs, including gills and skin. These damages may make the fish less resilient to withstand other impacts from climate change, secondary stressors arising from climate change or general stress from the production systems. Salmon exposed to jellyfish and increased temperature in combination had more damages in gills compared to fish kept at lower temperatures, while cod had weaker scales after combined exposure to increased temperature and reduced pH.

The results can further be used to better understand how changing climate conditions in combination with secondary stressors affect fish health. Understanding how multiple stressors affect the biological performance of farmed species is essential when planning for robust production in the coming years.

The work was supported by the Norwegian Troms and Finnmark county (TFFK2021-179, SecureCod), the Norwegian Research Council (No. 194050, Insight), and the UK Research and Innovation Future Leaders Fellowship (MR/V021613/1).

GENETIC IMPROVEMENT OF AQUACULTURE PERFORMANCE FOR TETRAPLOID PACIFIC OYSTERS *Crassostrea gigas* AND PORTUGAL OYSTER *C. angulata*

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Tetraploid oysters play an important role in the global oyster industry, especially as the core germplasm for producing triploid oysters. So far, the genetic improvement of tetraploid oyster is poorly understood. The viability, growth, ploidy and fertility of the tetraploid progenies were evaluated through four and three consecutive generations of mass selection in *C. gigas* and *C. angulata*, respectively.

In *C. gigas*, the viability of progeny has not been well improved, which is mainly affected by the quality of gametes. The growth traits of these progenies were significantly improved through mass selection over generations, similar to those of diploid oysters previous reported. The ploidy-level of tetraploid progeny differentiated and transformed into triploids, diploids, aneuploids, or mosaics in the early stage of gametogensis. Over generations, the proportion of tetraploid progeny increased significantly, and in the third generation (F3), the proportion of tetraploid reached more than 90%. Like diploid oysters, tetraploid progeny can undergo gonadal development to produce gametes and perform normal reproductive functions. Since the fourth generation (F4) have medium-high heritability, further genetic improvement is feasible.

In *C. angulata*, it's showed that there was no significant difference in the survival rate from larvae to adult between the selected and control groups. However, the growth of the selected group was significantly higher than that of control tetraploids, that was, the shell height and whole weight of F3 were significantly higher than that of control, and the selection advantage of shell height increased from 7.80% of F1 to 34.68% of F3. The ploidy composition of the two tetraploid groups was relatively stable before the 90th days, but on the 270th day, some tetraploids underwent chromosome loss and transformed into diploids or triploids, which might be related to gametogenesis of tetraploids. However, over generations, the number of individuals with chromosome loss in the selected group gradually decreased, and their ploidy became more stable than that in control. In addition, both groups were fertile, with a sex ratio of more males than females and a proportion of hermaphrodite individuals.

In conclusion, the growth rate and tetraploid stability of the two selected tetraploid lines were significantly promoted in *C. gigas* and *C. angulata*, respectively, promising further selection progress and better utilization in oyster industry.

ADVANCING AQUACULTURE FOR SUSTAINABLE FOOD SECURITY: 20 YEARS OF GENETIC ENHANCEMENT IN ASIAN SEABASS IN SINGAPORE

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Securing global food resources is of paramount importance, and aquaculture stands as a pivotal contributor to this endeavour. This presentation sheds light on the indispensable role of genetic enhancement in augmenting aquaculture production, with a focus on the 20-year journey of a selective breeding program targeting key traits in Asian seabass. Initiated in 2004 in Singapore, the program harnessed a comprehensive approach, integrating conventional selective breeding, molecular parentage analysis, marker-assisted selection, and genomic selection. A robust broodstock comprising 549 individuals sourced from the wilds of Indonesia, Thailand, Malaysia, and Singapore laid the foundation for this ambitious undertaking. Through four generations of family-based selection, the program successfully established three elite lines of Asian seabass, each comprising approximately 200 broodfish. These lines were meticulously chosen for their enhanced growth, elevated omega-3 content, and bolstered disease resistance. Notably, these improvements were achieved without compromising genetic diversity. The presentation will delve into the transformative impact of these elite lines on local seafood production, as they have been seamlessly integrated into commercial practices. The speaker will provide insights into the status of the 20-year breeding program, highlighting the strides made in genetic improvement for Asian seabass. Moreover, the presentation will explore the future directions envisaged for advancing the genetic potential of this crucial aquaculture species.

USE OF BLOOD BIOMARKERS TO DETERMINE SMOLTIFICATION STATUS IN ATLANTIC SALMON (*Salmo salar*)

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Objectives:
Determine the feasibility of biochemical profiling of the smoltification process in Atlantic salmon and establish a supervised machine learning model to predict the smoltification state.

Introduction:
Seawater tolerance is a fundamental and critical aspect that salmon must acquire when transferred from hatcheries to growing locations in the sea. The fish acquires this tolerance during smoltification, which is characterized by profound behavioral, biochemical, physiological and morphological changes. Insufficiently smoltified fish will exhibit osmotic problems when transferred to sea and are likely to die. Current techniques to determine the smolt status rely on measuring the plasma chloride level in seawater-challenged fish or the genetic expression analysis of Na+K+ATPases enzymes in the gill. The current methods are not the most effective in terms of being robust and cost-efficient.

Methods: In this study, Atlantic salmon parrs weighing around 10 grams were transferred to a 3 m^3 tank (salinity 4 ppm) for a smoltification trial. Weekly, 50 fish (control group) were extracted from the tank for blood and gill sampling. Concomitantly, 50 individuals (experimental group) were transferred to a seawater tank for a 72-hour seawater challenge. Smoltification was induced by increasing the photoperiod from LD 16:8 to LD 24:0 after 10 weeks. For both groups, blood samples were harvested, and plasma levels of 11 biomarkers were quantified (Crea, Bun, Phos, Mg, K, Ca, Na, Cl, Glu, Lac, tCO2) using the Seamaty SMT-120VP Point of Care system. For the experimental group, plasma chloride levels were additionally measured using a Sherwood 926S instrument. Gill expression levels of NKA1a and NKA1b, a from gill RNA, were obtained by qPCR analysis for both groups.

Results: According to the gene expression results, control fish from weeks 1 to 5 were considered non-smoltified, while fish from sampling points 9 to 11 were considered smolts. A receiver operating characteristics (ROC) curve analysis conducted on the individual analyte obtained by the point of care system indicated that plasma chloride exhibited the most significant predictive value in terms of discriminating smolt and non-smolt fish (AUC = 0.88, $p < 0.05$ (Mann-Whitney U)). Furthermore, calcium, sodium, glucose, and potassium also provided predictive values and exhibited significant differences between the groups. The compounded predictive values of all applicable biomarkers were deployed as independent feature variables for training a xgboost ML model in the statistic software R, and its output predictions corresponded to a ROC-AUC of 0.98 (unseen observations omitted in model training). A challenger ML regression model was used to predict the qPCR index (0-100 scale), and it achieved a root mean square error (RMSE) of 0.11. Increased plasma chloride levels in seawater-challenged fish indicated a transient de-smoltification during the experiment.

Conclusion: Chloride assessments of fish challenged with seawater produced conflicting results between qPCR and other methods, highlighting the limitations of relying solely on this approach. Correlation between the actual delta Ct and the predicted delta Ct based on the blood profile suggests that the biochemical profiling coupled with machine learning algorithms may provide better estimates for optimal seawater transfer and, in turn, decrease the mortality rate caused by osmoregulatory dysfunctions. This method can be used on-site with instant information on the state of the fish. The current ML models should be validated through new experiments and real-world testing to ensure reproducibility and representability.

This study was financially supported by the Regional Research Fund Nordland and Mabit.

(Continued on next page)

Table 1: Comparison of parrs and smolts based on the blood profile and gene expression.

Characteristic	Parr	Smolt	P-value
Weight (g)	24.3	91.5	0.000
Length (cm)	12.16	19.27	0.000
Chloride (mmol/L)	130.4	141	0.000
Calcium (mmol/L)	2.48	2.87	0.000
Potassium (mmol/L)	5.16	4.02	0.000
Sodium (mmol/L)	142.7	148.8	0.000
Magnesium (mmol/L)	0.97	0.76	0.000
Phosphorus (mmol/L)	5.36	5.34	0.218
Creatinine (umol/L)	143.2	83.8	0.000
Blood urea nitrogen (mmol/L)	1.79	1.55	0.019
tCO2 (mmol/L)	12.62	12.78	0.308
Glucose (mmol/L)	5.36	6.51	0.000
Lactate (mmol/L)	4.7	4.19	0.013
NKA1a (Ct)	14.72	17.33	0.000
NKA1b (Ct)	14.49	12.47	0.000
Delta Ct 1a/1b	-0.23	-4.86	0.000
Index	1.56	35.35	0.000

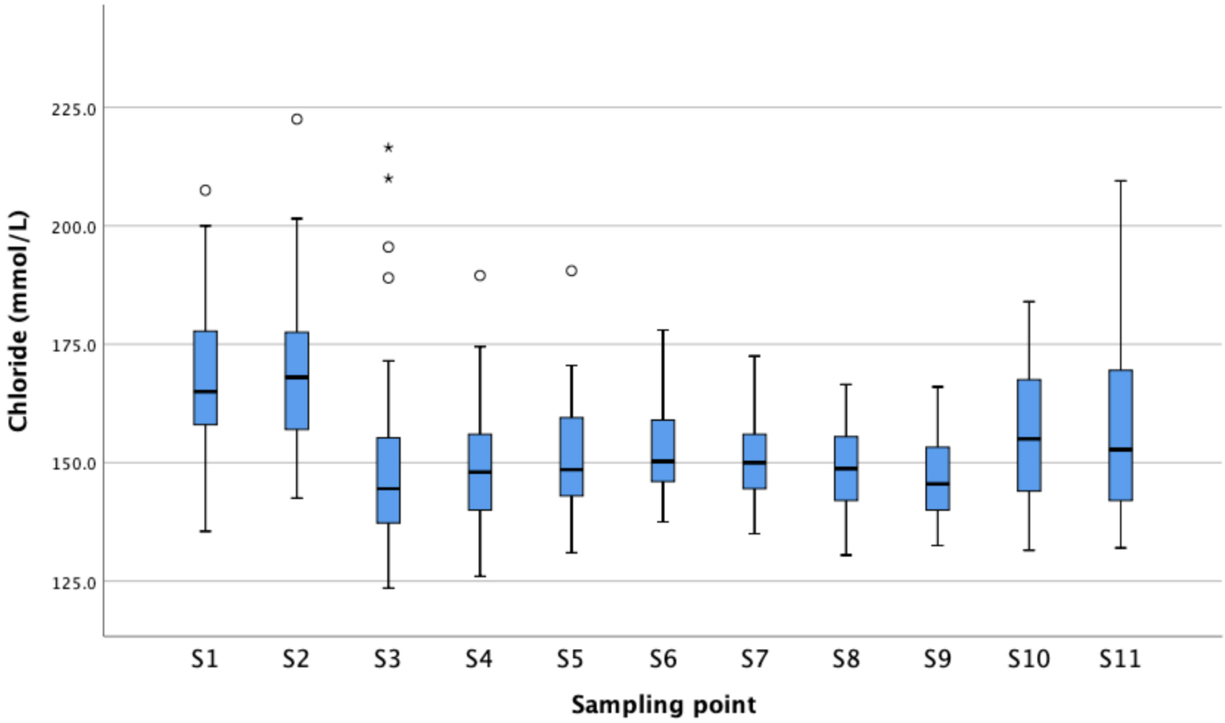

Figure 1: Plasma chloride levels of seawater-challenged fish.

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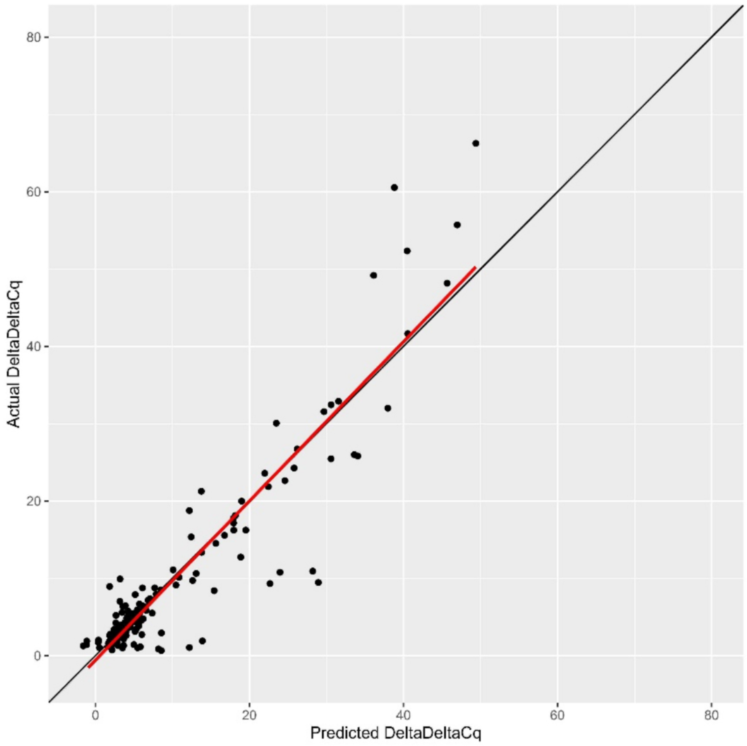

Figure 2: Correlation between ML-predicted and actual measured DeltaDeltaCq of NKA alpha1a and alpha1b.

PERFORMANCE DIFFERENTIALS OF COASTAL AND NONCOASTAL POND AQUACULTURE FARMS IN BANGLADESH

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Fish production environment and thereby the farm performance varies significantly between coastal and non-coastal regions in countries like Bangladesh. This study investigates technical efficiency and other performance indicators differences between farms in coastal and noncoastal regions using data obtained from 121 coastal and 479 noncoastal pond aquaculture farms in Bangladesh. The meta-frontier Data Envelopment Analysis (DEA) and Propensity Score Matching (PSM) have been employed to analyze the data obtained from face-to-face interviews for estimating technical efficiency and other performance indicators differences respectively.

It is seen from the table that the mean meta-technical efficiency score is around 41% when farms from both groups are projected onto a common frontier (pooled frontier) indicating that the outputs can be increased at best by 59% by appropriate management practices, technology, and knowledge. However, the mean technical efficiency is around 61% when coastal farms are projected onto their frontier i.e., frontier formed with observations only from coastal region. Besides, the mean technical efficiency is around 53% when non-coastal producers are projected against their own frontier i.e., frontier formed with observations only from non-coastal region.

On the other hand, the mean meta-frontier technical efficiency is around 49% when coastal farms are projected onto the pooled frontier i.e., the frontier formed with all observations from both coastal and non-coastal regions (Table 3). Besides, the mean meta-frontier technical efficiency is around 39% when non-coastal producers are projected onto the pooled frontier. Therefore, it is evident that coastal farms are technically more efficient than noncoastal farms. The difference is found significant at 1% level of significance (Kruskal-Wallis rank sum test, Kruskal-Wallis chi-squared = 8.05, df = 1, p-value = 0.0045).

Results show that farms located in coastal regions are more efficient than farms located in non-coastal regions. The average output per farm can be increased by relocating aquaculture farms from non-coastal to coastal regions with the same level of inputs.

The coastal farms are following industry best practice production technology. There are further economic benefits of locating pond aquaculture farms in coastal regions because of cost saving and higher return over investment; and also because of the possibility of reaching breakeven at a lower price and quantity than a non-coastal farm needed.

Location	Mean efficiency score		Mean TGR
	Group frontier	Meta frontier	
Coastal	.61	.49	.82
Non-coastal	.53	.39	.74
Overall	0.41		-

THE VALUE OF QUALITY: IMPLICIT PRICES FOR CONSUMER GROUPS PURCHASING PRAWN AT BANGLADESH FISH MARKETS

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In Bangladesh, the production of shrimp and prawn experienced an annual growth rate of 2.6% and 1.1% respectively over the past five years. Despite this growth, there has been a decline in exports, decreasing from 36,168 tons in 2017 to 30,571 tons in 2021 (DoF, 2021-22). As a result, the domestic market share has increased, accounting for 89% of the total shrimp and prawn production in 2021. The primary cause for the decline in exports is attributed to exporters failing to meet the safety and quality standards required by international export markets.

The purpose of this article is to disseminate insights regarding preferences concerning the quality of prawn in the local market of Bangladesh through the identification of implicit prices associated with various quality attributes across diverse consumer segments with differing socioeconomic profiles encompassing income, household size, profession, age, and residential location. The analysis of consumer segments' outcomes is accompanied by a discussion on strategies that prawn farmers can employ to boost both sales volume and pricing through the customization of their production processes in accordance with the varied quality preferences of distinct consumer segments. The research is underpinned by 310 surveys conducted among potential prawn consumers at various retail outlets in Bangladesh, from which a semi-logarithmic hedonic pricing model is derived.

The findings suggest that the size exerts a notable impact on the pricing. A rise of 1% in prawn size correlates with a 5.5% increase in implicit price. Factors like a bright/greenish body and head color, lack of roe, see-through legs, freshness, distinct and luminous eye quality, and an intact appearance with no body damage significantly elevate the price. Conversely, features like unpleasant odor, unprocessed (whole), broken shells, and farming origins negatively affect the price. Implicit prices for each attribute differ across various demographic segments, encompassing income, age, household size, and occupation. Keeping these research outcomes in perspective, players in the prawn value chain could adjust their tactics to boost sales and profitability, while consumers can relish prawns that align with their preferred quality standards.

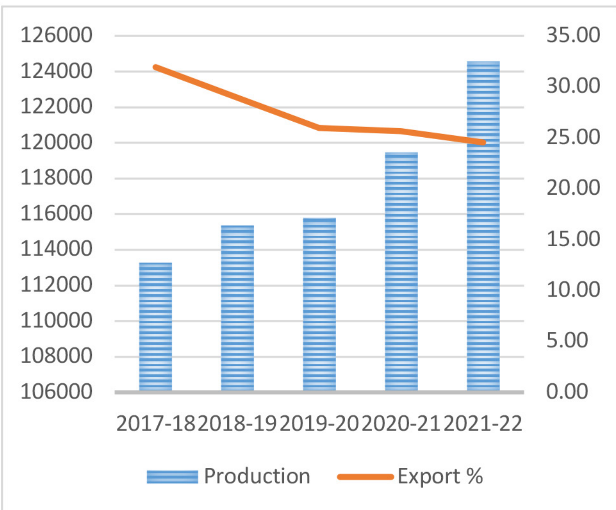

Figure: Production and Export quantity Comparison

REESTABLISHING SUSTAINABLE GROWTH OF SHRIMP PRODUCTION AND EXPORT FROM BANGLADESH BY HANDLING CLIMATE CHALLENGES AND THROUGH IMPROVEMENT OF QUALITY

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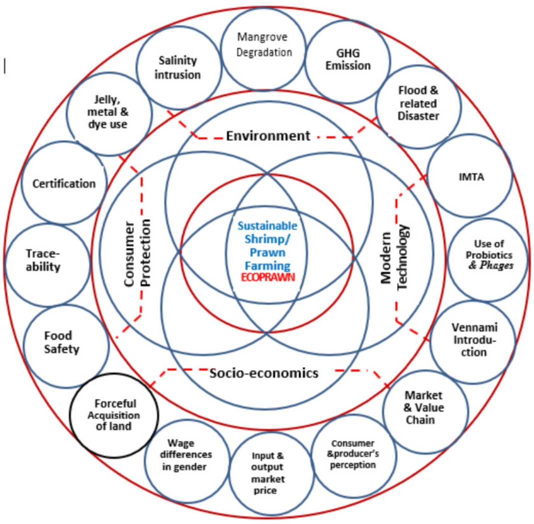
Bangladesh's geographical location, environment, and abundant water resources position the country as a major producer and exporter of shrimp/prawn. Frozen shrimp and prawn constitute Bangladesh's second-largest export category, following garments. However, recent years have witnessed a decline in production and export figures. This review aims to identify the key challenges impacting climate resilience and quality within the Bangladeshi shrimp/prawn industry in order to achieve sustainable growth. Data were gathered and categorized from primary sources (fish farmer's opinions, focus group discussions [FGDs], and key informant interviews [KIIs]) and secondary sources.

The study investigates the recent causes behind the declining trends in shrimp production and exports and discusses how to reverse this trend and reestablish growth. Climate change and quality issues are identified as the primary factors. It is seen from the figure that climate-related challenges include greenhouse gas (GHG) emissions, rising temperatures, sea-level rise, saltwater intrusion, fluctuations in rainfall patterns (floods, droughts), cyclones, and increased disease susceptibility. Quality challenges encompass a lack of food safety certification and traceability systems, chemical contaminants, antibiotic residues, biological/ microbial contamination, physical contaminants, inadequate personal hygiene and sanitation practices, environmental pollutants (including heavy metals), water soaking, jelly and dye injection practices, and notifications from the Rapid Alert System for Food and Feed.

Building resilience within the shrimp/prawn industry requires a multi-pronged approach. Mitigation strategies for climate change involve reducing GHG emissions through diversified farming practices (e.g., integrated multi-trophic aquaculture and introduction of Vennemei shrimp), improved farming infrastructure, implementation of new technologies, establishment of warning systems, and the use of probiotics and phages. Quality improvements can be achieved through international food safety certifications (HACCP), establishing traceability systems, implementing food safety regulations and training programs, raising food safety awareness, capacity building in quality assessment facilities, and continuous research and development efforts. These combined measures along with addressing the socioeconomic issues have the potential to expand Bangladesh's shrimp export earnings and promote sustainable farming practices.

Effective policy interventions are crucial for reestablishing sustainable growth with a climate-resilient, and high-quality export-oriented shrimp industry in Bangladesh and by that improve the socio-economic contribution of the shrimp sector to the national economy.



EFFECTS OF LYOPHILIZED DIETARY YEAST *Rhodotorula mucilaginosa* ON THE SKIN AND FILLET PIGMENTATION OF GILTHEAD SEABREAM *Sparus aurata*. A COMPUTER-BASED IMAGE ANALYSIS APPROACH

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Fish pigmentation assessment is commonly conducted via the CIE Lab model with a colorimeter. However, the non-homogeneous coloration of fish presents challenges for accurate measurement. Computer-based image analysis offers a promising alternative, providing homogeneous conditions without direct tissue contact. Gilthead sea bream, reliant on dietary carotenoids for coloration, can experience characteristic discoloration under intense rearing conditions. Carotenoid supplementation, including natural sources like yeasts, has been explored to mitigate this effect. *Rhodotorula mucilaginosa*, a yeast species capable of producing pigments, presents a potential solution. This study aimed to evaluate the efficacy of *R.mucilaginosa* as a pigmentation source for gilthead sea bream using computer-based image analysis.

One hundred twenty *Sparus aurata* juveniles were acclimatized to laboratory conditions and were randomly placed into 12 tanks. Four experimental diets (C:0%, RM1:1%, RM2:2% and RM3:3% inclusion) containing lyophilized *R. mucilaginosa* strain ACA-DC 5340 obtained by ACA-DC collection of Agricultural University of Athens. Each dietary treatment was triplicated. At the end of the experiment, 18 fish from each treatment were randomly selected for the assessment of skin and fillet pigmentation. The skin and fillet of the left side of the fish were photographed using a wide-angle camera with a resolution of 12 MP under homogeneous conditions. Adobe Photoshop Version 23.5.1 was used for the image analysis. After color correction 5 spots (forefront, operculum, muscle above and under the lateral line and andoment) across the specimen s' body and 3 spots on the fillet (2 on the white and 1 on the red muscle) were chosen for color assessment. The coordinates of the CIELAB, RBG and HSB colour spaces were recorded while Chroma and Whitness variables were calculated.

The results of the study indicated that the inclusion of the red yeast *Rhodotoroula mucilaginosa* could influence the skin pigmentation of gilthead seabream. Results become more noticeable at inclusion rates above 2%. Changes in the flesh pigmentation were noticeable mainly only on the red muscle part of the fillet and at an inclusion level of 3%.

Acknowledgements

Professor E. Tsakalidou and Mrs. E. Manolopoulou from the Laboratory of Dairy Research of the Agricultural University of Athens are kindly acknowledged for providing the experimental yeast strain from the ACA-DC microorganisms collection. The study was funded by the Operational Programme Maritime and Fisheries 2014-2020 and co-funded by the European Maritime and Fisheries Fund through the project "Use of yeasts and fungi in gilthead seabream diets towards improving external coloration and immune enhancement – BRIGHTFISH (MIS 5074567)".

EFFECTS OF FERMENTED GARLIC *Allium sativum* POWDER ON GROWTH PERFORMANCE, BLOOD BIOCHEMICAL, HEMATOLOGICAL FACTORS AND IMMUNE SYSTEM ON RAINBOW TROUT *Oncorhynchus mykiss* JUVENILES

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Fermented garlic powder is a natural growth promoter and immune stimulator. To investigate its effects, juvenile rainbow trout (*Oncorhynchus mykiss*) were distributed into twelve 150 L tanks, with 50 fish per tank, in a recirculation aquaculture system (RAS). Fish with an initial weight of 31.57±0.33 g were fed a pelleted diet containing 0 (Control), 10 (FG10), 20 (FG20), and 30 (FG30) g kg^{-1} of fermented garlic powder manually based on apparent satiation for 63 days. At the end of the feeding trial, growth performance, immune system response, hematological and blood biochemical factors were analyzed. No significant differences in growth performance and nutritional factors were observed among groups (p>0.05). Immune factor measurements demonstrated higher level of myeloid cell phagocytic activity in the control group compared to FG30 significantly (p<0.05). However, no significant difference was observed between in FG10, FG20, the control, and FG30 (p>0.05). Lymphoid cell phagocytic activity in the FG30 group increased significantly compared to FG10 and the control group (p<0.05) but no significant differences were observed between FG20 and the other groups (p>0.05). Hematological evaluations showed higher red blood cells in FG30 compared to other groups (p<0.05), whereas white blood cells demonstrated no significant difference among groups (p>0.05). Additionally, no significant difference in granulocytes, lymphocytes, and monocytes percent, and hemoglobin among groups was observed (p>0.05). The level of hematocrit % in FG30 was higher than other groups significantly (p<0.05). Blood biochemical analysis showed that AST enzyme significantly decreased in the FG30 group compared to FG20 group however no significant difference among control, FG10, and FG30 was found (p>0.05). In opposite, no significant difference in cholesterol, triglyceride, total protein, albumin, alkaline phosphatase, low-density lipoproteins, high-density lipoproteins, and glucose (p>0.05).

NAVIGATING THE COMPLEXITY: CHALLENGES AND SOLUTIONS IN IMPLEMENTING SUSTAINABLE RAW MATERIALS IN COMMERCIAL AQUAFEEDS

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The importance of sustainability in aquafeed is emphasized by increasing customer consciousness and demand for transparent and responsibly sourced raw materials. Central to this effort are standardized frameworks such as product environmental footprint (PEF), sustainability methods like carbon footprint, full life cycle assessment (LCA), and metrics like the forage-fish dependency ratio (FFDR) and fish-in fish-out (FIFO). Aquafeed production accounts for up to 80% of the scope 3 greenhouse gas emissions in salmon farming. It is therefore necessary to explore sustainable alternatives while ensuring optimal nutrition across a broad range of aquatic species. Transitioning to circular and restorative practices in aquafeed production by valorising waste streams and shifting to sustainable farming practices provides aquafeed producers a way to reduce scope 3 emissions in a manner that benefits multiple value chains. Novel ingredients such as insect meal, single cell proteins, low-impact agricultural crops, and by-products from food processing industries are gaining traction and hold promise to mitigate ecological footprints without compromising nutritional profile. However, cost, scalability, food safety, legislation, and consumer acceptance challenges widespread adoption; driving the importance to engage and educate consumers at an early stage. Overcoming these challenges requires collaborative efforts throughout the value chain including researchers, policy makers, producers, retailers, and consumers. To ensure holistic sustainability and avoid problem shifting, standardised methodologies and versatile tools for comprehensive assessments of raw materials are required. While LCA is a valuable industry tool, its limitations are known. Incorporating supply chain biodiversity indicators is essential to account for impacts not covered by an LCA. Furthermore, ensuring the quality, credibility, verification, and accountability of data is imperative to ensure the reliability of the assessment for accurate decision-making. Novel and standard raw materials should be designed with significant improvements over predecessors including both nutritional and environmental aspects. Promising ingredients for wide industrial adoption have low volume constraints, scalable potential, and are located near the supply chain. Raw materials with emphasis on renewable bioenergy feedstocks that leverage proximity to inputs or co-locate with renewable energy in a circular economy, display significant advantages over traditional materials and score highly on certain metrics. The complexity of shifting the global mindset toward implementing sustainable raw materials for aquaculture lies in creating standardised methodologies, navigating diverse stakeholder interests, overcoming deep-rooted practices and ideologies, and addressing economic and regulatory barriers across the value chain, with collaboration as the cornerstone.

PRODUCTION STATUS, CONSTRAINTS, AND OPPORTUNITIES IN EAST AFRICAN FRESHWATER CAPTURE AND CULTURE FISHERIES: A SYSTEMATIC REVIEW AND SYNTHESIS

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Demand for fish continued to grow worldwide while production from capture fisheries has decreased. In the Eastern African Region (EAR), the open-access nature of capture fisheries has resulted in illegal fishing. Within communities engaged in fishing, small-scale fisheries support food security strategies and sustain livelihoods. Despite the role of fisheries in EAR, inland fisheries are vulnerable to loss, and management solutions authorized for inland fisheries are inadequate. This review investigates production potential, challenges, opportunities, and management of inland fisheries in the EAR. It is therefore expected that in aquaculture promising areas, the EAR will turn to depend more on aquaculture to meet the extended needs and supply gaps created as a result of capture fisheries shortfalls. However, aquaculture is still in its infant stage in the region, and there exists no adequate aquaculture policy framework and funds in some EAR (e.g., Ethiopia, Somalia). Stakeholders at all levels should entertain the importance of fishery-based activities for food security in EAR. As a result, easily implemented and community-oriented fisheries legislative documents need to be prepared for advancing sustainable fisheries management. Legislative documents might consider techniques of continual catch statistics (consider small water bodies as much as possible) of inland fisheries and enforcing existing laws to manage illegal fishing activities to accustom sustainable development of inland capture fisheries.

THE USE OF THE ELECTROCARDIOGRAM AS A FIRST-LINE DIAGNOSTIC TOOL FOR MONITORING CARDIAC HEALTH IN FARMED SALMONIDS

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The salmonid fish industry faces a significant challenge with high mortality rates, leading to an annual loss of tens of millions of fish and posing a serious threat to the financial and environmental sustainability of the fish farming industry. While no particular event can be linked to the high mortalities, diseases affecting the heart may have a significant impact on fish performance and stress tolerance, as many fish die during stressful handling procedures when cardiac workload increases. Unfortunately, effective screening tools for early diagnosis and timely precautionary measures to achieve better productivity and economic performance, as well as improve fish welfare, are currently limited. In human medicine, an electrocardiogram (ECG) is often the first-line diagnostic tool for evaluating patients with suspected heart conditions. However, the ECG has not been widely applied in fish health thus far. The present study was designed to comprehensively evaluate the suitability of the ECG as a potential screening tool for cardiovascular disease risk in fish. Specifically, we mapped abnormalities in the ECG by inducing myocardial ischemia through coronary artery occlusion (coronary ligation) in anaesthetised rainbow trout, and used provocation maneuvers to manipulate the fish's heart rate (e.g., by changing artificial ventilation) to increase the sensitivity for detecting any cardiac abnormalities.

Myocardial ischemia significantly affected ECG characteristics. We found loss of QRS voltage and prolonged QRS duration as a consequence of myocardial ischemia, suggesting extensive myocardial injury and delayed ventricular activation. In addition, myocardial ischemia caused heart blocks, typically occurring at high heart rates, which could be alleviated during bradycardia (**Fig.1**). There was a 45% mortality rate among fish suffering from myocardial ischemic injury. At necropsy, some deceased fish showed hemopericardium (blood accumulation in the pericardial sac), likely due to atrial/ventricular rupture. In conclusion, the present study makes a substantial contribution to the development of new real-time diagnostic tools capable of advancing diagnostic and monitoring methods utilized in aquaculture. It also demonstrates that electrocardiography has the potential to be used for screening and diagnosing cardiac conditions in salmonid fish.

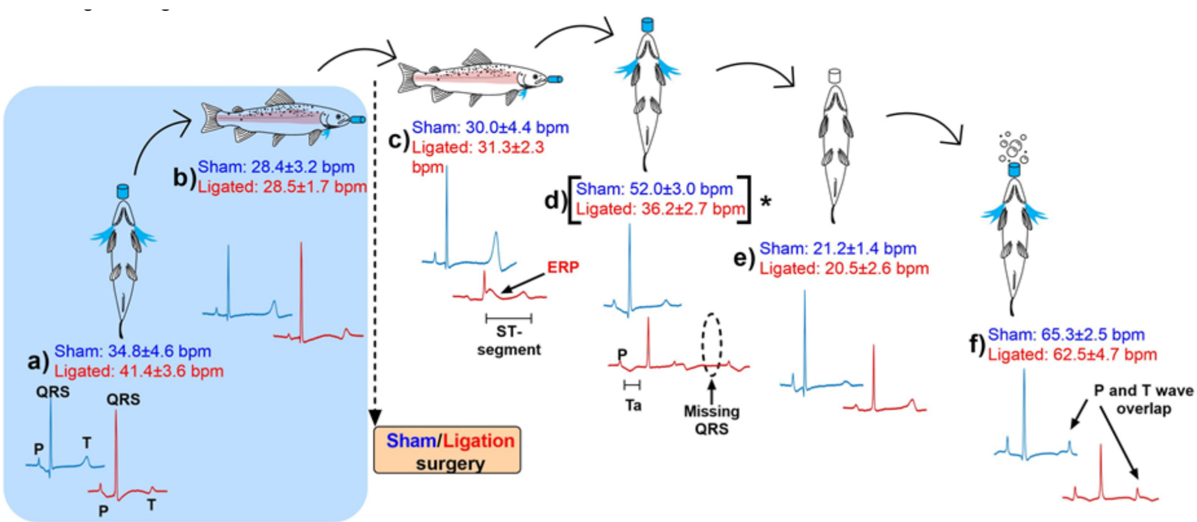

Figure 1. *Figure 1. Electrocardiography abnormalities following coronary occlusion or sham operation in trout. Schematic representations of each water flow regime [i.e., high (**a** and **d**), low (**b** and **c**), no flow (**e**), and high flow + hyperoxia (**f**)] in rainbow trout are depicted, along with representative ECG waveforms. Note the presence of an early repolarization pattern (ERP), causing changes in the ST-segment, and the absence of the QRS complex following the P wave (i.e., heart block). The P wave represents atrial depolarization, the QRS complex represents ventricular depolarization, the T wave represents ventricular repolarization, and the Ta wave represents atrial repolarization. (Modified from Zena et al., 2024).*

VARIATION OF INGESTED MICROPLASTIC SIZE AND QUANTITY IN DIFFERENT TISSUES OF JUVENILE EUROPEAN SEABASS *Dicentrarchus labrax*

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Tracking and quantifying the level of translocation of ingested microplastics into the tissues of fish is key to estimating MP impacts on fish physiology, performance and possible implications for consumers, including humans. Controlled studies on the passage and destination of MPs within higher marine organisms are few.

In this study, established methods were applied to quantify translocation of ingested microplastic (MP) into blood, intestine, gill, liver and fillet of juvenile European seabass, *Dicentrarchus labrax*. European seabass consumed fluorescent MP (1-5µm) particles for 16 weeks in a controlled feeding experiment before organs/tissues were collected and analyzed for quantitative MP contamination.

The average abundance of MPs differed significantly between tissues and was highest in blood samples (54.6 ± 46.3 MPs/g), the intestinal tract (26.8 ± 18.7 MPs/g) and gills (9.8 ± 9.4 MPs/g). In contrast, lower average MP amounts were found in liver with 0.6 ± 1.5 MPs/g and in fillet samples with 0.4 ± 0.3 MPs/g. A clear trend of MP size distribution was observed within the examined tissues. The highest relative abundance of 1 µm of MP was found in intestine, 2 µm in blood and gill, 4 and 5 µm in liver and fillet samples, respectively.

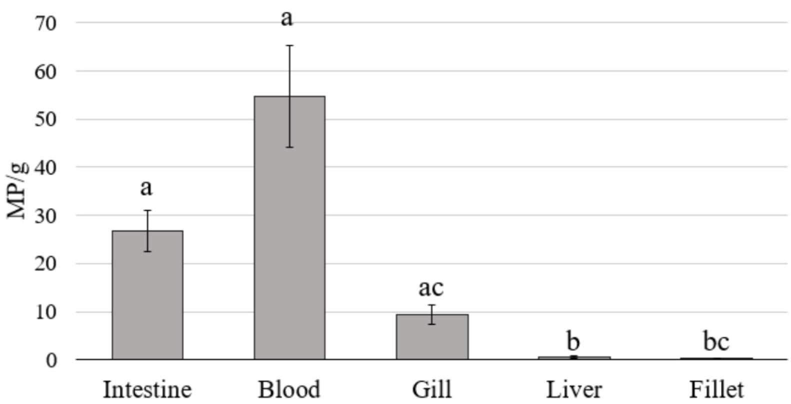

Figure 1: Amount of microplastics per gram (MPs/g) in intestine, blood, gill, liver and fillet samples of European seabass (*Dicentrarchus labrax*) under manual fluorescent microscopy.

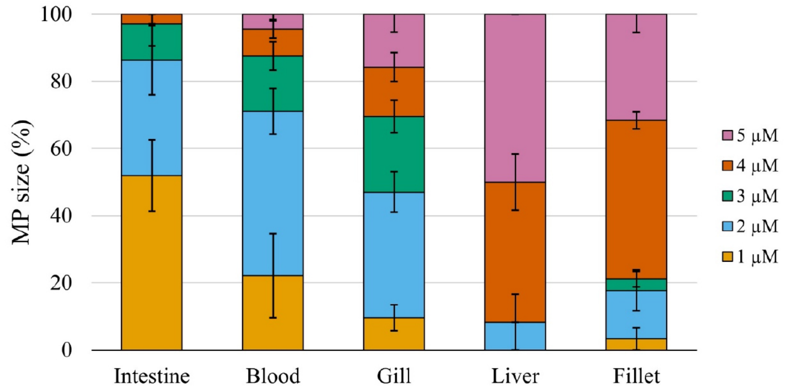

Figure 2. Percentage distribution of microplastic (MP) sizes (1-5 µm) found in all examined tissues of European seabass (*Dicentrarchus labrax*) (n = 20 for each tissue) under manual fluorescent microscopy.

CHARACTERIZATION OF THE MOLECULAR MECHANISMS OF SEXUAL MATURATION IN THE GREENLIP ABALONE *Haliotis laevigata*

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Abalone are economically important cultured species in many countries because of their highly palatable muscular foot. However, overfishing and depletion of wild abalone populations has led to a decrease in the global abalone population. Currently in abalone aquaculture the maturation of males and females is not synchronised, and artificial spawning induction methods are reported to be inefficient. This is unfavourable for rapid gains through selective breeding. Exploring reproductive mechanisms may lead to innovative intervention techniques to enhance abalone production. It has been established that sexual maturity of abalone is a complex process closely linked to the neuroendocrine system, however understanding the molecular signalling components and pathways required for gonad maturation remains unclear.

The greenlip abalone is a temperate species and the most commonly cultivated pure species in Australia. Here, transcriptomics was used to explore differential gene expression during gonad maturation of this species. RNA was extracted from the gonad and ganglia of both males and females at different maturation stages, assessed using the visual gonad index (VGI, Figure 1). The RNA was first used to generate new, high-quality (pooled) transcriptomes from these tissues. Transcriptome profiling was then conducted on individual samples using a low-input Next Generation Sequencing library preparation protocol (CEL-Seq^2). Differential gene expression analysis was conducted between samples from different VGI stages to identify genes likely to be associated with gonad maturation in greenlip abalone.

The data from this study will provide a solid foundation for the functional characterisation of these genes and will provide opportunities to develop innovative intervention methods to prevent or promote spawning. Intervention methods may include pharmacological, mechanical, or environmental mechanisms to control gonad maturation. The ability to intervene in maturation will assist the abalone industry to enhance production and delivery of a more robust product to market.

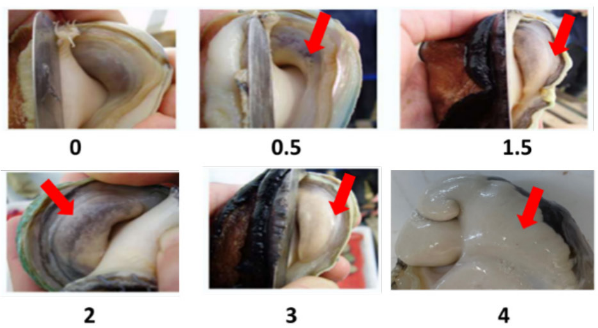

FIGURE 1. Different gonad developmental stages (VGI=0-4) in male greenlip abalone. The larger the number, the closer to sexual maturity. The red arrows indicate the location of the developing gonad.

MACHINE LEARNING-BASED SALMON LICE LARVAE DETECTION IN SEAWATER

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The welfare and economic implications of salmon lice (*Lepeophtheirus salmonis*) infestations on both farmed and wild salmon populations have prompted a growing need for efficient salmon lice detection methods. Accurate measurements of the density of pre-infective nauplius and infective copepodite stages of salmon lice in seawater are essential for farmers and policy makers to make timely interventions and alleviate the stress on salmon stocks. Traditional detection techniques, such as PCR and fluorescence microscopy, are reliable, but also labor intensive and do not allow the close to real-time salmon lice enumeration in sea water necessary for operational use on and between salmon farm sites. This study introduces a machine learning-based approach for automated detection of salmon lice in seawater. A setup for rapidly capturing images of salmon lice in seawater was developed, and a comprehensive dataset was created encompassing both the salmon louse nauplius and copepodite stages. Subsequently, in this study, 16 object detection models, including both CNN and transformer architectures, were trained, tested, and compared. The results demonstrate excellent performance of YOLO series models in this task, with YOLOv8 achieving notable metrics on the test dataset, including an inference speed of up to 416 FPS and a recall rate of 96.5%. During preliminary real seawater testing, the YOLOv8 model utilized in this study achieved a recall rate of 80% and accomplished real-time detection. In addition, to address the problem of limited training data from relevant sea water samples, not spiked with laboratory hatched lice larvae, a data synthesis method was proposed based on instance segmentation. The results indicate that the model can be trained and iteratively updated on a minimal amount of raw data using the synthetic data-based approach. The data synthesis method provides a basis for future model updates across time and environments. Due to morphological similarities, machine learning methods may not distinguish salmon lice from common sea lice as well as PCR. The machine learning method, however, has the potential to be combined with traditional methods, relying on its real-time ability to isolate larvae and facilitate genetic analyses, supporting for determining the relationship of salmon lice to *Caligus elongatus*. Compared to traditional methods, the machine learning approach employed in this study offers a more efficient and accurate detection solution, with the potential to achieve large-scale, cost-effective automation of salmon lice detection, supporting the enhancement of the welfare of both farmed and wild salmon.

Figure 1: Example of detection result of "wild" salmon lice in the filtered and concentrated seawater using YOLOv8n.

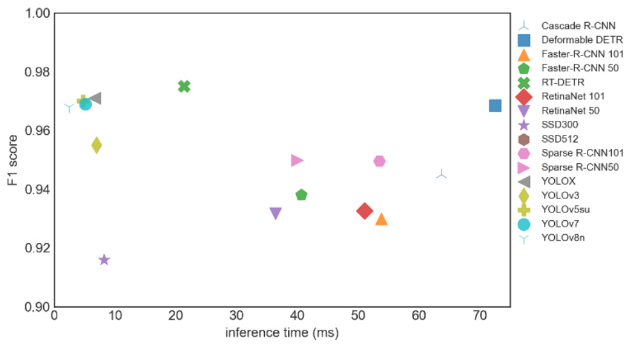

Figure 2: Results of the performance of each model on the salmon lice dataset. Models positioned closer to the top-left corner showed better performance.

INTESTINAL MICROBIOTA AND GENE EXPRESSION ALTERATIONS IN LEOPARD CORAL GROUPER (*Plectropomus leopardus*) UNDER ENTERITIS

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Enteritis poses a significant threat to fish farming, causing intestinal and hepatic inflammation, physiological dysfunction, and dysbiosis. Our study focused on the leopard coral grouper (*Plectropomus leopardus*) during an enteritis outbreak on a South China Sea farm. No abnormalities in feeding, water quality, or specific pathogen infections were identified. This study investigates intestinal flora alterations and host responses to uncover pathogenetic mechanisms and provide a foundation for devising effective disease surveillance and intervention strategies to mitigate the impact of enteritis on grouper aquaculture.

Enteritis-affected fish exhibited substantial differences in intestinal flora compared to controls (P = 0.001). *Norank_f_ Alcaligenaceae*, detrimental to fish health, predominated in affected fish (91.76%), while *Lactococcus*, a probiotic genus, dominated in controls (93.90%). Genera with pathogenic potential like *Achromobacter*, *Sphingomonas*, and *Streptococcus* were more abundant in diseased fish, whereas probiotic genera like *Enterococcus* and *Clostridium_sensu_stricto* were enriched in controls. Transcriptomic analysis revealed strong inflammatory responses, impaired metabolic functions, tissue damage, and iron death signaling in the intestines and liver during enteritis. Correlation analysis showed potential pathogen groups positively associated with inflammation and tissue damage genes, and negatively with metabolic function genes.

In conclusion, intestinal dysbiosis, particularly the high abundance of Alcaligenaceae with pathogenic potential, likely triggered the enteritis outbreak. Alcaligenaceae and genera *Achromobacter*, *Sphingomonas*, and *Streptococcus* were identified as biomarkers for enteritis, while some species of *Lactococcus*, *Clostridium_sensu_stricto*, and *Enterococcus* showed promise as probiotics. These findings enhanced our understanding of enteritis pathogenesis, highlight intestinal microbiota shifts, and propose biomarkers for monitoring, probiotic selection, and enteritis management in fish.

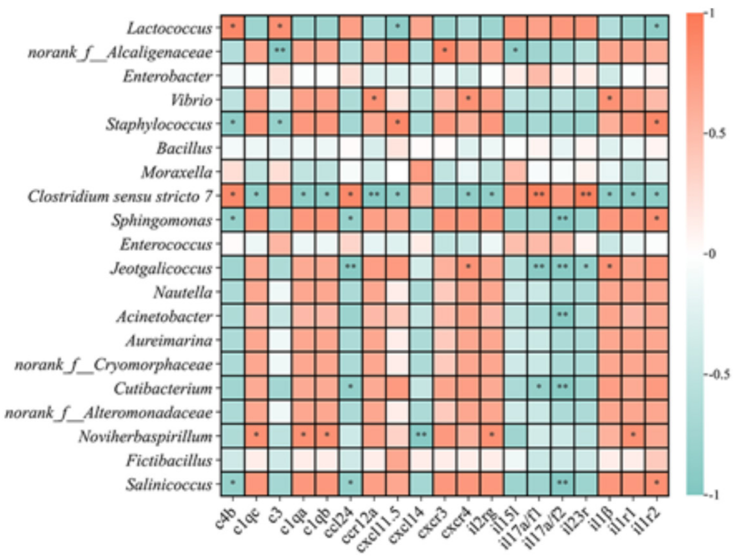

Fig. 1 Correlation between the top 20 abundant intestinal bacterial genera and differentially expressed metabolism-related genes in the liver between enteritis-affected leopard coral grouper and healthy controls. The significant differences at $p < 0.05$ and $p < 0.01$ are labeled as * and ** respectively.

EFFECT OF SEWAGE TREATMENT PLANT'S EFFLUENT ON EARLY LIFE STAGES DEVELOPMENT AND SEX DETERMINATION OF BROWN TROUT *Salmo trutta m. fario*

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Population consumption via treated wastewater is a significant source of pharmaceuticals and personal care products (PPCPs) in the aquatic environment. This pollution results in a high impact on exposed organisms when recipient dilution is low. Laboratory studies are limited by artificial conditions, making it challenging to describe the complex effects of real-world pollution on exposed organisms in the environment. This study aimed to evaluate the impact of contamination from the sewage treatment plant (STP) effluent on early life stages development and sex determination of brown trout (*Salmo trutta m. fario*) *in situ*. This fish is a native cold-water species with long embryo-larval development. Floating egg incubators were used to support salmonid species reproduction. Brown trout eggs were placed upstream (the control group) and downstream (the exposure group) of the STP Prachatice in the Zivny Stream, Czech Republic, maintained in incubators for about three months, and then grown in the natural environment for nearly one year. Up to 72 PPCPs were detected in passive samplers deployed downstream of the STP effluent. *In vitro* bioassays of the sampler extracts also showed the strong endocrine-disrupting potential of the polluted water. Compared with the control group, the mortality of brown trout in the exposed group was significantly higher. Also, the body size, growth, and metabolic rates of exposed fish were significantly lower after statistical correction for temperature. After several months of natural development in the stream, the sex ratio of randomly caught brown trout in the effluent-affected stretch was imbalanced, and sterile individuals were detected. These results suggest that STP effluents can negatively affect the early growth and development of fish in watersheds, and these adverse effects may further affect the population density of aquatic organisms and the balance of the whole freshwater ecosystem. The use of floating egg incubators proved to be a promising approach for studying the effect of pollution on the early developmental stages of fish in natural conditions.

NEW INSIGHTS INTO THE TETRODOTOXIN ACCUMULATION AND METABOLISM IN THE PUFFERFISH *Takifugu rubripes*

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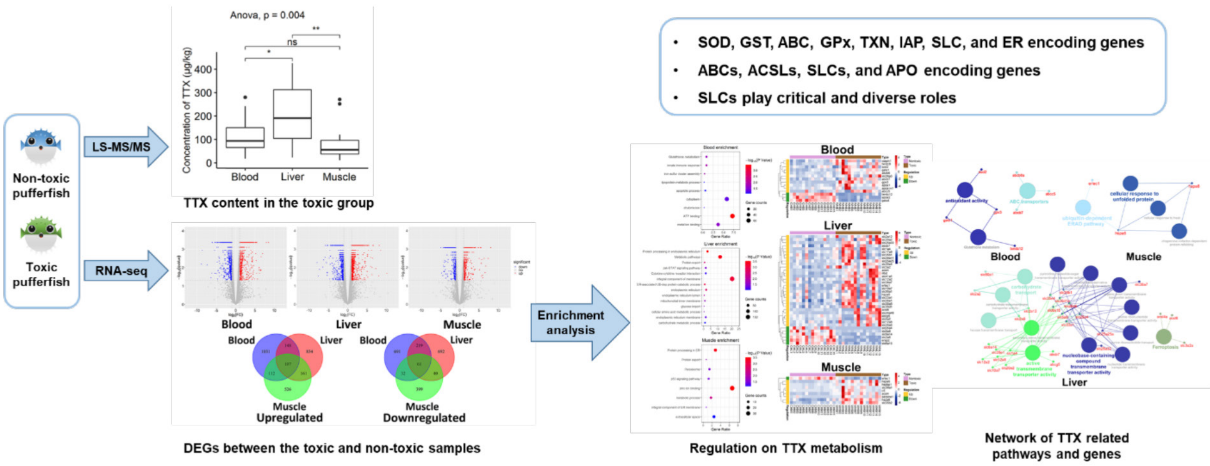
Tetrodotoxin (TTX) is a potent marine neurotoxin that exists in a variety of aquatic and terrestrial organisms. Pufferfish in different habitats show great variation in their TTX contents. Exploring the genes involved in TTX metabolism could contribute to our understanding of the molecular mechanisms underlying TTX accumulation, translocation, and detoxification in pufferfish. In this study, transcriptomic analysis was used to identify the functional genes related to TTX metabolism in the blood, liver, and muscle of the toxic and non-toxic tiger puffer (*Takifugu rubripes*). A total of 6,101 differentially expressed genes (DEGs) were obtained after transcriptomic analysis; of these, 2,401 were identified in the blood, 2,262 in the liver, and 1,438 in the muscle. After enrichment analysis, fourteen genes encoding glutathione S-transferases (GSTs), glutathione peroxidase (GPx), thioredoxins (TXNs), superoxide dismutase (SOD), ATP-binding cassettes (ABCs), apolipoproteins (APOs), inhibitors of apoptosis protein (IAP), and solute carrier (SLC), which are mainly antioxidant enzymes, membrane transporters, or anti-apoptotic factors, were revealed in the blood. Thirty-six genes encoding SLCs, ABCs, long-chain-fatty-acid-CoA ligases (ACSLs), interleukin 6 cytokine family signal transducer (IL6ST), endoplasmic reticulum (ER), and heat shock protein family A (Hsp70) were involved in transmembrane transporter activity and innate immune response. Notably, a large number of *slc* genes were found to play critical and diverse roles in TTX accumulation and translocation in the liver of *T. rubripes*. Nine genes from the *slc*, *hsp70*, complement C5 (*c5*), *acsl*, *er*, and serpin peptidase inhibitor (*serpin*) gene families were found to participate in the regulation of protein processing and anti-apoptosis. These results reflect the diverse functions of genes closely related to TTX accumulation, translocation, and detoxification in *T. rubripes*.

Graphical abstract:



UNVEILING THE TREND: GOOGLE SEARCH INSIGHTS ON SHIFTING PREFERENCES IN SALMON CONSUMPTION

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This study assesses preference shifts in salmon products at different processing levels during the recent pandemic in the UK. We hypothesize that consumers preferred less processed salmon over more processed salmon, as indicated by the significant increase in Google search results for ‘salmon recipe/salmon recipes’ and ‘how to cook salmon’ during the pandemic. For home consumption, the degree of processing is related to the time required for preparation, an indicator of convenience, which is one of the most important determinants of food acquisition and consumption practices. Based on household panel data, our descriptive analysis shows that consumers bought more natural, less processed salmon than prepared salmon, aligning with the internet search results. Among prepared salmon, the sales volume of fresh salmon products grew more than that of frozen salmon products, most of which are easily prepared for eating. We further estimate hedonic price models for natural and prepared salmon to investigate how Google search results for salmon-related queries affect price premiums of salmon product attributes during the pandemic.

DETECTION OF MORPHOLINO MOLECULE TRANSFER TO ATLANTIC SALMON OVULATED EGGS AND FERTILIZED EGGS

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Morpholino oligomers (MO) are short-stranded DNA analogous that are specifically designed to interfere with the targeted gene. By binding to the complementary sequence of target mRNA through a steric blockade, thereby blocking protein translation. MO offers effectiveness in developmental biology. Research involving gene knockdown, as well as a wide range of applications as specific and effective treatments for genetic disorders. Modified MO has been developed to conjugate with cationic liposomes or other carriers to alter the stability and solubility, allowing cell penetration and in vivo uptake. Vivo-morpholinos have been widely used in cell culture treatment through microinjection and bath-immersion.

Unfertilized salmonid egg quality can be extended in media celomic fluid or balanced salt solutions at low temperatures. Moreover, the pore channels in salmonid chorion potentially allow the uptake of biomolecules. Immersion-based method was applied to transport modified MO delivery systems into unfertilized salmon eggs and fertilized eggs. In addition, MO immersion-treated sperm were used for fertilization. Methods to assess MO inside cells remain limited due to the low intercellular concentration, lissamine, a fluorescence molecule was used to follow morpholino uptake, and distribution within the incubated egg.

Results showed MO in the medium was successfully transferred into both ovulated eggs and fertilized eggs, and pre-treated sperm is also capable of transporting MO to ovulated eggs. Microinjection is now still the most used method to deliver exogenous biomolecules into teleost eggs, even with the high cost and low throughput. Immersion-based method potentially provides a feasible approach for large-scale aquaculture applications.

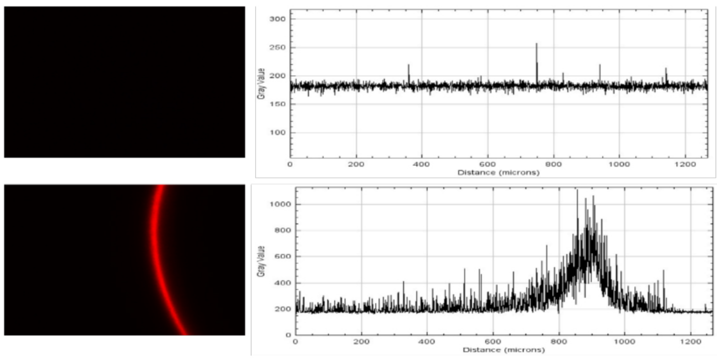

Fig 1. MO uptake in salmon eggs. The excitation wavelength for lissamine is 575.0nm, the control group (up) did not exhibit any fluorescence, and lissamine localization in the chorine and inside of the eggs was observed in MO-ZP9 treated eggs.

HYPOGONADOTROPIC HYPOGONADISM IN MALE TILAPIA LACKING A FUNCTIONAL *rln3b* GENE

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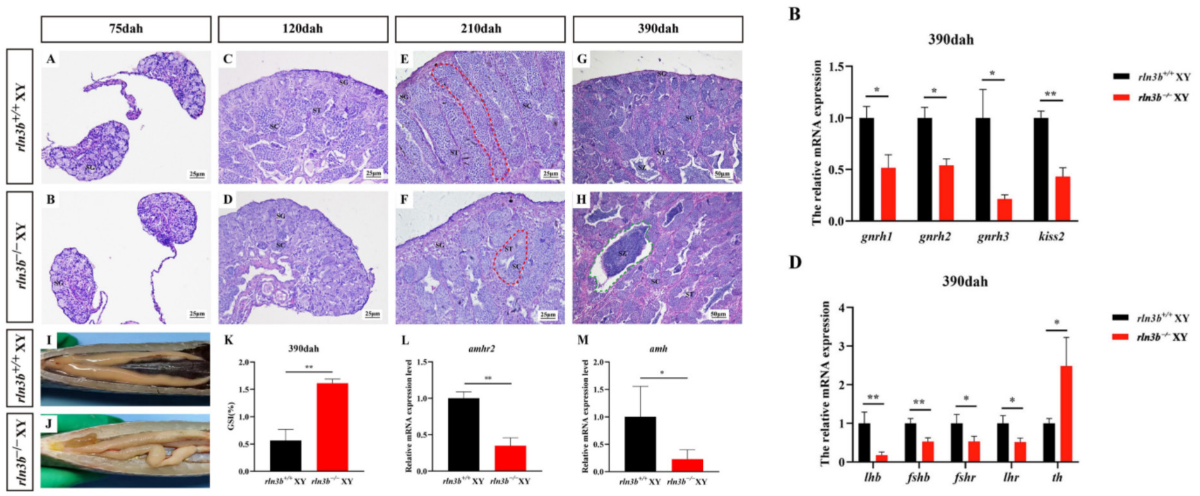
Relaxin 3 is a neuropeptide that plays a crucial role in reproductive functions of mammals, however, its roles in fish reproduction remain to be elusive. To understand the significance of rln3b gene in male fertility, we generated a homozygous mutation line of *rln3b* gene in Nile tilapia. Our findings indicated that rln3b mutation delayed spermatogenesis and led to abnormal testes structure. Knocking out rln3b gene resulted in a decrease in sperm count, sperm motility and male fish fertility. TUNEL detection revealed a small amount of apoptosis in the testes of *rln3b*$^{-/-}$ male fish at 390 days after hatching (dah). TUNEL detection revealed a small amount of apoptosis in the testes of rln3b−/− male fish at 390 days after hatching (dah). RT-qPCR analysis demonstrated that mutation of *rln3b* gene caused a significant downregulation of steroid synthesis-related genes such as Cyp17a1, Cyp11b2, germ cell marker gene, Vasa, and gonadal somatic cell marker genes of Amh and Amhr2. Furthermore, we found a significant down-regulation of hypothalamic-pituitary-gonadal (HPG) axis-related genes, while a significantly up-regulation of the dopamine synthetase gene in the rln3b−/− male fish. Taken together, our data strongly suggested that Rln3b played a crucial role in the fertility of XY tilapia by regulating HPG axis genes.



HEALTHY FEED TO HEALTHY AQUATIC FOOD VIA SINO-NORWEGIAN COOPERATION (*Feed2food*)

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This project builds on the original program to investigate the effects and mechanisms of feed additions of sodium propionate (SP) and ethoxyquin (EQ) on fish in the high-fat feed context, and the mechanisms of occurrence of gut microbial translocations in cultured fish on secondary biohazards in aquaculture and aquatic food products, respectively. Firstly, building on the previous finding that the mould inhibitor sodium propionate causes gut damage in fish in the high-fat feed context, it was further shown mechanistically that propionic acid addition leads to gut oxidative stress caused by propionylation of superoxide-dimutase-2 (Sod2), as well as inducing changes in the composition of the gut microbiota. Furthermore, the negative effects of sodium propionate on the gut epithelial barrier, gut microbiota and susceptible microorganisms in fish in the high-fat dietary background were further clarified on tilapia and rainbow trout. Additionally, the concomitant high dose EQ secondary biological hazards of high fat feeds in China were assessed. The effects of EQ on the gut epithelial barrier, gut microbiota and susceptible microorganisms of tilapia in the high-fat dietary context were clarified, as well as further demonstrating that the addition of SP to the flesh of tilapia fish raised with SP causes secondary biohazards of food for aseptic mice, which provides a theoretical basis for pushing for the enactment of a ban on EQ for feeding purposes in China. The project clarifies the mechanism of fish damage by SP and EQ in a high-fat dietary and the possibility of secondary biohazards, providing theoretical support for the safe production of aquaculture from feed to food. Based on the research results of this project and other research bases of the team, our team was approved to build the National Collection of Livestock and Aquatic Microbes (Beijing), and won the first prize of the 2022-2023 Shennong China Agricultural Science and Technology Award for Scientific Research Achievement.

COUPLING ANAEROBIC DIGESTION WITH AQUAPONICS TO PROMOTE THE CIRCULAR ECONOMY IN ARID ZONES

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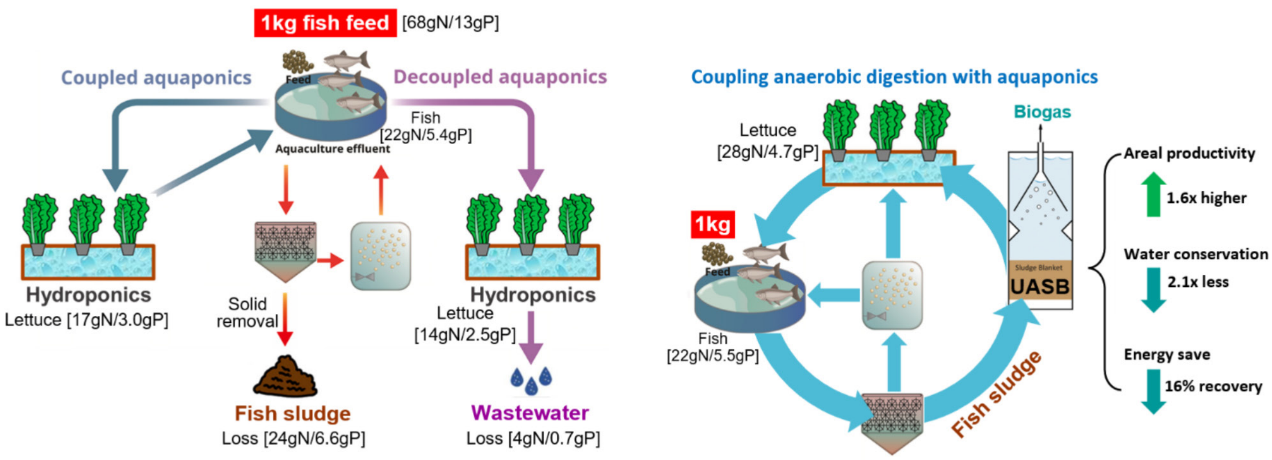
Aquaponics (integration of aquaculture and hydroponics) is gaining renewed interest in enhancing food security (FAO, 2022), with decoupled (DAP) and coupled (CAP) systems as their common types (Baganz et al., 2021). However, several limitations, including resource and energy demand, nutrient imbalances, and waste management, have limited their widespread application (Palm et al., 2023). Efficient onsite treatment of fish sludge and plant wastes as renewable resources to reuse nutrients (e.g., N and P), recover energy and recycle water is a promising solution (Zhu et al., 2023, 2022)focusing on the carbon cycle and energy recovery that was achieved by the addition of onsite anaerobic treatment of the solid waste streams. Following a stabilization stage, the system was closely monitored for four months. Fish tank water was recirculated via solid and nitrification reactors, from which 66% was recycled to the fish tank directly and 34% indirectly through the hydroponically grown plants. Fish solid waste was anaerobically treated, energy was recovered, and the nutrient-rich supernatant was recycled to the plants to enhance production. Plant waste was also digested anaerobically for further recovery of energy and nutrients. Fish stocking density was 15.3 and over time reached approximately 40 kg/m3 where it was maintained. Feed (45% protein content. Zhu et al. (2021) demonstrated that aquaponics wastes could be efficiently treated using anaerobic digestion (AD). We investigated the nutrient, water and energy balances in DAP, CAP, and a novel zero-discharge off-grid aquaponics system with UASB (CAP-AD). We also evaluated the efficacy of nutrient recovery from the UASB in improving crop yields and resource use efficiency. The experiments were conducted in a desert aquaponics facility in a greenhouse with RAS and an adjacent net-house containing deep water culture hydroponics at Ben-Gurion University of Negev, Israel. Catfish (*Clarias gariepinus*) were stocked density of ~50 kg/m^3 with daily feeding of 2% of the total stocking biomass. Lettuce seedlings (*Lactuca sativa cv. Noga*) were introduced on floating rafts of the hydroponics. Following a stabilization stage, the system was closely monitored for four months. An input-output model was developed to support balances for the three aquaponics (Zhu, 2023).

All systems demonstrated good performance in producing fish and vegetable products. Typical fish performance was observed with survival rates >97% and feed conversion ratios from 1.21 to 1.33. In addition, 33% of the total input N and 37% of the P were recovered from fish sludge via AD, yielding a high total nutrient utilization efficiency of 76% N and 80% P. The novel system also demonstrated 1.6× higher plant areal productivity, 2.1× lower water usage, and 16% less energy consumption per kg of feed relative to the (traditional) DAP and CAP systems. Biogas production was 0.84 m^3/kg for fish sludge and 0.67 m^3/kg for dry plant material, which supported about 84% of the energy demand for its operation. CO_2 sequestration was 1.4 higher than the feed carbon, which reduced the system's carbon footprint by 64%. Coupling anaerobic digestion with aquaponics has significant potential utility for increasing system efficiency and promoting the circular economy by treating and reusing waste streams, especially in arid zones.



DEVELOPMENT OF AQUACULTURE TECHNOLOGY BASED ON THE POTENTIAL SYNERGISTIC EFFECTS OF LIGHT WAVELENGTH AND SALINITY ON THE MALABAR GROUPER *Epinephelus malabaricus* GROWTH PHYSIOLOGY

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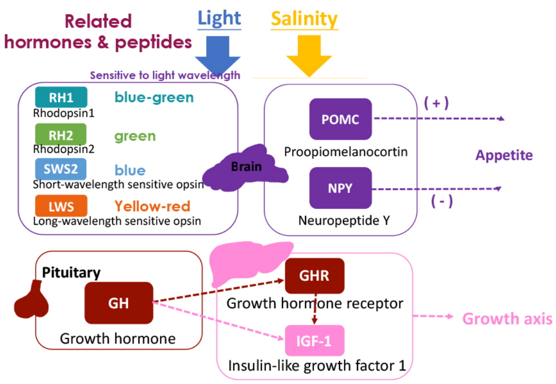
Environmental factors are critical factors influencing fish growth and reproduction. We hypothesized that optimizing these factors can stimulate endocrine systems, enhancing growth and reproduction. Our focus was on the Malabar grouper, a valuable species in Asian markets, and our goal was to understand how environmental factors affect its growth and reproduction for improved aquaculture. This study investigated synergistic effects of light wavelengths and salinity on this fish juvenile growth physiology.

Juveniles reared under various experimental conditions utilizing LEDs with different salinities (11psu blue - 463nm, 11psu red - 623nm, 34psu blue - 463nm, and 34psu red - 623nm) over a two-week period. Compared to red light, blue light increased condition factor (CF) at both 34psu and 11psu salinity, indicating potential lipogenesis promotion. Juveniles under blue light showed higher specific growth rate (SGR) than red light, suggesting consistent light wavelength impact across salinity levels. Best growth occurred under 11psu blue light, while diencephalon opsins expression levels varied under different salinities with the same light, emphasizing synergistic effects of light wavelength and salinities. Juveniles were reared under varied conditions using LEDs (11psu blue - 463nm, 11psu red - 623nm, 34psu blue - 463nm, and 34psu red - 623nm) for two weeks. While Feed conversion ratio (FCR) responded to salinity, *npy* transcript levels hinted at appetite promotion contributing to enhanced growth with blue light. The GH/IGF-1 axis didn't influence observed growth, suggesting complexity in blue light and salinity interaction.

The results emphasize the importance of considering both light wavelengths and salinity levels in optimizing the rearing conditions for aquaculture, offering valuable insights for the sustainable cultivation of Malabar grouper and potentially other marine species.



POTENTIAL BENEFITS OF GLYCINE, PROLINE AND HYDROXYPROLINE ON GROWTH AND FLESH QUALITY OF SONGPU MIRROR CARP, CYPRINUS SPECULARIS SONGPU

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Songpu mirror carp (Cyprinus specularis Songpu), a variety of common carp, is recognized for its notable attributes in growth rate, feed efficiency, and disease resistance. This investigation aims to explore the potential benefits of glycine, proline, and hydroxyproline in songpu mirror carp, seeking to provide empirical evidence that could inform and improve feed formulations. Such advancements are crucial for elevating aquaculture yields and enhancing product quality, thereby aligning with the escalating global demand for fish as a sustainable protein source.

This study aimed to assess the impact of dietary supplementation with glycine, proline, and hydroxyproline on the growth and flesh quality of Songpu mirror carp. A total of 240 fish were allocated to one control and three treatment groups, each receiving diet supplemented with 5 g/kg of either glycine, proline, or hydroxyproline. The findings indicated that while there were no significant differences observed in weight gain or feed conversion rates among the groups, notable changes in serum growth hormone and insulin-like growth factor-1 levels were evident, particularly in the groups supplemented with glycine and hydroxyproline. Dietary proline increased the muscle crude protein, whereas hydroxyproline notably enhanced muscle moisture and reduced drip loss, thereby suggesting improvements in flesh quality. Proteomics analysis revealed differential expression of proteins with clear separations among treatment groups. Targeted amino acid quantification highlighted changes in taurine, methionine, leucine, and glutamic acid, correlating with shifts in protein expression profiles. In summary, our findings suggest that dietary supplementation with these amino acids has beneficial effects on modulate growth hormone release and improving flesh quality in Songpu mirror carp, highlighting potential implications for aquaculture nutrition.

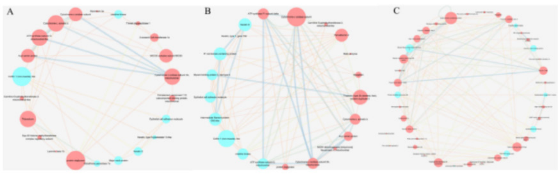

FIGURE 1. Protein-protein interaction network

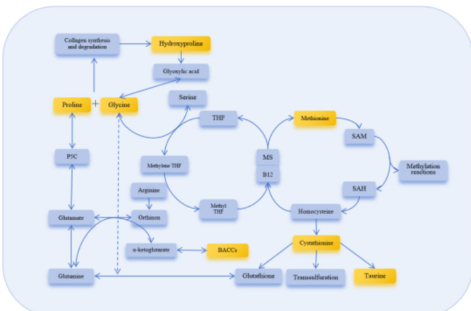

FIGURE 2. Amino acids metabolism pathways.

ADAPTING CNN-BASED INDIVIDUAL IDENTIFICATION TO IDENTIFY FISH INDIVIDUALS UNDER REAL CONDITIONS

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The interest in identifying individual fish within populations has grown in the fields of aquaculture and fisheries management in recent years. The common approach for individual identification is physical tagging, which can affect the fish's growth and welfare. Due to the issues associated with invasive individual fish identification, non-invasive methods are progressively superseding traditional tagging techniques to minimize or eliminate direct interaction with the fish. In this research field, several computer vision-based methods have been developed for individual fish identification. However, these methods have not presented a generalized model for individual fish identification, particularly for long-term applications and real-world conditions. The primary objective of the presented research was to investigate the capability of deep learning techniques as a generalized model for performing real-time individual fish identification task under real-world conditions.

For this study, a closed group of 34 individuals of rainbow trout juveniles (Oncorhynchus mykiss) were cultivated and kept in the fish tanks for over 6 months. After two months, all of the fish were PIT-tagged. One minute video record of the fish with known ID swimming in the aquarium was recorded. The other fish were visible behind (the background scene) the transparent separator to simulate the real-condition environment. The video sampling was conducted using an RGB camera, capturing footage of the aquarium at a resolution of 1280x720 pixels. The camera recorded at a frame rate of 80 frames per second, resulting in smooth motion. The PIT tag reader read the tag of the front fish and recorded it as the corresponding ID. For each fish, 10 frames of video were extracted when the fish was oriented to the left side and had less movement. The upper dorsal fin, eye and fish body detector were trained from the dataset. The detectors were applied to extracted frames, and the rectangular area of fish skin with the highest pigmentation pattern stability was automatically cropped as the region of interest for identification. The dataset for individual identification task consists of 680 images of regions of interest. The triplet network with resnet-50 architecture was used as a classifier to model the distinguishing features of individuals of fish.

The performance of the trained identifier model was 92% accuracy between the last two sessions (after 30 days of fish growth). The evaluation results and performance of the CNN-based model showed that the proposed method is able to learn the long-term invariant pigmentation patterns that provide precise identification of individual fish over a period of time in similar conditions for real-world application.

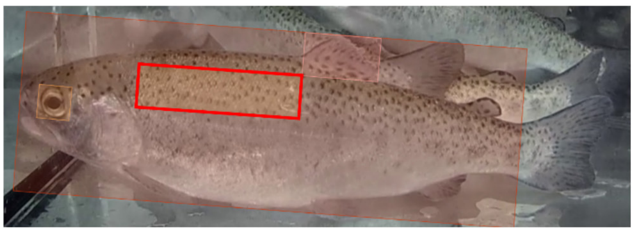

Figure 1. The red rectangle demonstrates the ROI with the highest pattern stability.

FISH REPRODUCTION IN AQUACULTURE - FIVE DECADES OF A FOUNDMENTAL AND TRANSLATIONAL JOURNEY

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As soon as intensive aquaculture platforms started to emerge in the early 1970s, it became clear that the fact that many commercially important fish fail to reproduce in captivity was a critical bottleneck in the industry. In order to enable efficient and cost effective aquaculture, the full life cycle of the fish of interest must be closed in confinement so eggs and juveniles ("seeds") can be available to the growers all year-round. Intensive research in fish reproductive biology ensued, which led to the discovery that captive fish fail to spawn because of a dysfunctional brain hormone, gonadotropin-releasing hormone (GnRH), which is the key regulator of reproduction. This understanding drove the development of a technology to induce spawning in farmed fish, consisting of the exogenous, sustained-release administration of super-potent GnRH analogs (GnRHa). The GnRHa were selected based on their resistance to enzymatic degradation in the fish tissues, increased binding to the pituitary GnRH receptors, and enhanced *in vitro* and *in vivo* bioactivity in triggering the pituitary-gonadal axis leading to ovulation and spawning. This technology has since been optimized for dozens of farmed fish species and used to induce/synchronize final oocyte maturation, ovulation, spermiation and spawning in commercial hatcheries around the world.

In an effort to further understand the fish GnRH system in the context of fish reproduction and aquaculture, many discoveries were made that established fish as a vertebrate reproductive model. Fish have been shown to possess 2-3 forms of GnRH, novel fish GnRHs were discovered, and the functional significance of GnRH multiplicity was studied. New functions of GnRH isoforms were described, including coordinating reproduction with feeding, which may have great relevance to aquaculture. Basic studies also led to the discovery of additional players in the control of reproduction that may ultimately lead to better control of the reproductive cycle in aquaculture, from sex differentiation to puberty and gonadal maturation. The advancement of novel platforms of molecular biology, genomics and biotechnology enables the application of recombinant and gene delivery technologies to the control of reproduction. Germ cell transplantation methods provide innovative strategies for gender control or spawning of fish that are difficult to culture in captivity until maturation.

As much as fertile broodstock are essential for successful seed production, reproductively sterile fish are desirable for optimal grow-out and for genetic containment. The emergence of gene silencing and genome editing platforms paves the way for highly efficient approaches for generating reproductively sterile fish for culture and harvest, while maintaining a fully fertile parent stock for efficient spawning.

STERILITY INDUCTION BY A TRANSIENT GENE SILENCING TECHNOLOGY WITHOUT INTRODUCING A GENETIC MODIFICATION TO FISH

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Farming reproductively sterile fish is the most proficient genetic containment strategy to prevent domesticated escapees from propagating or interbreeding with wild stocks. Sterility carries ecological significance along with economic benefits. Sterilization impedes energy input toward gonadal development, enhancing muscle growth (flesh/fillet yield) and promoting overall performance and health. Sexual maturation is accompanied by deterioration of flesh quality and suppresses the immune system, which increases susceptibility to stress and disease, causing significant economic consequences for aquaculture. Additionally, sterility is a practical means for producers to protect their selectively-bred IP strains from unauthorized propagation.

Aquaculture is becoming increasingly crucial to resolve the current and projected shortfalls in aquatic food production. Thus, an effective environmentally-friendly containment strategy for large-scale commercial aquaculture operations is desperately needed to achieve the environmental sustainability of this industry. We developed a bath-immersion method to produce sterile fish by irreversibly disrupting primordial germ cell (PGC) migration and development. We demonstrated that the Vivo molecular transporter effectively carries the transient gene silencing Morpholino oligomer (MO) across the chorion, entering the embryo and reaching the target PGCs. Indeed, immersion of salmonid eggs in the Vivo-conjugated MO targeting *deadend* (*dnd*), an essential gene in PGC development, effectively disrupted germ cell development and resulted in reproductively sterile fish with minimal gonads that are deprived of germ cells. This technology was successfully applied to salmonid species without introducing any genome modification. Using Dnd-MO-Vivo, 84% and 75% sterility induction was achieved in rainbow trout and Atlantic salmon, respectively. As a substitute for Vivo, we developed a ZP9 molecular transporter that enables the fluorescence labeling of the MO conjugate to advance the immersion technology. Using fluorescence screening, our ongoing experiments focus on optimizing bath immersion and developing a strategy to selectively sort eggs that have sufficient uptake of Dnd-MO to induce sterility (Fig 1).

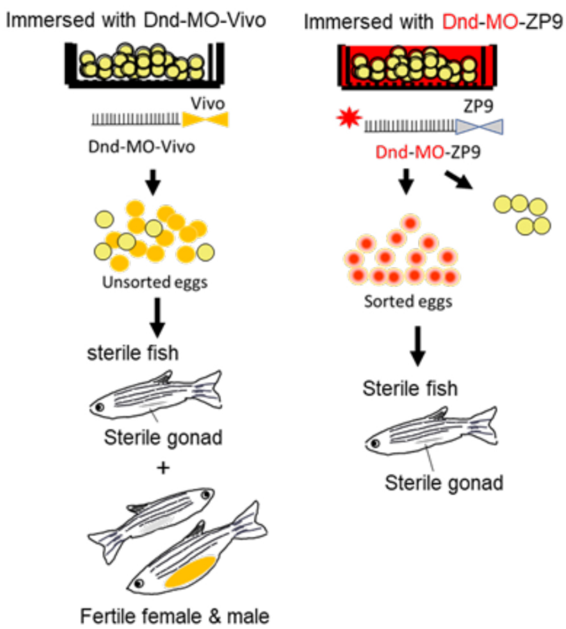

Figure 1. Fluorescence-labelled Dnd-MO-ZP9 assists the optimization of the bath immersion technology and enables the sorting of treated eggs that have sufficient uptake of Dnd-MO to induce sterility.

IMPROVING RAINBOW TROUT (*Oncorhynchus mykiss*) ENVIRONMENTAL FOOTPRINT THROUGH INSECT MEAL USE IN AQUAFEED

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Rainbow trout is one of the most important fish species raised in aquaculture in Europe. From an environmental point of view, one of the main critical points is the use of feed. Several alternative ingredients have been proposed in the feeding of rainbow trout, which have shown benefits from the point of view of zootechnical performance. However, an analysis of environmental impacts is essential to be able to assess the benefits of using these ingredients. The use of an environmental impact analysis based on life cycle assessment (LCA) is presented in this study. The study aims to evaluate the use of insect meal obtained from agricultural wastes and by-products in aquafeed formulation.

As functional unit, 1 ton of fish at the farm gate was used. The processes included in the environmental analysis (**Figure 1**) were: the processing of agricultural waste and by-products; production of BSF larvae; feed production; rainbow trout rearing; and consumption of several inputs such as electricity, oxygen, transports, infrastructures and equipment. The work also estimated the gaseous and wastewater emissions of the insect- and fish-rearing stages, respectively. Primary data were collected from insect rearing trials with different substrates and trout rearing in an Italian farm. Secondary data were used to estimate the impacts of the production of raw materials, materials, transportation, energy, oxygen and emissions, using specific databases (Ecoinvent and Agribalyse) and data from the scientific literature. As environmental impact analysis method, Environmental Footprint (EF) version 3.1 was used.

Feed use remains one of the main hotspots in rainbow trout farming, contributing between 70% and 40% in the impact categories analysed. However, the results show that depending on the type of substrate used for insect meal production, the overall impact of farming is reduced by approx. 5-15% in many of the impact categories analysed. The results of this study support the importance of alternative ingredients, and insect meal in particular to reduce the environmental footprint of rainbow trout aquaculture.

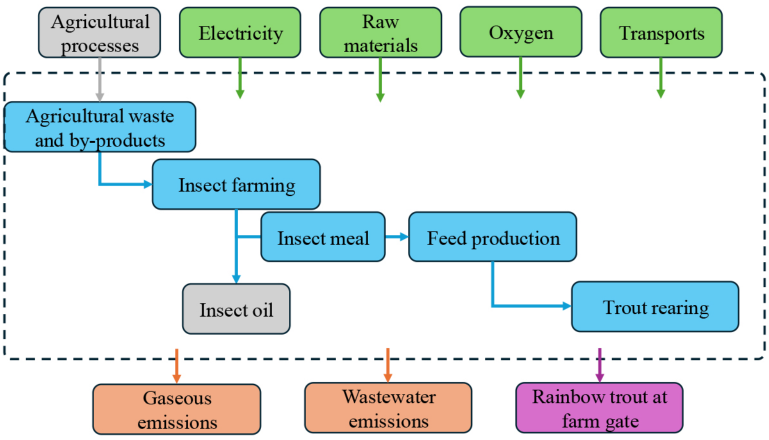

Figure 1. System boundaries considered. Light blue represents the core processes. Light green represents common inputs. Grey represents excluded processes. Orange represents emission. Purple represents output.

A NEW APPROACH TO HOLISTIC SUSTAINABILITY ASSESSMENT IN AQUACULTURE: A CASE STUDY OF AN ITALIAN LAND-BASED FARM

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The sustainability performance of the aquaculture sector, embracing environmental, economic, and social dimensions, is increasingly discussed concerning food production and resource management. To this purpose, the "DEXiAqua" evaluation model is a multicriteria decision analysis tool aiming at comprehensively assessing the environmental, economic, and social impacts of aquaculture systems. This model builds an attribute tree to delineate the complexities of sustainability, partitioning it into distinct branches for environmental, economic, and social aspects, further segmented into "sub-branches." Each branch is characterized by qualitative indicators, based on direct measurement or calculation. The model integrates established scientific methodologies such as Life Cycle Assessment, Life Cycle Costing, and Emergy accounting to calculate indicators, translated into qualitative scales using predefined thresholds. Weightings, termed utility functions, are then applied to aggregate sub-attributes into an overall sustainability score.

The aim of this study is to apply this methodology to an Italian land-based farm specialized in Gilthead sea bream and European sea bass in order to get an overall sustainability score.

The farm consists of several ponds and the production cycle starts with 3 g fry, for further growth till 400/600 g. Annual fish production reaches approximately 450 tonnes, with a Feed Conversion Ratio (FCR) of 2.4. Environmental, economic, and social data were collected by providing a questionnaire directly to the described company. Some of the main data collected include, for example, feed consumption, energy required, oxygen consumed, sales and purchase prices, gender equality, and employee workload.

The farm achieved a 'low' environmental score (2/5), which highlights the environmental impact of traditional land-based aquaculture, particularly due to the high consumption of energy and oxygen. In addition, the use of commercial feed further reduces eco-efficiency due to the lack of reuse of co-products or locally sourced feed. The economic and social sectors score medium (3/5), favored on one hand by average efficiency and viability and working condition, but disadvantaged on the other hand, by a low score in 'local development'. Finally, integrating the three dimensions of sustainability, the overall sustainability score of the company is classified as 'medium-low' (3/7).

Finally, the tool helps to define a strategy for the farm sustainability improvement.

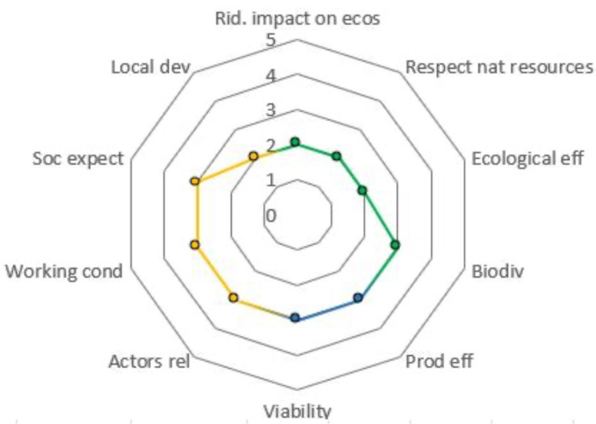

Figure 1: Graphical representation of the scores of the attributes of the penultimate aggregation level. Green refers to environment, blue refers to economic, yellow refers to social dimensions

ADAPTATION OF SEA CUCUMBER *Holothuria tubulosa* TO THE ENVIRONMENTAL CONDITIONS UNDER AND NEAR THE FISH FARM IN THE ADRIATIC SEA

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Sea cucumbers play an important role in the marine ecosystems due to their ability to process organic-enriched sediments, stabilizing the bacterial community and to improve sediment quality. Also, they have an increasing economic value, and therefore are considered as a potential candidate for introduction into IMTA. This research focused on the adaptations of *Holothuria tubulosa* (Gmelin 1791) to the environmental conditions under the influence of intensive aquaculture. Location of the research was a sea bass, sea bream, meagre and amberjack farm in Lamjana bay on the island of Ugljan (Middle part of the Eastern Adriatic). First site was directly under the farm, while the second was 100m away from the edge of the cages, and the reference site was chosen in are unaffected with aquaculture (1.200m away).

As a research method, a circular transect with a diameter of 20 meters was chosen, in which all individuals were counted and measured (total length), and samples were taken from each transect for laboratory analyses. The research was conducted on two occasions, once in July, before the intensive feeding of the fish, and once in November, after the major feeding season. The population structure and influence of organic matter sedimentation was compared between the affected sites compared to the reference site. Population density was significantly higher at the reference point compared to locations below and next to the cage in both periods. However, the average size of the sea cucumbers under and next to the cage was 38 – 45% larger than at the reference point, indicating that the population density is inversely proportional to the length of individuals. In July, the stable isotopes signal (□N15) in the tissue (meat) were significantly higher under and near the cages compared to the reference site (7.08 ‰, 6.78 ‰ and 5.37 ‰, respectively). Similar values were obtained in November, after the summer maximum feed uptake on the farm (6.93 ‰, 7.13 ‰, and 5.67 ‰, respectively).

This research lays the foundation for further research on the life cycle of *H. tubulosa*, especially in the context of the development of IMTA. The population structure and meat analyses indicate that the cucumbers are able to adopt under the fish farm cages. However, further research is necessary to evaluate the potential of sea cucumbers to mitigate the impact from the farm, as well in terms of designing the suitable farming technology integrated along the standard cage farming operations.